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VOLUME 10

ADVANCES IN GENETICS

VOLUME 10

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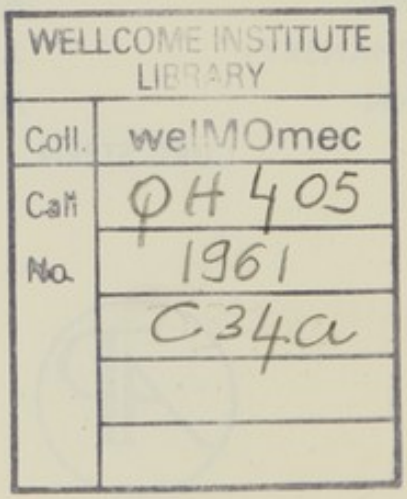
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CONTENTS

Contributors to Volume 10 v

Biochemical Aspects of Chromosome Breakage

B. A. KIHLMAN

I. Introduction 1
II. Oxygen, Respiration, and the Induction of Chromosomal Aberrations . 2
III. Discussion 40
References 51

The Genetics of Transformation

ARNOLD W. RAVIN

I. Introduction 62
II. Chemical Nature of the Transforming Agent 72
III. Competence 94
IV. Penetration 100
V. Genetic Integration and Phenotypic Expression 111
VI. Mechanism of Recombination Occurring in Transformation . . . 131
VII. Mechanism of the Heterocatalytic Function of the Transforming Agent 142
VIII. Interspecific Transfer of Bacterial Genes and Bacterial Evolution . . 147
References 151

Some Contributions to Population Genetics Resulting from the Study of the Lepidoptera

P. M. SHEPPARD

I. Introduction 165
II. Fluctuations in Selective Value 167
III. Industrial Melanism 170
IV. Protective Coloration 177
V. The Evolution of Dominance 188
VI. Sex-Controlled Inheritance 192
VII. The Evolution of Super-Genes 196
VIII. Disruptive Selection 197
IX. Polygenically Controlled Characters in *Euphydryas aurinia* . . . 200
X. Selection and Polygenically Controlled Characters in *Maniola jurtina* 203
XI. Polygenically Controlled Characters in *Panaxia dominula* . . . 209
XII. Summary 211
References 212

Origin and Cytogenetics of the Commercial Potato

M. S. Swaminathan and M. L. Magoon

I. Introduction 217
II. The Relationship between *S. tuberosum* L. and *S. andigena* Juz. et Buk. 219
III. The Nature of Polyploidy in *S. tuberosum* 224
IV. Probable Ancestor of *S. tuberosum* 240
V. Conclusion 247
References 249

Genetic Assimilation

C. H. Waddington

I. Introduction 257
II. Definitions 258
III. Experimental Evidence for Genetic Assimilation 260
IV. The Genetic Mechanisms of Assimilation 264
V. Genetic Assimilation and Developmental Canalization 269
VI. The Baldwin Effect 287
VII. Summary and General Conclusions 289
References 290

Genetics of *Habrobracon*

Anna R. Whiting

I. Introduction 295
II. Sex Types 297
III. Sex Determination 300
IV. Cell Size in Sex Types 304
V. Cytology 306
VI. Mutants 309
VII. Mosaics 310
VIII. Linkage Maps 315
IX. Radiations 316
X. Nitrogen Mustard 340
XI. Colchicine 342
XII. Concluding Remarks 343
References 343

Genetic Aspects of Ommochrome and Pterin Pigments

Irmgard Ziegler

I. Introduction 349
II. The Pigmentary System and Its Chemical Bases 350
III. The Ommochromes 356
IV. The Pterins 368
V. Pleiotropic Action of Genes Affecting Pigment 377

VI. Predetermination 389
VII. Modifications by External Factors 391
VIII. Pterin Mutants in Vertebrates 392
IX. Taxonomic Questions and Concluding Remarks 393
References 395

Author Index 405

Subject Index 415

BIOCHEMICAL ASPECTS OF CHROMOSOME BREAKAGE

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	Page
I. Introduction	1
II. Oxygen, Respiration, and the Induction of Chromosomal Aberrations	2
A. Oxygen-Dependent Chromosome Damage	2
B. Oxygen-Independent Chromosome Damage	34
III. Discussion	40
A. Classification of the Chromosome-Breaking Agents on the Basis of the "Oxygen Effect"	40
B. Delayed and Not-Delayed Breakage	43
C. Deoxyribonucleic Acid Synthesis, Chromosome Splitting, and Chromosome Breakage	44
D. Localized Chromosome Breakage	47
E. The Breakage-First Hypothesis and the Exchange Hypothesis	47
Acknowledgments	51
References	51

I. Introduction

In most cytological studies on the effects of chromosome-breaking agents in plants, the experimental material has been either microspores of the spiderwort, *Tradescantia paludosa* ($n = 6$), or root tips of the broad bean, *Vicia faba* ($2n = 12$). Thus, the effects are studied in haploid cells in *Tradescantia* and in diploid cells in *Vicia*.

Both these materials have their advantages and disadvantages. In *Tradescantia*, the developmental stage of the cells at the time of treatment can be determined rather accurately due to synchronized division of cells in an individual anther, but the material is less suitable for treatments with solutions of radiomimetic chemicals. The treatment of root tips with agents dissolved in water provides no difficulties, but the mitotic stage at the time of treatment can be determined satisfactorily only in cases where the cells have been "marked" before the treatment (Revell, 1953; Kihlman, 1955a; Evans *et al.*, 1957).

The six chromosomes in a *Tradescantia* microspore have a rather similar appearance. The chromosome complement in the root tips of *Vicia faba* consists of five pairs of chromosomes with subterminal centromeres (S-chromosomes) and one pair with median centromeres (M-

chromosomes). In an M-chromosome, a large satellite is separated from the rest of the chromosome by a nucleolar constriction. An M-chromosome is about twice as long as an S-chromosome, the ratio of the total metaphase lengths of the M-chromosomes and the S-chromosomes being 1:2.2 (Revell, 1953). Thus, if breaks occurred at random, the ratio of the total number of breaks in M-chromosomes and in S-chromosomes would be 1:2.2. After treatments with radiomimetic chemicals, the resulting ratios usually diverge greatly from 1:2.2, indicating that breakage is nonrandom.

Other differences exist between the two experimental materials in addition to those already mentioned. The requirements of the problem to be studied would necessarily determine the investigator's choice of the two.

In the past, studies on chromosome breakage were performed mainly from a biophysical point of view (see, e.g., Lea, 1946), when they could not be classified as descriptive cytology. During the last few years, however, the biochemical aspects of chromosome breakage have come to the front. It has been realized that the chromosome is a dynamic rather than a static system, and that biochemical processes are involved in the formation of structural chromosomal changes. Of those biochemical processes, oxidative phosphorylation seems to be one of the most significant.

In the present review article, the main theme has been the effects of oxygen, respiration, and oxidative phosphorylation on the production of chromosomal aberrations by radiations and chemicals. Accordingly, of the known cases of agents with chromosome-breaking effects, only those in which the influence of oxygen has been studied have been included.

II. Oxygen, Respiration, and the Induction of Chromosomal Aberrations

A. Oxygen-Dependent Chromosome Damage

1. *X-rays*

a. *Effect of Oxygen on Breakage.* The significance of oxygen concentration in the chromosome-breaking effects of X-rays appears in studies performed by Thoday and Read (1947) with root tips of *Vicia faba* as experimental material. They found that the frequencies of chromosomal aberrations produced by a given X-ray dosage in nitrogen were about one-third of those produced by the same dosage in oxygen. The fact that the replacement of nitrogen by nitrous oxide, carbon dioxide, or hydrogen had no influence on the effect indicated that it

was the lack of oxygen and not the presence of nitrogen which was responsible for the reduction of the frequencies of X-ray-induced chromosomal aberrations.

The results of Thoday and Read were soon confirmed by a series of investigations performed by Giles and his collaborators with microspores of *Tradescantia paludosa* as the experimental material. The aberrations were studied in the first microspore mitosis. When the irradiations were performed in air or in oxygen, the frequencies of both chromatid (Riley *et al.*, 1952) and chromosome aberrations (Giles and Riley, 1949; Giles and Beatty, 1950) were considerably higher than those obtained when the same X-ray dosage was given in nitrogen, argon, or helium. It was not possible, however, to suppress the X-ray effect completely by removing the oxygen. Even under completely anaerobic conditions, the effect obtained was considerable. The studies by Giles and his collaborators further showed that it was the oxygen concentration during the treatment itself which was significant in the effect; pre- and post-treatments with various oxygen concentrations were without influence on the frequencies of chromosomal aberrations (Giles and Riley, 1950). The effect of oxygen was an immediate one, which appeared from the fact that it was possible to affect the aberration frequency by altering the oxygen concentration during the treatment.

Giles and his collaborators also made attempts to determine in more detail the influence of oxygen concentration on the X-ray effect. As appears from Fig. 1, the experiments showed that the frequency of aberrations increased rapidly when the oxygen concentration in the gas phase was increased from 0 to 10%. Above this level the increase became gradually less marked, being insignificant between 21 and 100% oxygen (Giles and Riley, 1950; Giles and Beatty, 1950; Riley *et al.*, 1952). Since similar relations between oxygen concentration and X-ray effect were obtained using other experimental materials and methods, it seemed as if this relationship was characteristic of the X-ray effect.

Recently, Alper and Howard-Flanders (Alper and Howard-Flanders, 1956; Howard-Flanders and Alper, 1957) found that the lethal effect of X-rays on microorganisms was enhanced by such low concentrations of oxygen as 0.07%. The maximum enhancing effect was reached when the oxygen concentration in the gas phase was about 2.5%. In the experiments of Alper and Howard-Flanders, the gas mixture was passed through the treatment vessel both for a period before and during irradiation. The gas mixture was introduced into the vessel through a sintered glass filter so that it passed through the solution as a vigorous stream of fine bubbles. By this method, a rapid stirring and a large area of interface between liquid and gas was ensured.

Alper and Howard-Flanders (1956) found that the sensitivity of microorganisms to X-rays at different oxygen concentrations could be fitted by the equation:

$$\frac{S}{S_N} = \frac{m[O_2] + K}{[O_2] + K}$$

where S is the sensitivity at the oxygen concentration $[O_2]$ and S_N the sensitivity in the absence of oxygen. The factor m represents the maximum enhancement ratio, or the ratio between a given dose and that dose which in oxygen produces the same effect as the given dose in the absence of oxygen. K is that oxygen concentration in μM which gives

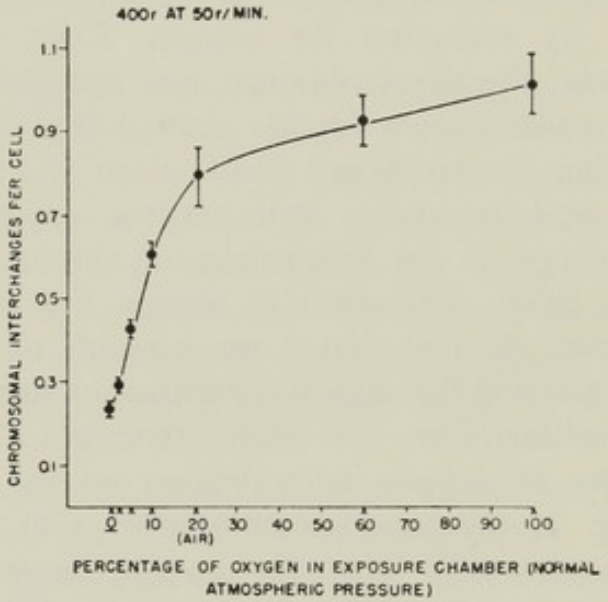


Fig. 1. Relation between percentage of oxygen and yield of X-ray-induced chromosome interchanges in *Tradescantia* microspores. (From Giles and Beatty, 1950.)

a sensitivity of $(m + 1)/2$ times the radiosensitivity in nitrogen. When $m = 3$, as is usually the case when X-rays are used, the sensitivity is increased by the oxygen concentration K to a level halfway between the maximum sensitivity and the nitrogen sensitivity.

In the experiments of Howard-Flanders and Alper (1957), an m-value of 2.92 and a K-value of 4.0 ± 0.4 μM were obtained for the bacterium *Shigella flexneri*. Values of the same order of magnitude were obtained by Howard-Flanders and Alper in experiments with haploid yeast, *Saccharomyces cerevisiae*. The K value for *Shigella flexneri* has subsequently been corrected by Howard-Flanders to 1 μM, however (Howard-Flanders, 1958; Howard-Flanders and Jockey, 1960).

In a study of the influence of oxygen concentration on the frequencies of X-ray-induced abnormal anaphases in mouse ascites tumors, Desch-

ner and Gray (1959) obtained a K value of $5 \pm 2\ \mu M$. Dewey (1960) found $K = 8.5\ \mu M$ for human cells in tissue culture. The K values of Deschner and Gray, and of Dewey are of the same order of magnitude as those obtained by Howard-Flanders and Alper. In all these cases, where the experimental material consisted of suspensions of single cells, oxygen proved to be active in concentrations about thirty times lower than those effective in the experiments of Giles and collaborators. According to Howard-Flanders and Alper (1957), this difference is probably due to the fact that when the experimental material consists of organized tissues, the available oxygen is to a large extent consumed in the outermost cell layers as a result of their respiratory activity. Therefore, at low oxygen concentrations anaerobic conditions are likely to prevail in the central parts of the tissue.

This suggestion of Howard-Flanders and Alper was supported by results obtained by Kihlman (1958b, 1959c, 1961c) in studies on the influence of respiratory inhibitors on the frequencies of chromosomal aberrations produced at different oxygen concentrations by a given X-ray dosage in the root tips of *Vicia faba*. In most experiments cupferron (*N*-nitrosophenylhydroxylamine—ammonium salt) was used as the respiratory inhibitor. Cupferron, a chelating agent, was found to inhibit bean root respiration effectively at pH values between 5 and 6. Some of the results obtained in these studies appear in Fig. 7A.

It was found that in the presence of cupferron an increase of the X-ray effect was obtained at oxygen concentrations as low as 0.1% (corresponding to 1.3 μM in solution at 22°C.). As a rule, the maximum effect was obtained when the oxygen concentration in the gas phase reached 2% (26 μM in solution). In experiments performed in the absence of cupferron, oxygen concentrations below 5% did not produce a significant increase in the frequencies of chromosomal aberrations. A very marked increase was obtained in these experiments at oxygen concentrations between 5 and 21%, and the maximum effect was obtained when the gas phase contained about 50% oxygen. A K value of $4.2 \pm 0.9\ \mu M$ was obtained in the presence of cupferron as compared to about 130 μM in the absence of the inhibitor.

The results indicate that when respiration is inhibited, the same relationship exists between oxygen concentration and X-ray effect in experiments with organized tissues as in experiments with suspensions of single cells. Apparently, oxygen is able to diffuse freely into the central parts of the tissue only when respiration is inhibited.

In addition to cupferron, the respiratory inhibitors azide, cyanide, and carbon monoxide were tested (Kihlman, 1959c, 1961c). Most of the experiments were performed in the presence of 1% oxygen in the gas

phase (about 14 μM in solution at 20°C.). This oxygen concentration, in the absence of respiratory inhibitors, had no effect on the radiosensitivity of bean roots. When respiration was inhibited by azide (at pH 5), cyanide, or carbon monoxide, the frequency of chromosomal aberrations produced by a given dosage of X-rays was of approximately the same order of magnitude in the presence of 1% oxygen as in air.

If the effect of respiratory inhibitors on X-ray sensitivity is to abolish the oxygen gradient existing in organized tissues, they should be effective only in the presence of oxygen. This appears to be the case for all the inhibitors tested (Kihlman, 1959c, 1961c). Furthermore, they should be effective only at oxygen concentrations below 21%, since at higher oxygen concentrations the gradient should have no influence on X-ray sensitivity. However, as appears from Fig. 7A, the maximum effect in the presence of cupferron is considerably higher than that obtained in the absence of the inhibitor. No satisfactory explanation has as yet been obtained for this increase in maximum effect, but apparently it has nothing to do with the oxygen effect.

Recent experiments have shown that cupferron treatments are effective only when given before and during the irradiation (Kihlman, 1961c). Post-treatments with this inhibitor have no effect. Similar results were obtained when carbon monoxide was used as a respiratory inhibitor (Kihlman, 1961c). These results are entirely in agreement with the explanation given above for the effect of respiratory inhibitors on X-ray sensitivity.

Respiratory inhibitors such as azide, cupferron, and cyanide are convenient to use when the experimental material is immersed in an aqueous solution during irradiation, as in the case of bean roots. The difficulties are considerably greater when *Tradescantia* microspores are the experimental material. Of the inhibitors discussed above, only carbon monoxide can be conveniently and effectively applied in this case.

Evans and Neary (1959) have shown, however, that it is possible to do without the inhibitors in *Tradescantia* if isolated germinating pollen tubes are used as experimental material. In their experiments the irradiations were performed 4 hours after the pollen had been sown on slides coated with a lactose-agar medium containing 0.01% colchicine. At this time, the generative nucleus of the pollen grain had passed into the pollen tube. In this material, the oxygen concentration cannot be markedly reduced by respiration and, consequently, no oxygen gradient exists.

Evans and Neary determined the X-ray sensitivity at different oxygen concentrations and obtained a curve (Fig. 2), which is considerably steeper than that obtained by Giles and Beatty (Fig. 1). The K value

found by Evans and Neary was $10.2 \pm 2.8\ \mu M$. According to the calculations of Alper (1956), the curve published by Giles and Beatty (1950) corresponds to a K value of $135\ \mu M$.

How then is the striking ability of low concentrations of oxygen to enhance X-ray sensitivity to be explained?

A reasonable explanation seemed to be that oxygen influences X-ray sensitivity by means of the respiratory chain, i.e., that the oxygen effect is tied up with oxidative metabolism. Actually, such an explanation

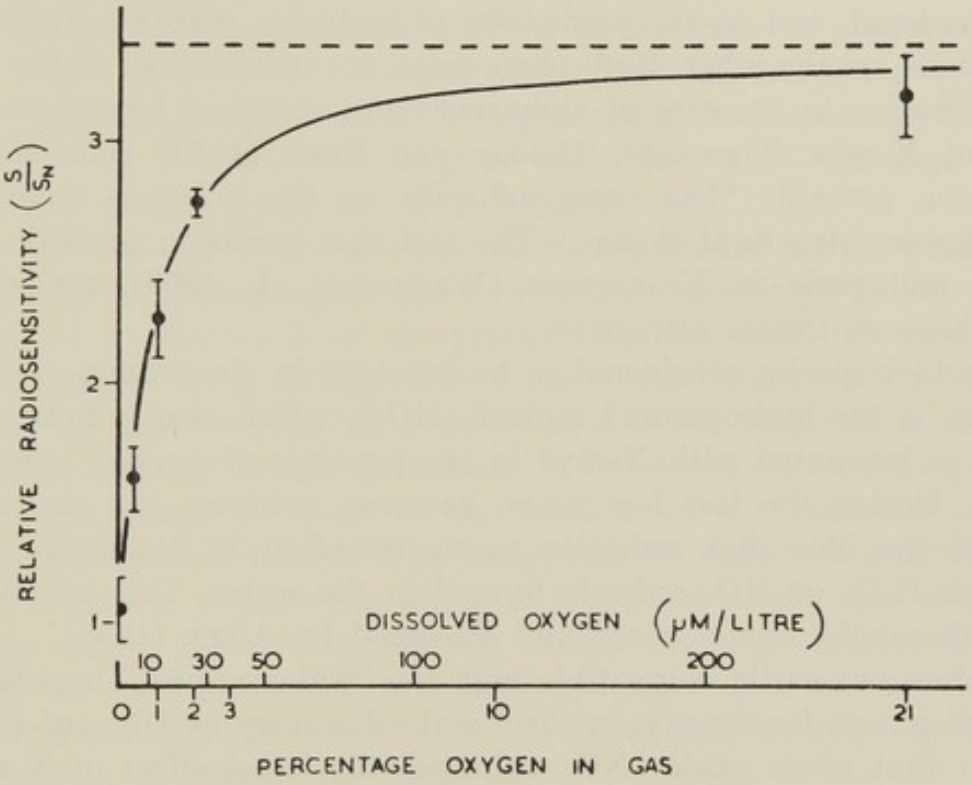


FIG. 2. Relation between percentage of oxygen and the relative yield of X-ray-induced isochromatid aberrations in *Tradescantia* pollen tubes. (From Evans and Neary, 1959.)

appears to be valid for the oxygen effects observed in connection with the production of chromosomal aberrations by certain radiomimetic chemicals (Kihlman, 1955c, 1956; Rieger and Michaelis, 1960a). In the cases of the radiomimetic chemicals referred to, the chromosome-breaking effect was inhibited to the same extent by respiratory inhibitors as by anoxia. However, the X-ray effect on chromosome structure is not suppressed by respiratory inhibitors (Kihlman, 1955c, 1959c), nor is it inhibited by agents such as 2,4-dinitrophenol, which uncouple phosphorylation from respiration (Merz, personal communication; Kihlman, unpublished). Thus, it must be concluded that, in the case of X-rays, oxidative metabolism is not involved in the oxygen effect.

Thoday and Read (1949) found that the production of chromosomal aberrations by alpha rays in root tips of *Vicia faba* was influenced very slightly by oxygen tension. At that time it was known that irradiation of water with X-rays resulted in the production of hydrogen peroxide (H_2O_2) only if the water contained dissolved oxygen. In the case of alpha rays, on the other hand, hydrogen peroxide was formed regardless of whether the water contained dissolved oxygen or not. Thus, there appears to be a striking correlation between the influence of oxygen on the production of chromosomal aberration by ionizing radiations, on the one hand, and on the production of hydrogen peroxide by the same radiations, on the other. Both phenomena are independent of the oxygen concentration in the case of alpha rays and enhanced by oxygen in the case of X-rays. Therefore, Thoday and Read (1949) concluded that hydrogen peroxide "has some influence on the processes involved in chromosome structural change." The fact that hydrogen peroxide proved to be mutagenic in *Neurospora* (Wagner *et al.*, 1950), rendered the hypothesis still more attractive.

Another species considered to be involved in chromosome structural damage is the hydroperoxyl radical (HO_2), which also is formed when water is irradiated with X-rays in the presence of oxygen (e.g., Gray, 1953). During the last few years, however, evidence has accumulated against the idea that radiation exerts its effect in biological systems through H_2O_2 or HO_2-radicals formed in the water. This evidence has been thoroughly summarized and discussed by Alper (1956).

A fact not easily compatible with the "water radical" hypothesis as an explanation for the oxygen effect is the discovery by Howard-Flanders (1957) that nitric oxide (NO) enhances the lethal effect of X-rays on bacteria to the same extent as oxygen. Subsequently, Howard-Flanders also studied the enhancing influence of NO on the radiation sensitivity of yeast (Howard-Flanders and Jockey, 1960). He points out (Howard-Flanders, 1958; Howard-Flanders and Jockey, 1960) that NO reproduces the influence of oxygen on the radiation effect both qualitatively and quantitatively.

These results have been confirmed by Gray *et al.* (1958), Kihlman (1958a, 1959d), and Dewey (1960), using mouse ascites tumor cells, *Vicia* root tips, and human cells, respectively, as experimental materials.

In dry systems NO seems to have the opposite effect. Powers *et al.* (1959, 1960) found that NO protected dry spores of *Bacillus megaterium* against X-ray damage, and a similar effect of NO was found by Sparrman *et al.* (1959) for dry seeds of *Agrostis stolonifera*.

Howard-Flanders (1958, 1959) has also provided a possible explanation for the oxygen and nitric oxide enhancement of X-ray damage, and

for the fact that the effect of alpha radiation is independent of oxygen concentration. According to this hypothesis, two types of primary changes are induced by ionizing radiation in those molecules of the cell which are essential for multiplication. One of the two types results in a permanent and lethal damage only in the presence of oxygen (or nitric oxide), whereas the other results in a lethal effect regardless of whether oxygen is present or not. In principle, the oxygen-dependent change may be produced when the number of ions formed (i) along a segment of length t of the track of the ionizing particle, fulfills the condition $r \leqslant i < n$, while an oxygen-independent injury may be produced when $i \geqslant n$. Which of the two types will predominate is, therefore, dependent on the ion density of the radiation. Alpha radiation is a densely ionizing radiation, and, therefore, the relative probability that a change of the oxygen independent type will occur is high.

The reasons that n or more ionizations are needed for the production of an oxygen-independent change may be the following:

Apparently, the result of the primary change, which Howard-Flanders assumes to be a carbon radical, has a very short lifetime so that the original configuration of a radiation-affected molecule in the cell is usually restored within a fraction of a second. A prerequisite for permanent damage to arise is the reaction of the radiation-induced radical with another radical. The latter may be radiation-induced, such as an OH-radical produced in water, or it may be stable, such as oxygen or nitric oxide. When the radiation is sparsely ionizing, the distance between the radicals formed is usually too great for a reaction between them to occur. Therefore, the effect is dependent in this case on the concentration of oxygen (or nitric oxide) molecules in the cell. In the case of alpha rays, on the other hand, the radicals are formed closely enough for reactions between them to occur, and the effect becomes more or less independent of oxygen concentration. Oxygen and nitric oxide owe their radical properties and ability to react with carbon radicals to the fact that they possess unpaired electrons in π molecular orbitals.

b. *Effect of Oxygen on Rejoining.* It was stated above that the effect of oxygen on the production of chromosomal aberrations by X-rays is not tied up with the oxidative metabolism of the cell (see page 7). However, Wolff and Luippold (1955) have shown that, under certain conditions, the frequencies of chromosomal aberrations produced by a given dose of X-rays are influenced by oxidative metabolism. Such an influence is observed when the dosage is given in fractions or when the dose rate is low.

The fundamental studies by Sax (1939, 1940, 1941) demonstrate that

the frequency of exchanges produced by a given X-ray dosage is dependent on the dose rate. When the duration of the irradiation period is prolonged beyond a certain limit, the frequency of exchanges decreases. The time of irradiation necessary to produce this decrease appears to be different in different organisms, types of cells, and division stages. Studies by Sax (1939, 1941) and Fabergé (1940) have shown that a similar decrease of the X-ray-induced exchanges can be obtained by fractionating the dose.

The decrease in frequency of exchanges obtained when the dose is fractionated or the dose rate decreased may be explained on the basis of the breakage-first hypothesis in the following way:

Exchanges can be formed between breaks that are open at the same time and close enough in space. Breaks remain open only for a relatively short time. When the dose rate is high, all the breaks produced are open at the same time and numerous exchanges occur. When the dose rate is low, on the other hand, the chances for exchanges to occur are less, since breaks produced at the beginning of the irradiation period have had time to rejoin before the irradiation is finished and are, therefore, unable to take part in exchanges with breaks produced toward the end of the irradiation period. The decrease obtained by dose fractionation is similarly explained as due to the rejoining of breaks produced by the first fraction of the dose before the second fraction is given. The fact that dose-rate and fractionation effects are found in the case of X-rays indicates that the two breaks involved in an exchange are, as a rule, formed by two separate ionizing particles at high dose rates. In the case of fast neutrons, the two breaks involved in an exchange appear to be induced by the same particle, since no dose-rate or fractionation effects have been observed.

Wolff and his collaborators (Wolff, 1954, 1960a; Wolff and Atwood, 1954; Wolff and Luippold, 1955, 1956a, 1956b) have performed detailed studies on the effects of dose rate and dose fractionation on the frequencies of "two-hit aberrations" in the root tips of *Vicia faba*. In these experiments, seeds were irradiated after having been soaked in water for 18 to 24 hours. The effect was studied in the first root tip mitosis. All the aberrations produced by this method were of the chromosome type.

The effect of the duration of irradiation on the number of "two-hit aberrations" is shown in Fig. 3. When the time of irradiation was prolonged from ½ to 1 minute, the number of aberrations was diminished by approximately one-third. This happened both in the presence and in the absence of oxygen. The frequency of "two-hit aberrations" was unaffected when the duration of exposure was prolonged from 1 to 120

minutes under aerobic conditions and from 1 to 30 minutes under anaerobic conditions. When the time of irradiation for the constant dosage was prolonged from 120 to 180 minutes in the presence of oxygen, and from 30 to 60 minutes in the absence of oxygen, the yield of aberrations was diminished by one-half in both cases. According to Wolff and Luippold (1956b), the results indicate that two types of breaks are produced by radiation: a fast rejoining type, which rejoins within one minute, and a more slowly rejoining type. Breaks of the latter type rejoin faster when induced under anaerobic conditions. A

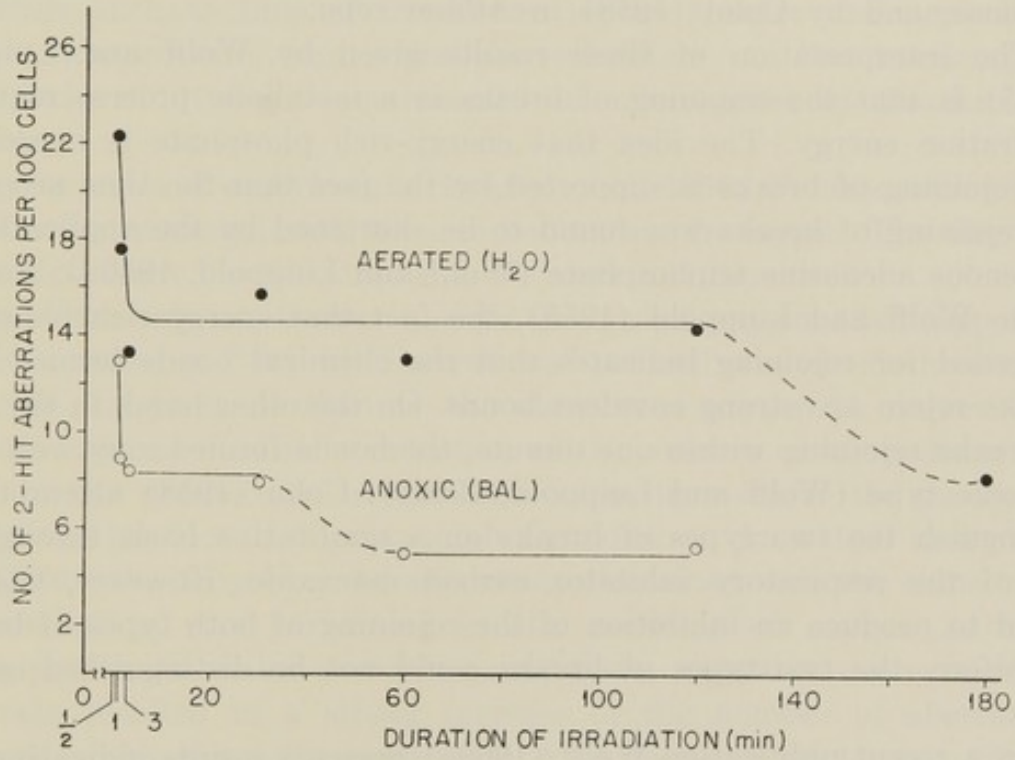


FIG. 3. Two-hit aberrations produced in *Vicia* by 600 r of X-rays at different dose rates in the presence and absence of oxygen. (From Wolff and Luippold, 1956b.)

more rapid rejoining of breaks produced under anaerobic conditions, compared to those induced in the presence of oxygen, can also be observed when the dosages have been adjusted so as to produce effects of the same magnitude in both cases. Wolff and Atwood (1954) concluded that X-ray irradiation produces two independent effects, both of which are dose-dependent. One is chromosome breakage, the other is a damage to the "rejoining system." The protection afforded by anoxia is greater in the latter case.

Wolff and Luippold (1955) have further shown that the time during which breaks of the slowly rejoining type stay open is dependent on the oxidative metabolism of the cell. In their experiments the dosage

was divided into two fractions. The first fraction was given *in vacuo* to produce breaks that normally remain open for 30 minutes. The other fraction was given 75 minutes later in air. Wolff and Luippold then found that when the soaked bean seeds were treated between the two dose fractions with respiratory inhibitors, such as carbon monoxide (CO) in the dark and cyanide, or with agents which uncouple phosphorylation from respiration, such as 2,4-dinitrophenol, the fractionation effect disappeared, i.e., breaks produced by the first dose fraction interacted with breaks produced by the second. Qualitatively similar results were later obtained by Wolff and Luippold (1958) in *Tradescantia paludosa,* and by Cohn (1958) in *Allium cepa.*

The interpretation of these results given by Wolff and Luippold (1955) is that the rejoining of breaks is a metabolic process requiring respiration energy. The idea that energy-rich phosphate is needed for the rejoining of breaks is supported by the fact that the time necessary for rejoining of breaks was found to be shortened by the application of exogenous adenosine triphosphate (Wolff and Luippold, 1956a). According to Wolff and Luippold (1955), the fact that energy-rich phosphate is needed for rejoining indicates that the chemical bonds formed when breaks rejoin are strong covalent bonds. On the other hand, in the cases of breaks rejoining within one minute, the bonds formed may well be of an ionic type (Wolff and Luippold, 1956b). Cohn (1958) attempted to distinguish the two types of breaks on a qualitative basis through the use of the respiratory inhibitor carbon monoxide. However, CO was found to produce an inhibition of the rejoining of both types of breaks. Therefore, the two types of breaks could not be distinguished on this basis.

In a recent publication Wolff (1960a) presents results indicating that protein synthesis is necessary for rejoining to occur. The synthesis of ribonucleic acid and deoxyribonucleic acid does not appear to be involved. These results seem to indicate that the bonds formed when breaks rejoin are protein links.

Wolff's findings are most interesting, and during the last years several authors have based the interpretations of their results on them. It should be pointed out, however, that the interpretation of Wolff's results is dependent upon whether the breakage-first hypothesis or the exchange hypothesis of Revell (1959) is accepted as the basis. Wolff has interpreted his results in terms of breakage and rejoining according to the breakage-first hypothesis. If this hypothesis is accepted, there can hardly be any objections to his interpretations.

The implications of Wolff's results are different, however, if they are interpreted on the basis of the exchange hypothesis. According to

this hypothesis (see pages 48–50), the process requiring respiration energy (and protein synthesis) will be the repair of the primary event. Since the primary event is not a break, but some other kind of localized disturbance in the chromosome, Wolff's results, if interpreted according to the exchange hypothesis, do not permit any conclusions regarding the nature of the bonds broken in a chromosomal break.

Of those studies in which the results have been interpreted on the basis of Wolff's findings, those by Beatty and his collaborators should be discussed.

Their main research efforts have been to determine the influence of factors such as time of irradiation in the absence and presence of oxygen, temperature, and metabolic inhibitors on the frequency of X-ray-induced "two-hit aberrations" in microspores of *Tradescantia paludosa*.

In one series of experiments (Beatty *et al.*, 1956) *Tradescantia* microspores were irradiated with 400 r in helium and in oxygen. The dose rate was varied from 1 r per minute to 50 r per minute.

At a dose rate of 50 r per minute the typical oxygen effect appeared, i.e., the number of "two-hit aberrations" per cell was about three times higher in the presence than in the absence of oxygen. A reduction of the dose rate resulted in a reduction of the number of aberrations produced in the presence of oxygen and in an increase of the number of aberrations produced under anaerobic conditions. At a dose rate of 2 r per minute the frequency of aberrations obtained was the same when the irradiation was performed in the presence of oxygen as when it was performed under anaerobic conditions. A further decrease of the dose rate resulted in a strong increase of the number of aberrations produced under anaerobic conditions. At a dose rate of 1 r per minute, 2.5 times more aberrations were obtained under anaerobic conditions than in oxygen.

Furthermore, Beatty *et al.* (1956) were able to show that the effect obtained in helium was dependent on the time during which the microspores were kept in helium. When the microspores were exposed to 400 r of X-rays at a dose rate of 50 r per minute, after pretreatments in helium for periods from 16 to 400 minutes, the effect obtained increased with the duration of the period in helium. The effect obtained when the duration of the pretreatment was 200 minutes, and the dose rate 50 r per minute, was of the same magnitude as that obtained when the microspores were irradiated in helium with 400 r at a dose rate of 2 r per minute.

Beatty *et al.* (1956) have explained their results on the basis of the findings of Wolff and Luippold (1955). They believe that the frequency

of "two-hit aberrations" is increased when the microspores are pretreated in helium for periods of 80 minutes or more, because rejoining of breaks is inhibited as a result of energy lack. As a result of this inhibition of rejoining, all the breaks produced by the irradiation are open at the same time and are able to interact when close enough in space.

Subsequently, Beatty and Beatty (1957) studied the influence of carbon monoxide (CO) on the frequency of "two-hit aberrations" produced by X-rays in helium and in the presence of various concentrations of oxygen. The CO had no effect on the frequency of aberrations produced in the absence of oxygen. In the presence of oxygen, on the other hand, CO enhanced considerably the frequency of aberrations produced by 400 r at a dose rate of 50 r per minute. The enhancement was most marked at low oxygen concentrations. The curves obtained when the effects in the presence and absence of CO are plotted against oxygen concentration (Fig. 4) are, in fact, very similar to those obtained by Kihlman (1959c) in experiments where root tips of *Vicia faba* were exposed to X-rays at various oxygen concentrations in the

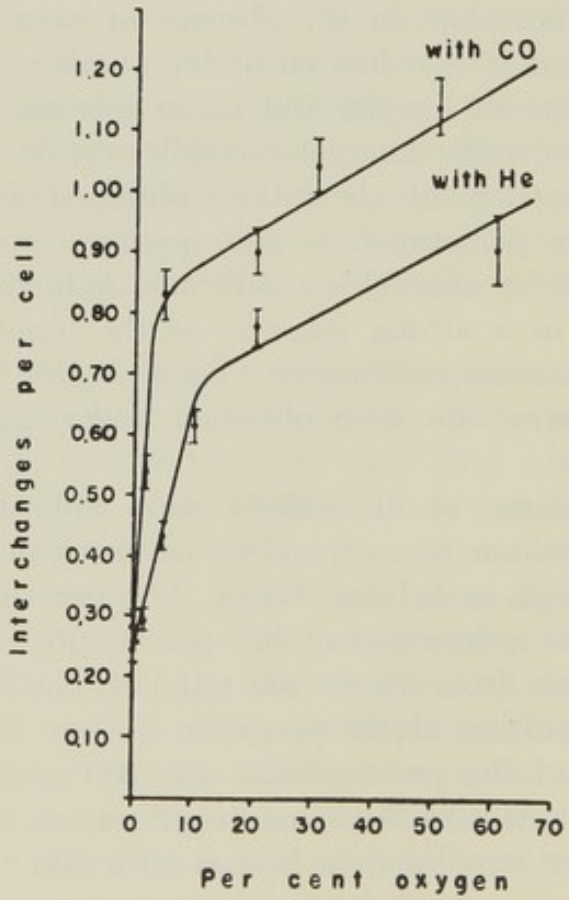


Fig. 4. Relation between percentage of oxygen and X-ray-induced chromosome interchanges in the presence and absence of carbon monoxide (*Tradescantia* microspores). (From Beatty and Beatty, 1957.)

presence and absence of the respiratory inhibitor cupferron (compare Fig. 7A). Subsequently, Kihlman (1961c) found that CO has a similar effect on the X-ray sensitivity of *Vicia* roots.

Kihlman (1959c) has explained his results as due to the fact that cupferron, by inhibiting respiration, prevents the formation of an oxygen gradient in the roots at low oxygen concentrations. Beatty and Beatty (1957) believe, however, that their findings are best explained as a result of an inhibition of the rejoining of breaks. As a result of the CO inhibition of respiration, the energy supply of the cell is decreased. The rejoining of breaks would then be delayed because of the lack of energy. The CO enhancement of the frequency of "two-hit aberrations" was light-reversible, which indicated that cytochrome oxidase was involved.

The explanation for the CO effect given by Beatty and Beatty is supported by their finding that the CO treatment is as effective when given immediately after, as when given before and during, the irradiation Beatty and Beatty, 1957). In a recent publication, Beatty and Beatty (1960) report that the CO post-treatment does not become ineffective until the period between irradiation and CO treatment is 10 minutes or more. In Kihlman's (1961c) experiments, cupferron and CO were ineffective when given as post-irradiation treatments.

The findings of Beatty and Beatty that CO is effective also when given as a post-treatment indicate that the rejoining hypothesis, rather than the oxygen-gradient hypothesis, accounts for the CO effect on the frequency of X-ray-induced "two-hit aberrations" in *Tradescantia* microspores. However, since the experiments of Evans and Neary (1959) suggest that the true K value for *Tradescantia* is considerably lower than that which can be calculated on the basis of the X-ray data obtained by Beatty and Beatty in the absence of CO, the existence of an oxygen gradient in the anthers should be considered.

Beatty and Beatty (1959) also studied the influence of temperature on the frequency of "two-hit aberrations" produced by 400 r of X-rays (50 r per minute) under anaerobic conditions and in the presence of 5% oxygen in the microspores of *Tradescantia*. Previously, Giles *et al.* (reference by Giles, 1954) had found that at high temperatures the oxygen effect was reversed, i.e., the effect of a given dose was stronger in the absence than in the presence of oxygen.

In those experiments of Beatty and Beatty (1959) which were performed in the presence of 5% oxygen, the pressure was adjusted in such a way that the oxygen concentration in solution was the same at all temperatures. They then found that the temperature had no effect between 45 and 20°C. At temperatures below 20°, however, the

effect increased when the temperature was lowered. In the series of irradiations performed in the absence of oxygen, on the other hand, the effect increased with the temperature.

According to Beatty and Beatty (1959), only the rejoining of breaks is affected by temperature. In the presence of oxygen the increased effect obtained at low temperatures would be due to the inhibition of oxidative phosphorylation by these temperatures. The reduced energy supply is believed to delay the rejoining of breaks, a condition which would favor new reunions over restitutions. CO in the dark, like low temperatures, inhibits respiration. When the irradiations were performed in the presence of 5% oxygen, the effect obtained at 0.3°C. was the same in the presence as in the absence of CO. At 10° the effect obtained in the presence of CO was of the same magnitude as the effect obtained at 0.3°, whereas that obtained in the absence of CO was lower.

These experiments show that respiration is involved in the temperature effect. However, as pointed out by Deschner and Gray (1959), the influence of respiration on X-ray-induced chromosome damage in organized tissues may be not only that it provides the energy necessary for rejoining, but also that it causes an oxygen gradient to develop within the tissue at such low oxygen concentrations as 5%. Because of this oxygen gradient, it is possible that a considerable number of the microspores in the anthers are irradiated under anaerobic conditions, even though the surrounding gas phase contains 5% oxygen. At 0.3°C., or in the presence of CO, there is very little respiration and the oxygen gradient disappears. Experimental materials which lack oxygen gradients have only a slight positive temperature effect, i.e., the X-ray sensitivity increases somewhat with temperature (Deschner and Gray, 1959).

The explanation suggested by Deschner and Gray is very plausible but appears to be contradicted by the findings of Beatty and Beatty (1957, 1960) that CO is as effective when given as a post-treatment as when given before and during the irradiation.

Beatty and Beatty (1959) believe that the increase of aberration frequency obtained under anaerobic conditions by raising the temperature also can be explained as an effect on the rejoining of breaks. In this case, the enhancing effect is not obtained as a result of a decrease in the generation of energy, but as a result of an increased utilization of energy. According to Beatty and Beatty, there exists in the cell a competition for the available energy. Such processes as are necessary for the maintenance of the life of the cell have priority. At low temperatures all the available energy is not consumed by the vital processes and the excess can be used for the rejoining of breaks. Therefore, the effect

produced by the given dose is low. When the temperature is raised, the metabolic activities and energy requirements of the cell are also increased and there will be less energy left over for the rejoining of breaks. Since more breaks are open at the same time, the frequency of "two-hit aberrations" will increase.

In connection with a review of the influence of oxidative phosphorylation on the rejoining of breaks, the enhancing effect of infrared (e.g., Swanson, 1949; Yost, 1951) and far-red (Withrow and Moh, 1957) on chromosome damage by X-rays should be mentioned, since this effect has been explained on the basis of the findings of Wolff and Luippold.

It has been suggested that the enhancing effect of far-red is due to its ability to inhibit phosphorylation and, as a result, the rejoining of breaks (Gordon and Surrey, 1958, 1960; Moh and Withrow, 1959a, 1959b). This idea has been criticized by Yost and Robson (1959) on the ground that the inhibition of phosphorylation produced by far-red is far too slight to account for the substantial enhancement of X-ray breakage.

According to Wolff (1960b), the effect of far-red on chromosome breakage in *Vicia* is simply a reflection of the mitotic inhibition produced by the far-red radiation.

2. *Visible Light-Acridine Orange*

Since the beginning of this century, visible light has been known to induce inhibitory and lethal effects on living organisms when oxygen and some sensitizing substance are present. This phenomenon has been called "photodynamic action." The function of the sensitizing substance, which is usually a dye, is to absorb the light energy. The photodynamic action has been interpreted as a photosensitized oxidation of organic material (Blum, 1941; Clare, 1956).

Recent experiments have shown that structural chromosomal changes can be induced by visible light in the root tips of *Vicia faba* (Kihlman, 1959a, 1959e). The sensitizing substance used in these studies was acridine orange (G. T. Gurr, London). Like other acridine derivatives (Bauch, 1949; DeBruyn *et al.*, 1953), acridine orange has a strong affinity to nucleic acids (Armstrong, 1956; Beers *et al.*, 1958), one of the main components of the chromosome. For structural chromosomal changes to be obtained, oxygen had to be present in addition to light and acridine orange. A significant effect was not obtained unless the gas phase contained more than 20% oxygen. The effect of visible light in the presence of acridine orange and oxygen appears to be independent of temperature.

Since it had been found that chromosome damage by X-rays was

influenced by much lower concentrations of oxygen when the respiration of the bean roots was inhibited (see pages 5 and 6), a number of experiments with light were performed in the presence of 400 μM of the respiratory inhibitor cupferron. The rather surprising result of these experiments was that the strongest effect was obtained under anaerobic conditions. When the gas phase contained 20% oxygen or more, the effect obtained was the same in the presence as in the absence of cupferron (Fig. 7D). When the roots were not pretreated with acridine orange, or when the experiments were performed in the absence of light, the cupferron treatments did not result in any chromosomal aberrations under anaerobic conditions.

As described above (page 8), nitric oxide (NO) has proved to be as efficient an enhancer of the X-ray effect as oxygen. In experiments where chromosomal aberrations are produced by visible light, it also appears as if oxygen can be replaced by NO.

Furthermore, the experiments of Kihlman showed that only such wavelengths of visible light as were absorbed by acridine orange were able to induce structural chromosomal changes. This was found irrespective of whether oxygen, nitric oxide, or cupferron was the "third" factor (the other two necessary factors being light and acridine orange).

It was pointed out above that a photodynamic action is believed to occur in the presence of oxygen only, and that it has been interpreted as a photosensitized oxidation of organic material. But if a photodynamic action can occur in the presence of oxygen only, then the light-induced chromosomal aberrations cannot have arisen as a result of a photodynamic action, because a similar effect was obtained when oxygen was replaced by nitric oxide or by cupferron.

The effect of NO, cupferron, and oxygen was also studied in connection with the light-induced inactivation of *E. coli* phage T3 in the presence of acridine orange. This photodynamic effect, which had previously been studied by Yamamoto (1958), is extremely sensitive to oxygen. In the complete absence of oxygen the inactivation is negligible; but even in the presence of traces of oxygen a strong inactivating effect is obtained. In this typical photodynamic reaction, NO and cupferron were unable to replace oxygen (Kihlman, 1961a).

As long as it was not known that NO and cupferron were able to replace oxygen in connection with the induction of structural chromosomal changes by visible light, it was reasonable to assume that the chromosomal aberrations had been produced by organic peroxides formed photodynamically in the cell (Kihlman, 1959a). It had previously been shown that organic peroxides have radiomimetic effects in *Vicia* root tips (Loveless, 1951; Kihlman, 1957) and it is also known

that organic peroxides are formed in connection with photosensitized oxidations of organic compounds (Schenck, 1955; Schenck *et al.*, 1957). However, the fact that cupferron and NO appear to have the same effect as oxygen renders the peroxide hypothesis less attractive.

In order to investigate the mechanisms underlying the activities of oxygen, NO, and cupferron in the light-acridine orange system, a number of compounds with some chemical property or properties in common with the active agents were tested (Kihlman, 1961a). The agents tested included radical scavengers such as iodine, substituted ethylenes, and quinones (Schenck *et al.*, 1959); paramagnetic salts; chelating agents; nitroso compounds; respiratory inhibitors; and radiomimetic agents such as X-rays, nitrogen mustard, maleic hydrazide, and 8-ethoxycaffeine. In these experiments the radical scavengers, chelating agents, radiomimetic agents, respiratory inhibitors, and paramagnetic salts tested were all inactive, as were six of the eight nitroso compounds tested. The two active nitroso compounds were the nitrosamines, methylphenylnitrosamine and diphenylnitrosamine. Among the inactive nitroso compounds were the two related nitrosamines, dimethylnitrosamine and diethylnitrosamine. A slight effect was obtained with sodium nitrite.

Some typical effects obtained in the presence of oxygen, methylphenylnitrosamine, or cupferron appear in Table 1.

TABLE 1

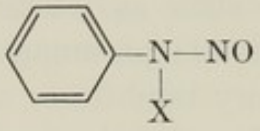
The Induction of Chromosomal Aberrations in *Vicia faba* by Visible Light-Acridine Orange in the Presence of Oxygen, Methylphenylnitrosamine, or Cupferron *

Active chemical present (conc. in μM)	Pretreatment with acridine orange (+ or −)	Illumination (+ or −)	Abnormal metaphases (%)	Aberrations per 100 cells		
				I Isolocus breaks	II Exchanges	I + II
Oxygen (1300)	+	+	22	24	3	27
	−	+	0	0	0	0
	+	−	0	0	0	0
Methylphenyl-nitrosamine (400)	+	+	31	17	26	43
	−	+	1	1	0	1
	+	−	1	0	1	1
Cupferron (400)	+	+	47	45	33	78
	−	+	1	1	0	1
	+	−	0	0	0	0

* Duration of illuminations = 30 minutes. The roots were fixed 26 hours after the treatment. One hundred cells were analyzed after each treatment. (From Kihlman, 1961a and unpublished data.)

The fact that methylphenylnitrosamine and diphenylnitrosamine were as active as cupferron shows that the effect of cupferron in the light-acridine orange system has nothing to do with its chelating properties. Methylphenylnitrosamine and diphenylnitrosamine are not chelating agents. The same conclusion can be drawn from the fact that the chelating agents tested were inactive.

The three nitroso compounds active in the light-acridine orange system are all phenylnitrosamines with the general structure:



where X is methyl in methylphenylnitrosamine, phenyl in diphenylnitrosamine, and hydroxyl in nitrosophenylhydroxylamine, the acid corresponding to the ammonium salt cupferron. The significance of the NO-group appears from the fact that when a methyl group is substituted for the NO-group in methylphenylnitrosamine, an inactive compound (dimethylaniline) is obtained.

It was mentioned above that the effect of cupferron in the light-acridine orange system is counteracted by oxygen. A similar antagonism was observed between oxygen and the other phenylnitrosamines.

It seems possible that the effect of the phenylnitrosamines, as well as that of sodium nitrite, is due to the formation of nitric oxide in the cell from these compounds. Perhaps the light energy absorbed by acridine orange can be used for the cleavage of the N–NO bond in the phenylnitrosamines. The possibility cannot be excluded, however, that the phenylnitrosamines act as such with organic radicals in the cell.

Thus, the experimental results indicate that there are only two active compounds in the light-acridine orange system: oxygen and nitric oxide, i.e., the same molecules which are able to enhance the X-ray sensitivity. The experiments have also shown that the light-acridine orange system does not cause the chromosomes to become more sensitive to the effects of chromosome-breaking agents. As mentioned above, the effects of the eight chromosome-breaking agents tested were unaffected by the light-acridine orange system. The negative results obtained in the experiments with the paramagnetic salts indicate that the effect of oxygen and NO is not due to their paramagnetic properties.

At present, the working hypothesis is that the effect of oxygen and NO in the light experiments, as in the X-ray experiments, is due to their reaction with radicals produced in the cell by the radiation. However, a difference between the effect of visible light and the effect of X-rays is that the former is delayed, i.e., the structural chromosomal changes

produced in *Vicia faba* root tips do not begin to appear in metaphase until about 8 hours after the treatment. As always seems to be the case when the effect is delayed, the aberrations are exclusively of the chromatid type. As a matter of fact, the effect of the light-acridine orange system is very similar to that obtained after treatments with radiomimetic chemicals such as nitrogen mustard, maleic hydrazide, and potassium cyanide. In contrast, the effect of X-rays is not delayed, i.e., chromosomal aberrations appear about 2 hours after irradiation and the aberrations are of the chromatid or chromosome type, depending on the stage of the mitotic cycle at which the cells were irradiated. The explanation for this difference between the effects of visible light and X-rays could be that the effect of visible light is, in contrast to that of X-rays, dependent on the localization and mode of reaction of the sensitizing substance, the chemical acridine orange.

3. *Potassium Cyanide*

The radiomimetic effect of potassium cyanide (KCN) was discovered by Lilly and Thoday (1956) and was described in more detail by Lilly (1958) and by Kihlman (1957, 1959b). These authors found structural chromosomal changes in root tips of *Vicia faba* after 30-minute treatments with solutions containing 500 μM KCN. No effect was obtained under anaerobic conditions.

The effect of KCN is delayed and the aberrations are of the chromatid type (Kihlman, 1957). KCN produces some stickiness, which can be seen 0 to 4 hours after the treatment. A few isolocus breaks (Thoday, 1951) can be seen during the first 8 hours, but the majority of the aberrations occur about 24 hours after the treatment. The breaks induced by KCN appear to be localized in heterochromatic segments of the chromosomes.

Like other radiomimetic agents, KCN appears to be active only when its concentration exceeds a certain threshold value. In the case of KCN, however, an upper limit also seems to exist. When this limit is reached, an increase of the KCN concentration or of the time of exposure is without influence on the magnitude of the effect (Kihlman, 1957).

In contrast to the effect of the majority of the radiomimetic agents, the effect of KCN seems to be independent of the temperature during the treatment (Kihlman, 1957).

In the absence of oxygen KCN appears to be inactive. At oxygen concentrations below 10% in the gas phase the effect is negligible; above 10% the effect increases with oxygen concentration (Fig. 7C).

When the radiomimetic effect of a chemical compound is dependent

on the oxygen concentration, the explanation is often that oxidative phosphorylation is required for the activity of the agent. In these cases, the effect is inhibited by 2,4-dinitrophenol (DNP). Pretreatments with DNP were without influence on the effect of KCN, however, which indicates that the oxygen effect in the case of KCN has nothing to do with oxidative phosphorylation. This was not to be expected, either, because KCN itself as a respiratory inhibitor suppresses phosphorylation.

Lilly and Thoday (1956) suggest that the chromosomal aberrations obtained after treatments with KCN are produced by hydrogen peroxide, a compound which is believed to accumulate in the cell in connection with cyanide treatments. According to Wyss *et al.* (1948) and King *et al.* (1952), an increase of the hydrogen peroxide concentration in the cell is to be expected as a result of the cyanide inhibition of the cytochrome oxidase, catalase, and peroxidase enzymes.

There are, however, results which are not compatible with the idea that the KCN effect is mediated by hydrogen peroxide. Such results are the findings of Loveless (1951) and Kihlman (1957) that hydrogen peroxide does not produce any chromosomal aberrations in the root tips of *Vicia faba*.

The fact that hydrogen peroxide is not likely to be the agent responsible for the radiomimetic effect of KCN does not exclude the possibility that organic peroxides are involved. As mentioned when the effect of visible light was described, organic peroxides are known to have radiomimetic effects. The active principle in the case of organic peroxides could possibly be an alkyl radical (Loveless, 1951).

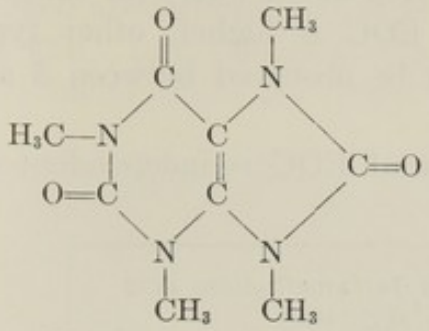
4. *N-Methylated Oxypurines*

N-Methylated oxypurines have proved to be able to produce structural chromosomal changes in several plant species, e.g., onion, *Allium cepa* (Kihlman, 1951, 1952), broad bean, *Vicia faba* (Kihlman, 1955c), pea, *Pisum sativum* (Kihlman, 1952), and barley, *Hordeum sativum* (Moutschen-Dahmen and Moutschen-Dahmen, 1958).

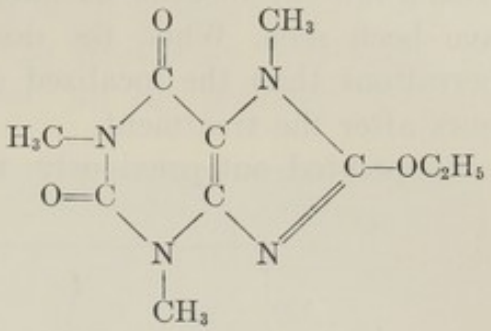
As regards their mode of action, it is possible to distinguish between two types of methylated oxypurines. One type is represented by compounds such as caffeine (1,3,7-trimethylxanthine), theophylline (1,3-dimethylxanthine), and 1,3,7,9-tetramethyluric acid. The effects of these compounds are dependent on the mitotic activity during the treatment: the larger the number of cells dividing during the period of treatment, the larger the number of cells containing structural chromosomal changes when the roots are fixed 24 hours after the treatment.

The other group of methylated oxypurines consisted of 8-ethers and 8-thioethers of caffeine. The effects of these oxypurines are independent

of the mitotic activity during the period of treatment. Of the oxypurines belonging to this group, 8-ethoxycaffeine (EOC) is the most interesting.



1,3,7,9-Tetramethyluric acid (TMU)



8-Ethoxycaffeine (EOC)

Treatments of root tips of *Allium* and *Vicia* with EOC result in a cytological effect resembling in many respects that obtained after irradiation with X-rays (Kihlman, 1955a, 1955c, 1956, and unpublished work). Immediately after a 1¾-hour treatment with 10^{-2} *M* EOC, a strong stickiness effect can be found. One to 3 hours after the treatment, when the reversible stickiness effect has disappeared almost completely, numerous "pseudochiasmata" (Levan, 1949) can be seen in anaphase cells. The corresponding metaphase changes are chromatid associations, which seem to have arisen as a result of a partial chromatid exchange. These half- or sub-chromatid exchanges are also found after irradiations with X-rays (Swanson, 1947; LaCour and Rutishauser, 1954; Crouse, 1954). In comparison with an X-ray irradiation producing the same number of chromatid aberrations, the EOC treatment results in both a stronger stickiness effect and in a higher frequency of subchromatid exchanges.

Both in X-ray and in EOC experiments, chromatid aberrations can be observed in metaphase cells fixed about 2 hours after the bean roots are exposed to the agent (Kihlman, 1955c, also unpublished data; Revell, 1959). The aberrations consist of chromatid breaks, isochromatid breaks, and chromatid exchanges. In addition, 2 to 6 hours after treatment, numerous false chromatid breaks or gaps occur both in the case of X-rays (Revell, 1959) and in the case of EOC.

In contrast to the X-ray effect, the effect of 1¾-hour treatments with EOC has a distinct maximum 4 to 5 hours after the treatment. When this maximum has been reached, the frequency of the nonlocalized chromatid aberrations decreases rapidly (Fig. 5). At the same time, a new type of aberration begins to appear. This type consists of isolocus breaks localized in the nucleolar constriction (Kihlman and Levan, 1951) (Fig. 6A). Chromatid interchanges in the nucleolar constriction also occur, but less frequently.

Twelve hours after the treatment, almost all of the aberrations which occur are of the localized type. Between 16 and 20 hours after the treatment, a few chromosome exchanges localized in the nucleolar constriction have been seen. When the dosage of EOC is higher, other types of aberrations than the localized ones can be observed between 5 and 20 hours after the treatment.

As pointed out previously, the effect of EOC is independent of the

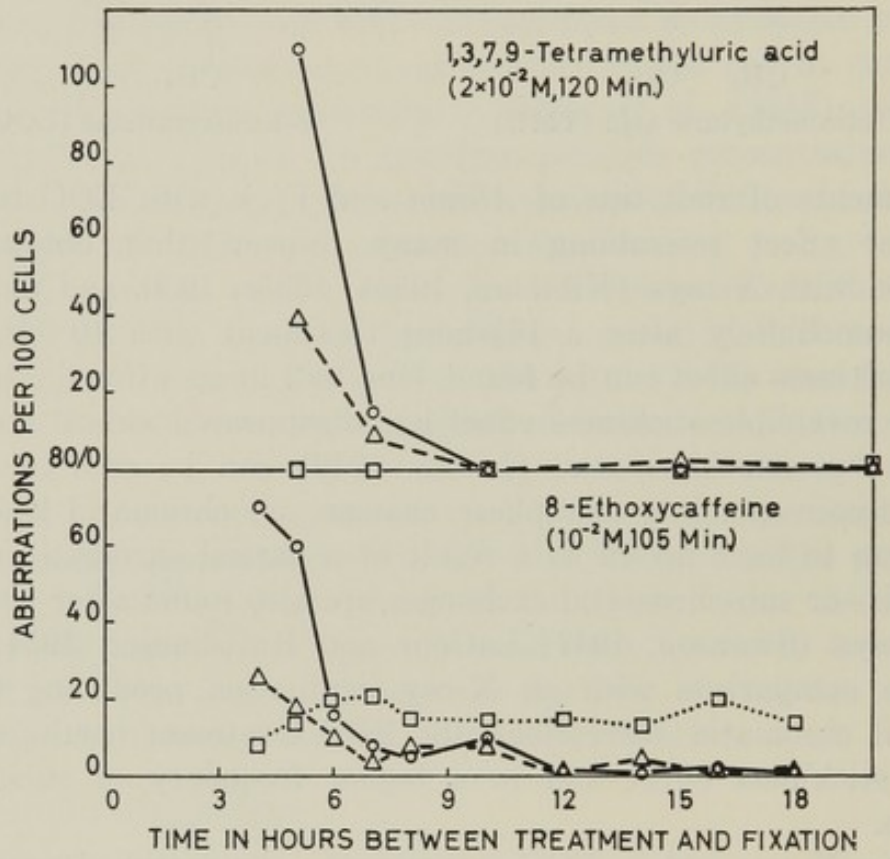


Fig. 5. The frequencies of exchanges (–○——○–), isolocus breaks in the nucleolar constriction (...□.....□...), and other isolocus breaks (–△--△–) obtained at various times after treatments of *Vicia* root tips with tetramethyluric acid and ethoxycaffeine. (From Kihlman, unpublished data.)

mitotic activity during the period of treatment. Both the number of cells containing structural chromosomal changes and the number of aberrations per abnormal cell are proportional to the concentration of EOC (Kihlman, 1951).

The effect of tetramethyluric acid (TMU) is, in the first hours after the treatment, very similar to that of EOC (Kihlman, unpublished). A strong stickiness effect can be observed immediately after a 2-hour treatment with TMU. About 1 hour after the treatment, the stickiness effect is replaced by pseudochiasmata, and 2 hours after the treatment the pseudochiasmata are replaced by real chromatid aberrations. The struc-

tural chromatid changes occur with a maximum frequency about 4 hours after the treatment, whereupon they decrease rapidly (Fig. 5). The localized aberrations, which at this time begin to appear after treatments with EOC, have not been observed after treatments with TMU. Eight to 10 hours after the TMU treatment, all the dividing cells appear

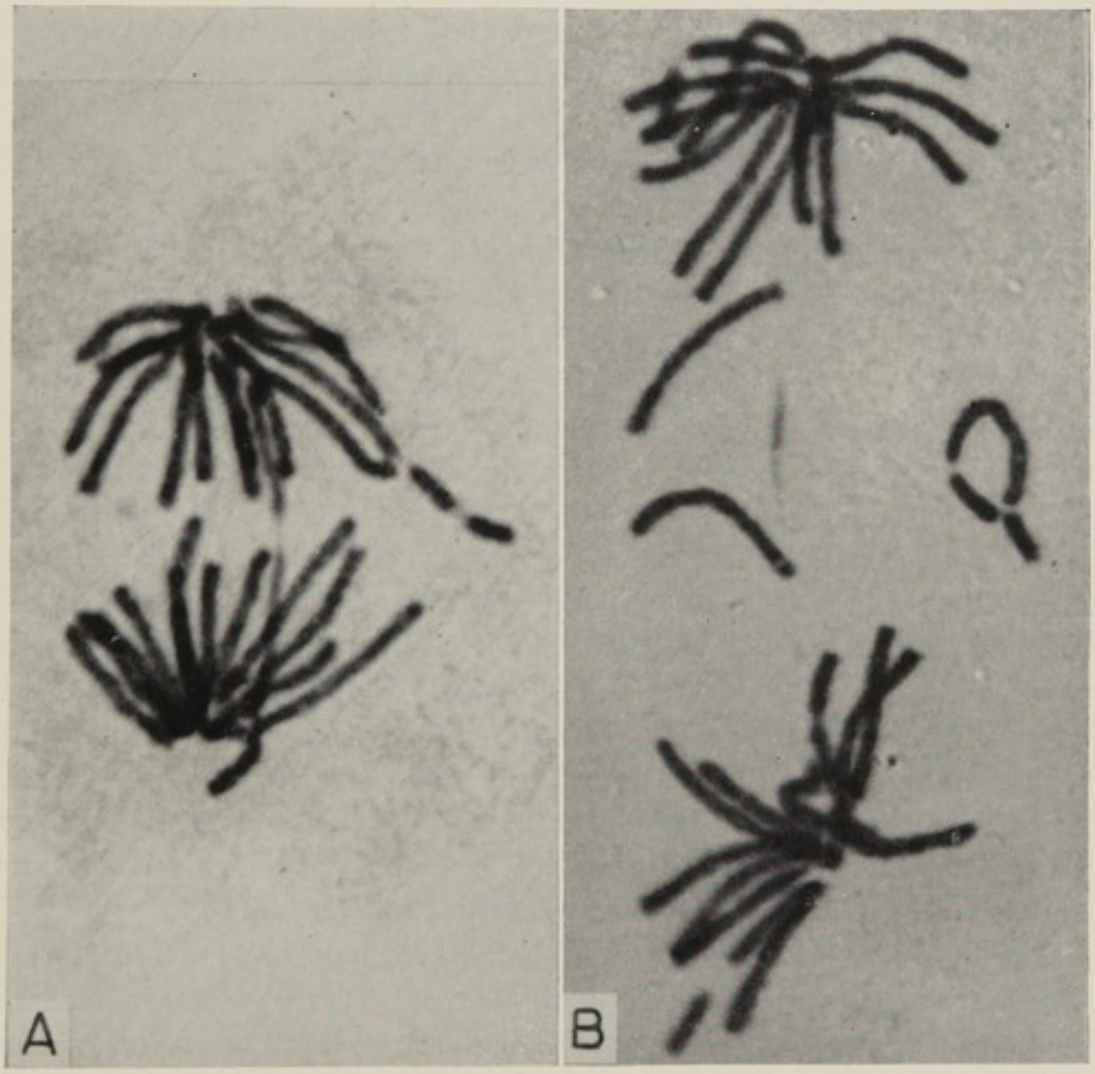


FIG. 6. Anaphase cells of *Vicia faba* showing typical dicentrics and acentrics which have arisen as a result of the localized chromosome breakage produced by ethoxycaffeine (A) and maleic hydrazide (B). From McLeish, 1954.)

normal. A new wave of cell divisions containing chromosomal aberrations occurs 20 to 24 hours after the treatment when such cells as were treated in late interphase or early prophase divide for the second time after the treatment.

In contrast to the effect of EOC, the effect of TMU is dependent on the mitotic activity during the period of treatment (Kihlman, 1951). Thus, TMU treatments result in a high frequency of abnormal cells only when the period of treatment is 24 hours or more and the concen-

tration low enough not to suppress cell division seriously. At a TMU concentration high enough to inhibit mitosis completely, the percentage of abnormal cells is not increased when the period of treatment is prolonged. Therefore, the percentage of abnormal cells obtained after 24-hour treatments with TMU is inversely proportional to the concentration (Kihlman, 1951). The number of aberrations per abnormal cell, on the other hand, is directly proportional to the TMU concentration. The effect of TMU is also inhibited by low temperatures, which reduce the rate of cell division.

How then, is the difference between agents of the EOC and TMU types to be explained?

It has appeared from determinations of the relative lipoid solubilities of *N*-methylated oxypurines that purines of EOC type have a comparatively high relative lipoid solubility, whereas such purines as act in the same way as TMU have a low relative lipoid solubility (Kihlman, 1951). According to the lipoid theory of Overton, the penetration ability of neutral organic compounds, such as EOC and TMU, is related to their relative lipoid solubility: the higher the relative lipoid solubility of such compounds, the more easily they penetrate biological membranes. Like other membranes in the cell, the nuclear membrane contains lipoids (Baud, 1948; Callan and Tomlin, 1950). It seemed, therefore, reasonable to explain the difference between the effects of purines of the EOC and TMU types as due to a difference in their ability to penetrate the membrane of the interphase nucleus. Purines of the EOC type should be able to penetrate this membrane, whereas purines of the TMU type enter the cell nucleus only during cell division when the nuclear membrane is changed and, finally, completely dissolved.

More recently (Kihlman, unpublished) it has been found that the ability of oxypurines to produce chromosomal aberrations independently of the mitotic activity is dependent not only on their relative lipoid solubilities but also on structural factors. Thus, it was found that 2,6-dimethoxy-7,9-dimethyl-8-oxypurine, which has the same relative lipoid solubility as EOC, had an effect of the TMU type when tested in the same concentration as EOC. Structural factors significant for the mode of action of *N*-methylated oxypurines appear to be the position of the lipophilic groups in the molecule and the balance between lipophilic and hydrophilic groups.

An alternative explanation to the difference between the effects of purines of the EOC and TMU types is that they are adsorbed differently inside the nucleus. According to this hypothesis, chromosomal aberrations are produced by *N*-methylated oxypurines during early or middle interphase only when the purines are adsorbed onto the chromosomes.

This does not necessarily mean that the adsorption process itself is of significance, but only that the concentration of the purines in the interphase nucleus is high enough to produce chromosomal aberrations solely in places where they have been adsorbed. Purines of the TMU type would not be adsorbed strongly enough to cause chromosomal aberrations during early or middle interphase. The surface-active and lipoid-soluble purines of EOC type would, however, be adsorbed more easily. The adsorption of these purines would be expected to occur mainly on well-developed interfaces, such as that between the nucleolus and the nuclear sap, and on structures containing high concentrations of lipoids. Since the nucleolar constrictions of the M-chromosomes of *Vicia faba* during interphase are in contact with the nucleolus, and since these same structures are known to be particularly rich in lipoids (Albuquerque and Serra, 1951), the nucleolar constrictions would be exposed to higher concentrations of the purines in question than other parts of the chromosomes. This would explain why aberrations induced by EOC during early or middle interphase are localized in the nucleolar constriction.

The effects of methylated oxypurines are influenced by the temperature and the oxygen concentration during the treatment. The effect of EOC is increased when the temperature is raised from 3 to 12°C. When the temperature is raised above 12° the effect decreases. At 25° the effect is only about one-third of that at 12° (Kihlman, 1956).

Both EOC and TMU are almost completely inactive in the absence of oxygen (Kihlman, 1955b, 1955c). Between 1 and 10% oxygen in the gas phase, the EOC-induced chromosomal aberrations increase rapidly with the oxygen concentration (Fig. 7B). At oxygen concentrations higher than 21%, the effect of EOC is independent of oxygen tension. In Fig. 7 oxygen concentration-effect curves are also shown for a number of other agents. Of the radiomimetic chemicals, the effects of which are shown in Fig. 7, EOC has by far the steepest curve. The different shapes of the oxygen-effect curves indicate that different enzymatic systems and/or mechanisms are involved.

In contrast to the effects of X-rays, visible light, and KCN, the effect of EOC is inhibited not only by anoxia but also by respiratory inhibitors and uncoupling agents (Kihlman, 1955c). Pretreatments of bean roots with sodium azide or 2,4-dinitrophenol make them almost completely insensitive to EOC (Kihlman, 1955c; Read and Kihlman, 1956).

These experiments indicate that energy-rich phosphate is required for the activity of EOC. The effect of EOC is dependent on oxygen because in the absence of oxygen no effective synthesis of energy-rich phosphate occurs in bean roots. Why energy-rich phosphate is required for the radiomimetic effect of *N*-methylated oxypurines is not known.

That it is not the absorption of EOC which requires respiration energy is indicated by the studies of Fredga and Nyman (1961). They found that tritium-labeled EOC was absorbed by the roots as rapidly under anaerobic as under aerobic conditions.

It has been suggested that the mutagenic effect of *N*-methylated oxypurines is due to their incorporation into the nucleic acids of the chromosomes (Biesele *et al.*, 1952; Kalckar, 1954) or to their possible effect on nucleic acid synthesis (Novick, 1956). However, these hypotheses can hardly explain the chromosome-breaking effects of the *N*-methylated oxypurines. One of the reasons is that the oxypurines are most active at a stage of the mitotic cycle when the nucleic acid synthesis in *Vicia* is already completed (Howard and Pelc, 1953; Deeley *et al.*, 1957). Furthermore, there is the fact that no correlation exists between the ability of a purine derivative to be incorporated into nucleic acids and its radiomimetic (Kihlman, 1955a) or mutagenic (Koch, 1956) effect. Finally, there is the fact that enzymatic reactions appear to be very little if at all affected by methylated oxypurines. Among the enzymes studied were several involved in nucleotide and nucleic acid metabolism, e.g., polynucleotide phosphorylase (Beers, personal communication), nucleoside phosphorylase, deoxyribonuclease, xanthine oxidase (Kihlman and Overgaard-Hansen, 1955), and phosphatase (Kihlman, 1951).

Because of the chemical and biochemical inactivity of the *N*-methylated oxypurines, it seems as if the reason for their biological effect has to be sought in their physical properties. Characteristic of the methylated oxypurines as a group is their solubilizing power (e.g., Weil-Malherbe, 1946; Neish, 1948; Booth and Boyland, 1953). A comparison between the ability to produce chromosomal aberrations, on the one hand, and the solubilizing power, on the other, has shown that a very good correlation exists between these two properties of the methylated oxypurines. Such oxypurines as are effective as solubilizers also have a strong radiomimetic effect (Kihlman, 1952). The solubilizing power of the methylated oxypurines is correlated with their ability to form molecular complexes (Weil-Malherbe, 1946) and is probably due to their electron-donor properties (Pullman and Pullman, 1958).

It seems reasonable to assume that the radiomimetic effect of the *N*-methylated oxypurines is also determined by their electron-donor properties.

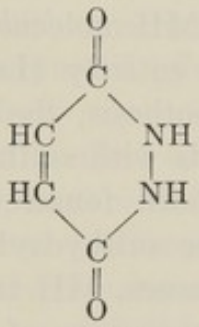
5. *Maleic Hydrazide*

Darlington and McLeish (1951) have shown that maleic hydrazide (MH), or 1,2-dihydro-3,6-pyridazinedione, has a strong radiomimetic effect in the root tips of *Vicia faba*.

The aberrations induced by MH are localized in heterochromatic segments of the chromosomes. In *Vicia faba* it is mainly a heterochromatic segment close to the centromere in the nucleolar arm of the M-chromosome which is broken (Fig. 6B) (Darlington and McLeish, 1951; McLeish, 1953).

According to McLeish (1953), the localization is most pronounced at low concentrations of MH. In *Zea mays* the frequency of structural chromosomal changes is related to the number of heterochromatic "knobs" occurring in the corn varieties used; the larger the number of knobs, the more aberrations obtained (Graf, 1957).

The effect of MH is delayed (see page 44). Stickiness does not occur and the cell divisions are quite normal the first 6 to 8 hours after the treatment (McLeish, 1953; Kihlman, 1956). At moderate MH dosages (e.g., 10^{-4} *M*, 2 hours, 20°C., pH 5.8) the highest frequency of



Maleic hydrazide (MH)

aberrations is obtained between 24 and 36 hours after the treatment (McLeish, 1953; Kihlman, 1956). The chromosomal aberrations induced by MH in *Vicia faba* are of chromatid type.

The radiomimetic effect of MH is dependent on the temperature, the hydrogen ion concentration, and the oxygen concentration during the treatment (Kihlman, 1956). The maximum effect obtained at 25° is about five times stronger than that obtained at 3°C. At a constant temperature the effect of MH is more than four times stronger at pH 4.7 than at pH 7.3 (Kihlman, 1956). It seems likely that the influence of the hydrogen ion concentration on the radiomimetic effect of MH is due to the fact that the ability of the MH molecule to enter the root-tip cells is affected by the pH of the treatment solution (Naylor and Davis, 1951). MH is a weak acid, and it is possible that the undissociated molecule penetrates the plasma membrane more easily than does the dissociated one.

When the MH treatments are performed under anaerobic conditions, fewer aberrations are obtained than when they are performed in the presence of oxygen (Kihlman, 1956). In contrast to EOC and KCN, MH is not entirely inactive in the absence of oxygen. When the oxygen

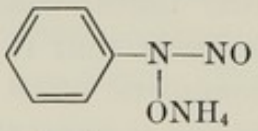
concentration is increased from 0 to 100%, the frequency of aberrations is increased approximately threefold (see Fig. 7E).

Pretreatments with sodium azide and 2,4-dinitrophenol suppress the radiomimetic effect of MH to the same extent as does anoxia (Kihlman, 1956). This fact indicates that the MH effect is dependent on oxygen concentration because it is tied up with oxidative phosphorylation.

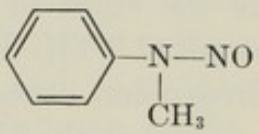
It has been suggested that the radiomimetic effect of MH is due to its being a structural isomer of uracil (Loveless, 1953). According to this idea, MH would produce chromosomal aberrations by acting as an antimetabolite in connection with nucleic acid synthesis and metabolism. However, all attempts to counteract the radiomimetic effect of MH with uracil, thymine, and orotic acid were unsuccessful (Loveless, 1953). Nor has it been possible to induce chromosomal aberrations with other structural isomers of uracil. Thus, no evidence has been obtained in favor of the idea that MH produces chromosomal aberrations because it is an antimetabolite. If the MH molecule is modified, the radiomimetic effect decreases or disappears entirely (Loveless, 1953).

According to another hypothesis, the biological effects of MH are due to the fact that MH reacts with sulfhydryl groups in the cell (Muir and Hansch, 1953). It has been found that MH irreversibly inhibits certain enzymes requiring free sulfhydryl groups (Hughes and Spragg, 1958). In actively dividing tissues, MH treatments result in an increase of reduced glutathione at the expense of oxidized glutathione (Hughes and Spragg, 1958). Very likely the inhibition of mitosis produced by MH is due to the ability of MH to react with sulfhydryl groups. However, this ability of MH can hardly be responsible for the radiomimetic effect, since no correlation has been found between the ability of various agents to react with SH-groups and their radiomimetic or mutagenic effect (Loveless, 1951; Auerbach, 1951).

6. *Phenylnitrosamines*



Cupferron



N-Methylphenylnitrosamine (MPNA)

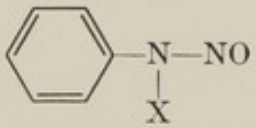
In connection with a study of the radiomimetic effects of cyanide and heavy metal complexing agents, the effect of cupferron (the ammonium salt of *N*-nitrosophenylhydroxylamine) was also tested (Kihlman, 1957). This chelating nitrosamine proved to have a radiomimetic effect, which, in regard to the localization of the aberrations between and within the chromosomes, is very similar to the radiomimetic effect of KCN.

Subsequent, more detailed studies (Kihlman, 1959b) showed that the effect of cupferron is strongly influenced by the pH and temperature of the treatment solution. The effect was enhanced by high temperature and low pH. Since cupferron is the ammonium salt of the acid *N*-nitrosophenylhydroxylamine, the explanation for the influence of hydrogen ion concentration on the radiomimetic effect may well be the same as in the case of MH, i.e., that the undissociated molecule penetrates the plasma membrane more easily. The effects of cupferron on respiration, on X-ray sensitivity at low oxygen tensions, and on light-induced chromosome damage are influenced by pH in a similar way (Kihlman, 1959c, 1961a).

Between 1 and 100% oxygen in the gas phase, the effect of cupferron appears to be independent of oxygen concentration. In the complete absence of oxygen, cupferron is inactive (Kihlman, 1959b).

After cupferron treatments, a high frequency of chromosomal aberrations is always combined with a strong toxic effect, since the same factors which favor the radiomimetic effect, high temperature and low pH, also enhance the toxic effect.

Previously, the radiomimetic effect of cupferron was believed to be due to its ability to form complexes with heavy metals. It was suggested (Kihlman, 1957) that cupferron produces chromosomal aberrations by removing heavy metals from the chromosomes. The metals were believed to occur in the chromosomes as stabilizing factors. Another possibility considered was that cupferron acted in a way similar to that suggested in the case of KCN (see page 22). Results recently obtained (Kihlman, 1961b) indicate, however, that the chemical properties responsible for the radiomimetic effect of cupferron are the same as those responsible for its activity in the visible light-acridine orange system. In this system the activity of cupferron has nothing to do with its inhibitory effect on respiration or with its chelating properties. The configuration necessary for activity was found to be:



The same configuration also appears to be necessary for the radiomimetic effect. *N*-Methylphenylnitrosamine (10^{-3} *M*, 1 hour) proved to have a strong radiomimetic effect, whereas dimethylnitrosamine, diethylnitrosamine, and dimethylaniline were inactive. It is true that no chromosomal aberrations were found after treatments with diphenylnitrosamine, but since it has not been possible to test this nitrosamine in higher concentrations than 40 μM because of its slight solubility

in water, the results are not significant. Methylphenylnitrosamine (MPNA), too, is inactive in such low concentrations.

The toxic effect of MPNA is negligible and it is, therefore, more suitable than cupferron for studies on the radiomimetic effect.

The radiomimetic effect of MPNA, like that of cupferron, is delayed and localized in heterochromatin. The aberrations obtained were exclusively of the chromatid type. Although the aberrations tended to be localized in heterochromatin, they were otherwise rather evenly distributed between the chromosomes. Of 838 breaks, 627 were in S-chromosomes and 211 in M-chromosomes, which gives a ratio of S:M = 2.97:1. After treatments with KCN, the ratio obtained was very similar, or 3.2:1 (Kihlman, 1957). Other similarities between KCN and MPNA are that the effect in both cases increases with oxygen concentration and is almost independent of the temperature and pH during the treatment.

In the absence of oxygen, MPNA is almost inactive. The curve obtained when the frequencies of isolocus breaks and exchanges are plotted against oxygen concentration (Fig. 7F) resembles the corresponding curve for KCN (Fig. 7C), but differs from that for EOC (Fig. 7B) in that no plateau is reached, i.e., the effect increases with oxygen concentration up to 100% oxygen.

When sodium azide is present during the MPNA treatment, the radiomimetic effect is strongly suppressed. Cupferron, being a respiratory inhibitor, has the same inhibiting action as sodium azide on the radiomimetic effect of MPNA. The influence of the uncoupling agent 2,4-dinitrophenol on the radiomimetic effect was also tested. A slight, hardly significant suppression was obtained in these experiments. The inhibitor experiments seem to indicate (1) that the radiomimetic effect of MPNA, like that of EOC, and MH, is dependent on the respiratory activity of the cell, and (2) that in contrast to the effect of EOC and MH, the effect of MPNA is not dependent upon oxidative phosphorylation. The results indicate that the active agent is not the intact MPNA molecule, but some decomposition product formed as a result of the activity of the respiratory enzymes.

The studies on the radiomimetic effect of MPNA (Kihlman, 1961b) have revealed several differences between the effects of MPNA and cupferron. How are these differences compatible with the idea that a similar mechanism is underlying the radiomimetic effects of both these compounds?

The fact that MPNA is not influenced by pH is only what one would expect, since in contrast to cupferron, MPNA is a neutral compound. Nor is it difficult to understand that the radiomimetic effects of MPNA and cupferron do not respond to oxygen in exactly the same way. The radiomimetic effect of MPNA is inhibited by respiratory inhibitors such

as cupferron. Thus, if the mechanisms underlying the effects of MPNA and cupferron are the same, cupferron should inhibit its own radiomimetic effect. The different influence of treatment temperature on the effects of the two agents is, perhaps, more difficult to explain. It may be

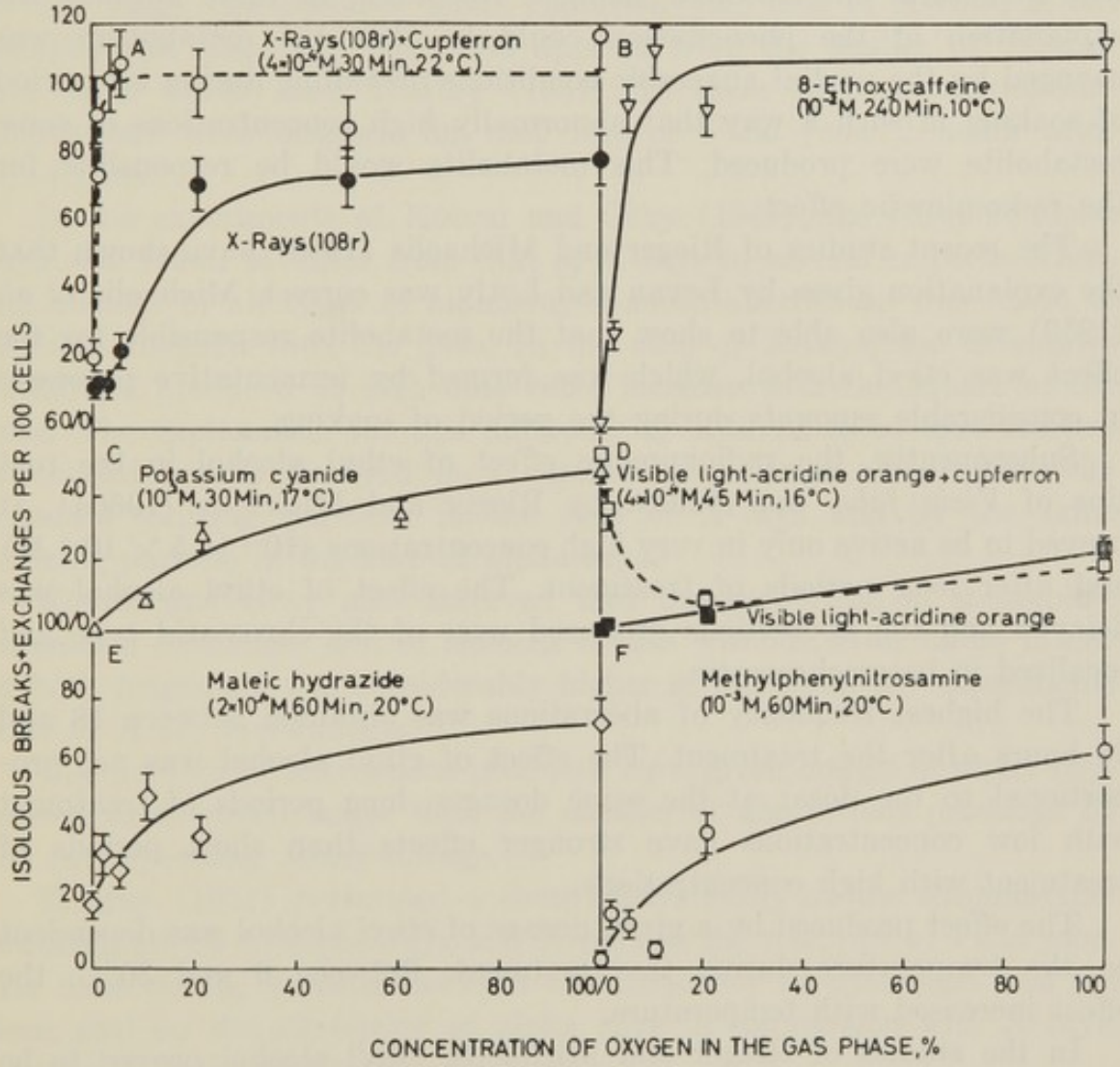


FIG. 7. Relation between percentage of oxygen and the frequencies of isolocus breaks and exchanges obtained after treatments of *Vicia* root tips with various chromosome-breaking agents. (The curves are based on data from Kihlman, 1955c, 1957, 1959a,c, 1961b, and unpublished data.)

that this difference is also a reflection of the fact that oxidative metabolism is inhibited by cupferron but not by MPNA.

The mechanism responsible for the radiomimetic effects of MPNA and cupferron is not as yet understood. Perhaps the agents are decomposed by oxidative metabolism to yield free radicals and/or organic peroxides, which then may react with chromosome precursors in a way similar to that suggested in the case of alkylating agents (see pages 39 and 40).

7. *Ethyl Alcohol*

Levan and Lotfy (1950) observed that if *Vicia faba* seeds were soaked in water for 24 hours before they were allowed to germinate on moist filter paper, the first root-tip mitosis contained numerous cells with structural chromosomal changes. According to these authors, the explanation of the phenomenon could be that the metabolism was changed by the partial anaerobic conditions prevailing during the period of soaking in such a way that abnormally high concentrations of some metabolite were produced. This metabolite would be responsible for the radiomimetic effect.

The recent studies of Rieger and Michaelis (1958) have shown that the explanation given by Levan and Lotfy was correct. Michaelis *et al.* (1959) were also able to show that the metabolite responsible for the effect was ethyl alcohol, which was formed by fermentative processes in considerable amounts during the period of soaking.

Subsequently, the radiomimetic effect of ethyl alcohol in the root tips of *Vicia faba* was studied by Rieger and Michaelis (1960a). It proved to be active only in very high concentrations (10^{-3} to 5×10^{-1} *M*) and after long periods of treatment. The effect of ethyl alcohol was delayed and the aberrations produced were of the chromatid type and localized in heterochromatin.

The highest frequency of aberrations was obtained between 18 and 24 hours after the treatment. The effect of ethyl alcohol was not proportional to the dose: at the same dosages, long periods of treatment with low concentrations gave stronger effects than short periods of treatment with high concentrations.

The effect produced by a given dosage of ethyl alcohol was dependent on the temperature during the treatment. Between 6 and 30°C. the effect increased with temperature.

In the studies of Rieger and Michaelis, ethyl alcohol proved to be entirely inactive under completely anaerobic conditions. The radiomimetic effect was strongly suppressed when 2,4-dinitrophenol was present during the treatment. Rieger and Michaelis concluded, therefore, that oxidative phosphorylation is involved in the production of structural chromosomal changes by ethyl alcohol.

B. Oxygen-Independent Chromosome Damage

1. *Alpha Radiation*

Kotval and Gray (1947) studied the structural chromosomal changes obtained 24 hours after treatments of *Tradescantia bracteata* microspores with known dosages of alpha radiation. The method used in

these experiments consisted in immersing the inflorescences in radon solutions of known concentrations.

Twenty four hours before metaphase the microspore nuclei are in early prophase (Beatty and Beatty, 1953) and the chromosomes are effectively split. Since alpha radiation induces chromosomal aberrations at this stage, the effect of alpha radiation, like that of X-rays, is not delayed (see page 43). This also appears from the studies of Thoday and Read (1949) and Thoday (1951). In these studies chromosomal aberrations were obtained in root tips of *Vicia faba* 2 hours after irradiation.

In the experiments of Kotval and Gray (1947), the effect of alpha rays was found to differ from that of X-rays in several respects. Thus, the number of all types of alpha-ray-induced aberrations was found to increase linearly with the dose. In the case of X-rays, the chromatid exchanges produced at high dose rates increase with the square of the dose. The explanation for this difference is believed to be that the two chromatid breaks involved in a chromatid exchange are produced by separate ionizing particles in the case of X-rays and by the same ionizing particle in the case of alpha rays.

Kotval and Gray also observed that the proportion of incomplete chromatid exchanges and of isolocus breaks without sister union in one or both fragments was considerably higher in the alpha-ray experiments than in X-ray experiments.

The number of aberrations produced by a given dosage of alpha rays proved to be much larger than the number of aberrations produced by the corresponding X-ray dosage.

Thoday (1951) performed a comparative study on the chromosome-breaking effects of alpha rays and X-rays in the root tips of *Vicia faba*. His data on the relation between the frequency of exchanges and the dose, and on the effectivity of alpha rays in comparison with X-rays, agree with those of Kotval and Gray.

Thoday and Read (1949) studied the influence of oxygen concentration on the frequency of structural chromosomal changes produced by a given dosage of alpha rays in the root tips of *Vicia faba*. In contrast to the effect of X-rays, the effect of alpha rays proved to be almost independent of the oxygen concentration during the period of irradiation (compare page 8).

2. *Ultraviolet Light*

Since ultraviolet light is absorbed very strongly by the tissues and therefore does not penetrate very far, the production of chromosomal aberrations with ultraviolet light has been possible only in cases where the cells to be irradiated are in one layer and not covered by overlying

tissues. Materials used in studies of this type are the pollen and pollen tubes of *Tradescantia paludosa*.

The pioneer work with this material was done by Swanson (1940, 1942). In his experiments, dry pollen was sown on a sugar-agar-gelatin medium and the generative nucleus irradiated after it had passed over into the pollen tube. The effect was analyzed in metaphase of the pollen tube mitosis. X-ray experiments have shown that the chromosomes are effectively split at least a day before anthesis (Swanson, 1942). Thus, the effect obtained in the pollen tube mitosis after irradiation of mature pollen must be of the not-delayed type (see page 43).

The aberrations obtained in Swanson's experiments were mainly chromatid breaks. The frequency of these aberrations increased linearly with dose. The chromatid breaks "showed all gradations from free fragments to achromatic lesions" (Swanson, 1942). Swanson also observed half-chromatid breaks after irradiation of pollen tube chromosomes with ultraviolet light. Chromatid exchanges and isolocus breaks were not obtained in Swanson's experiments, and it seemed as if aberrations of this type could not be produced by ultraviolet light.

However, both chromatid exchanges and isochromatid breaks were obtained by Lovelace (1954) after irradiation of dry pollen with ultraviolet light. In addition to chromatid breaks, isochromatid breaks, and chromatid exchanges, Lovelace observed a localized, extreme fragmentation ("shattering") of chromosomes after high dosages of ultraviolet light at a wavelength of 2650 A.

An extensive and careful study of the effect of ultraviolet light on the chromosome structure in *Tradescantia* pollen was performed by Kirby-Smith and Craig (1957). In these experiments, as in those of Lovelace, dry pollen was irradiated. After irradiation, the pollen was sown on a lactose-agar medium containing colchicine and cultured for 18 to 20 hours. The chromatid aberrations produced were scored in metaphase of the pollen tube division.

Their work confirmed the observation of Lovelace that ultraviolet light is able to produce isochromatid breaks and exchanges. These types of aberration were rather rare, however. The proportion of isochromatid breaks that were incomplete, i.e., not showing sister-chromatid union in both fragments, was considerably higher after irradiation with ultraviolet than after irradiation with X-rays.

The action spectrum for ultraviolet-induced chromosomal aberrations was obtained by plotting the relative frequency versus wavelength. The resulting curve coincided with the nucleic acid absorption spectrum. Thus, the highest frequency of aberrations was obtained at a wavelength corresponding to the absorption maximum of nucleic acid.

TABLE 2
Aberration Frequencies for *Tradescantia* Pollen Irradiated in Air and in Nitrogen *

Experiment number	Treatment	UV dose, at 2650 A [(ergs/cm^2) $\times$ 10^6]	Cells scored	Normal cells, percentage	Number of aberrations per cell			Incomplete isochromatid breaks, percentage
					Chromatid	Isochromatid	Exchange	
4	Air	1.18	135	52	0.36	0.41	0.01	44
	N_2	1.15	150	53	0.37	0.39	0	66
17	Air	1.01	200	58	0.42	0.22	0.01	46
	N_2	0.98	200	56	0.37	0.24	0	65
34	Air	0.98	250	44	0.37	0.22	0.02	48
	N_2	1.00	300	35	0.45	0.21	0.01	62

* From Kirby-Smith and Craig (1957).

The frequencies of chromosomal aberrations induced by a given dosage of ultraviolet light were not affected by oxygen concentration (Table 2). The percentage of incomplete isochromatid breaks was significantly higher when the irradiations were performed in nitrogen than when they were performed in air. But if the same number of breaks is produced in nitrogen as in air, and if the breaks produced in nitrogen are less likely to rejoin than those produced in air, more aberrations should have been obtained when the irradiations were performed in nitrogen than when they were performed in air. To account for the fact that the same number of aberrations was obtained in nitrogen and air, Kirby-Smith and Craig assume that fewer breaks were produced in nitrogen. This assumption is unnecessary if the exchange hypothesis of Revell (1959) is accepted (see pages 48–50).

3. *Alkylating Agents*

This group consists of agents such as sulfur and nitrogen mustards, epoxides, and β-lactones (Ross, 1958). *N*-Nitroso-*N*-methyl urethan, the radiomimetic effect of which appears to be due to its conversion into the alkylating agent diazomethane (Kihlman, 1960), should also be included in this group.

The radiomimetic effects of the mustards were first studied by Darlington and Koller (1947). The effect of nitrogen mustard (NM), or 2,2′-dichloro-*N*-methyldiethylamine, has been analyzed more in detail by Ford (1949), using root tips of *Vicia faba* as the experimental material.

The radiomimetic effects of the epoxides were discovered by Loveless and Revell (1949). Owing to the studies of Revell (1953), the effect of 2,3,2′,3′-diepoxypropyl ether (DEPE) in *Vicia* is particularly well known. The radiomimetic effect of DEPE in root tips of *Tradescantia paludosa* has been studied by Lane (1955).

The radiomimetic effect of β-propiolactone (BPL) was discovered by Smith and Srb (1951), and has subsequently been studied by Smith and Lotfy (1955), and by Swanson and Merz (1959). The experimental materials were root tips of *Allium cepa* and *Vicia faba* in the studies of Smith and his collaborators, and root tips of *Vicia faba* in the studies of Swanson and Merz.

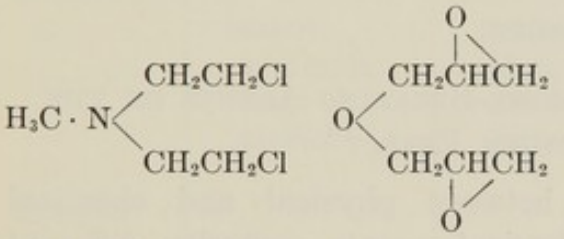
The radiomimetic effect of *N*-nitroso-*N*-methyl urethan (NMU) in *Vicia faba* was studied by Kihlman (1960).

Treatments of *Vicia* root tips with NM (10^{-6} *M*, ½ hour, 20°), DEPE (2×10^{-4} *M*, 1 hour, 20°), or NMU (10^{-4} *M*, 1 hour, 20°), result in few or no aberrations the first 8 hours after the treatment (Ford, 1949; Revell, 1953; Kihlman, 1956, 1960). Ten to 12 hours after the

treatment, structural chromosomal aberrations begin to appear, but the maximum effect is not obtained until 24 to 36 hours after the treatment (Revell, 1953; Kihlman, 1956, 1960). A delayed effect is also found after ½-hour treatments with $7 \times 10^{-3}\,M$ BPL at 17° (Swanson and Merz, 1959).

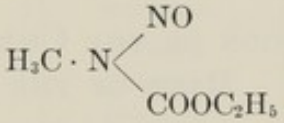
The aberrations obtained with alkylating agents are of the chromatid type. After treatments with DEPE, all types of aberrations seem to increase linearly with the dose.

The effects of alkylating agents are to some extent localized in the heterochromatin. Breaks induced by NM, DEPE, and BPL are usually localized in a segment situated in the middle of the long arm of the S-chromosomes (Ford, 1949; Loveless and Revell, 1949; Revell, 1953; Swanson and Merz, 1959). The ratio of breaks in S-chromosomes and

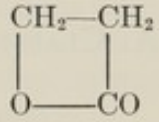


2,2′-Dichloro-*N*-methyldiethylamine (NM)

2,3,2′,3′-Diepoxypropyl ether (DEPE)



N-Nitroso-*N*-methyl urethan (NMU)



β-Propiolactone (BPL)

M-chromosomes was found to be 24.8:1 in the case of NM (Ford, 1949), 18.5:1 in the case of DEPE (Revell, 1953), and greater than 6:1 in the case of BPL (Swanson and Merz, 1959). If aberrations had been produced at random, a ratio of 2.2:1 would be obtained (Revell, 1953).

After treatments with NMU, an S:M ratio of 2.2:1 was found, which indicated that the breaks were produced at random between the chromosomes. Within the chromosomes, the aberrations appeared to be localized in heterochromatic segments (Kihlman, 1960).

The radiomimetic effects of DEPE, NMU, and BPL increase strongly with temperature, but are independent of the oxygen concentration during the treatment (Kihlman, 1956, 1960; Swanson and Merz, 1959). The radiomimetic effect of NM is also independent of the oxygen concentration (Kihlman, 1955c).

The chemical and physiological properties of the alkylating agents have been described by Ross (1953, 1958). They are all chemically very reactive agents, which readily combine with nucleophilic centers in other molecules. In biological systems, sulfhydryl groups, ionized acid groups, and non-ionized amino groups are nucleophilic. Carboxyl groups in proteins and phosphoryl groups in nucleic acids appear to be particularly reactive at physiological pH values. As a result of the reactions

between the latter groups and the alkylating agents, esters are formed (Ross, 1953, 1958; Stacey *et al.*, 1958).

According to Ross (1953, 1958), the fact that radiomimetic alkylating agents are electrophilic, i.e., have an affinity toward nucleophilic groups, is due to their ability to form positive carbonium ions in polar solvents, e.g.:

$$R_2NCH_2CH_2Cl \rightleftharpoons R_2N\overset{+}{C}H_2CH_2 + \overset{-}{Cl}$$

Some of the alkylating agents produce chromosomal aberrations in very low concentrations. Thus, a high frequency of chromosomal aberrations was obtained in *Vicia* root tips with NM concentrations as low as 10^{-6} *M* (Revell, 1953).

III. Discussion

A. Classification of the Chromosome-Breaking Agents on the Basis of the "Oxygen Effect"

It is customary to distinguish between physical and chemical chromosome-breaking agents. The physical agents comprise different types of radiation which are classified as corpuscular and electromagnetic. On the basis of their effect on matter, the radiations are also classified as ionizing, such as X-rays, gamma rays, protons, neutrons, etc., and non-ionizing, such as ultraviolet and visible light. The chemical agents may be classified as reactive and unreactive agents. A further classification of chromosome-breaking or radiomimetic chemicals may be obtained on the basis of their chemical structure and reactions.

Along with classifications based on the physical and chemical properties of chromosome-breaking agents, it may be useful to apply a classification which is based on the cytological effect and which takes into account the biochemical aspects of chromosome breakage. The classification suggested below is based on the influence of oxygen on the cytological effects of the agents, and on the duration of the interval between the treatment and the appearance of the chromosomal aberrations at metaphase.

It has appeared, from the survey of the mode of action of various chromosome-breaking agents given above, that the effects of certain agents are strongly enhanced by the presence of oxygen during the treatment, whereas other agents are as active in the absence as in the presence of oxygen. On the basis of whether the effect is dependent on oxygen concentration or not, it is possible to distinguish between two main groups of chromosome-breaking agents (Table 3).

TABLE 3
Classification of Chromosome-Breaking Agents on the Basis of the Influence of the Oxygen Concentration on the Effect

Type of effect (delayed or not delayed)	I. The effect dependent on oxygen concentration			II. The effect independent of oxygen concentration
	A. The effect inhibited by respiratory inhibitors		B. The effect not inhibited by respiratory inhibitors (oxygen can usually be replaced by nitric oxide)	
	1. The effect inhibited by uncoupling agents	2. The effect not inhibited by uncoupling agents		
Delayed	Maleic hydrazide, ethyl alcohol	*N*-Methyl-phenyl-nitros-amine	Visible light-acridine orange, potassium cyanide	Alkylating agents (nitrogen mustard, diepoxypropyl ether, *N*-nitroso-*N*-methyl urethan, Myleran, β-propiolactone)
Not delayed	*N*-Methylated oxypurines (e.g., 8-ethoxycaffeine, 1,3,7,9-tetramethyluric acid)		X-rays	Alpha rays, ultraviolet light

Oxygen-independent effects are obtained with agents such as alkylating chemicals, ultraviolet light, and alpha rays. All the chemicals belonging to this group[1] are very reactive agents, the effects of which are probably due to a chemical reaction between the agent and some chromosomal constituent or precursor. Such a reaction is to be expected

[1] Recent experiments by Michaelis and Rieger (1960) have shown that in addition to the chemicals discussed in Section II,B,3, the alkylating agent Myleran should be included in this group.

to have a positive temperature coefficient and to be independent of oxygen concentration, in agreement with the experimental findings.

The agents which produce oxygen-dependent effects can be further divided into two subgroups depending on whether or not the effect is suppressed by respiratory inhibitors, as well as by anoxia. A suppression of the radiomimetic affect has been obtained in the case of *N*-methylated oxypurines, maleic hydrazide, and *N*-methylphenylnitrosamine. The degree of suppression varies. The radiomimetic effects of 8-ethoxycaffeine and *N*-methylphenylnitrosamine are completely, or almost completely, inhibited by anoxia or by respiratory inhibitors, whereas that of maleic hydrazide is strongly, but not completely suppressed (see Fig. 7). As a rule, agents which uncouple phosphorylation from respiration, such as 2,4-dinitrophenol, have the same effect as respiratory inhibitors, a fact which indicates that the physiological process of significance for the radiomimetic effect is oxidative phosphorylation. However, the radiomimetic effect of *N*-methylphenylnitrosamine was only very slightly suppressed by dinitrophenol, which indicates that respiration, rather than phosphorylation, is the significant physiological process in this case. Therefore, with some hesitation, methylphenylnitrosamine is placed in a class separate from that of ethoxycaffeine and maleic hydrazide.

According to Rieger and Michaelis (1960a), both anoxia and dinitrophenol suppress the radiomimetic effect of ethyl alcohol. Thus, although the effect of respiratory inhibitors has not been tested in the case of ethyl alcohol,[2] there can be little doubt that it belongs to the same group as ethoxycaffeine and maleic hydrazide.

The role of oxidative phosphorylation in the effects of ethoxycaffeine, maleic hydrazide, and ethyl alcohol is not known. In the case of ethoxycaffeine and maleic hydrazide, the different appearance of the oxygen concentration-effect curves in Fig. 7, as well as the fact that the effect of maleic hydrazide is delayed whereas that of ethoxycaffeine is not, indicates that oxidative phosphorylation influences the effects of these agents in different ways. The energy-requiring process involved in the production of chromosomal aberrations by maleic hydrazide may be the synthesis of deoxyribonucleic acid, whereas ethoxycaffeine may produce chromosomal disturbances by affecting the transport and distribution of respiration energy within the chromosomes.

Chromosome-breaking agents, the effects of which are classed as oxygen-dependent but not influenced by respiratory inhibitors, are X-rays, the visible light-acridine orange system, and potassium cyanide.

[2] In a recent article, Rieger and Michaelis (1960b) report that the radiomimetic effect of ethyl alcohol is suppressed by respiratory inhibitors.

In the case of X-rays and visible light-acridine orange, nitric oxide seems to be able to replace oxygen as an enhancing agent. Conclusive evidence is lacking for an enhancement of the potassium cyanide effect by nitric oxide. It seems likely that oxygen and nitric oxide influence the chromosome-breaking effects of X-rays and visible light-acridine orange by reacting with free radicals produced in the cell by the radiations in question. The oxygen dependence of the potassium cyanide effect has been attributed to the mediation of the radiomimetic effect by peroxides, which are believed to accumulate in the cell as a result of the inhibitory effect of cyanide on cytochrome oxidase and peroxide-destroying enzymes (Lilly and Thoday, 1956).

Although the effect of X-rays is correctly classified as oxygen-dependent but not influenced by respiratory inhibitors, oxidative phosphorylation does play an important role in connection with the formation of chromosomal aberrations after treatments with X-rays. As shown by Wolff and Luippold (1955), the dose-rate effect and fractionation effect disappear in the presence of respiratory inhibitors and uncoupling agents. According to Wolff, oxidative phosphorylation is required for the rejoining of chromosomal breaks. Irrespective of whether or not Wolff's interpretation will prove to be correct, the fact remains that oxidative phosphorylation somehow appears to be involved in the repair of chromosomal damage. This is probably true for aberrations produced by all chromosome-breaking agents, although so far, it has been possible to demonstrate this for X-ray-induced aberrations only. The processes influenced by oxidative phosphorylation are in this case those following upon the "primary event." Thus, the effect of oxidative phosphorylation discovered by Wolff has nothing to do with the usual "oxygen effect," which operates in the formation of the primary event, and on which the classification above is based. In the case of ethoxycaffeine, maleic hydrazide, and ethyl alcohol, however, oxidative phosphorylation seems to be of significance in the primary event, as well.

Since oxidative phosphorylation appears to be so important in connection with the formation of chromosomal aberrations in plant cells, it might be well to point out that the enzyme systems involved in oxidative phosphorylation in plant cells are similar to those in animal cells (for references, see Hackett, 1959).

B. Delayed and Not-Delayed Breakage

Table 3 shows the chromosome-breaking agents classified on the basis of the influence of oxygen, as discussed above. Within each group, a further classification has been made on the basis of whether the effect is delayed or not. In *Vicia faba* root tips, the effect is regarded as not-

delayed when structural chromosomal changes are found in metaphase as soon as this stage is reached by such cells as were in late interphase at the time of the treatment. Practically, this means that chromosomal aberrations produced by agents with not-delayed effects appear in metaphase about 2 hours after the treatment. Ionizing radiation has an effect of this type, and so have *N*-methylated oxypurines. It has not been possible to study the effect of ultraviolet light in *Vicia* root tips, but experiments with *Tradescantia* pollen indicate that the chromosome-breaking effect of ultraviolet light is not delayed. All the other radiomimetic agents in Table 3 have delayed effects.

In *Vicia* the delayed effect is characterized by the fact that the cell divisions are, as a rule, completely normal the first 6 to 8 hours after the treatment. Between 8 and 12 hours after treatment, structural chromosomal changes begin to appear. The time at which the maximum effect occurs depends on the agent and on its dosage. If the treatment produces a strong inhibition of mitosis, it may take 48 hours or more before the effect is maximal, but usually the aberrations occur with a maximum frequency between 18 and 36 hours after the treatment.

Radiomimetic chemicals with delayed effects seem to be able to induce aberrations of the chromatid type only. In contrast, agents with effects of the not-delayed type produce both chromatid and chromosome aberrations. In *Vicia* root tips, chromosome aberrations are induced by X-rays mainly during the first third of interphase. The aberrations produced during the last third of interphase are exclusively of the chromatid type (Revell, 1960).

C. Deoxyribonucleic Acid Synthesis, Chromosome Splitting, and Chromosome Breakage

Howard and Pelc (1953) have divided the interphase in *Vicia faba* root-tip cells into three stages on the basis of results obtained from studies on the incorporation of radioactive phosphorus (P^{32}) into chromosomal deoxyribonucleic acid (DNA). They found that P^{32} was incorporated during the middle third of these stages, which indicated that the synthesis of DNA occurs during this stage (interval *S*). The duration of *S* is about 6 hours. The time interval (G_2) between the end of synthesis and the beginning of division was estimated by Howard and Pelc to be 8 hours, and the time interval (G_1) between telophase and the beginning of synthesis at 12 hours. The duration of G_1 was obtained by subtracting G_2 and *S* from the total interphase time, given as 26 hours. The results of Howard and Pelc have been confirmed by Deeley *et al.* (1957) in a cytochemical study of the DNA-synthesis in root tips of *Vicia faba*. Deeley *et al.* point out, however, that the total interphase time of 26 hours given by Howard and Pelc is subject to

a rather large uncertainty. The results of Deeley *et al.* are more in agreement with a time of 20 hours. A total interphase time of approximately 20 hours has also been found by Revell (1953), and by Evans *et al.* (1957). When the total interphase time is 20 hours, G_1 is 6 hours. Thus, we may distinguish between three interphase periods of approximately the same duration: (1) a presynthesis period of about 6 hours, (2) the synthesis period itself, lasting about 6 hours, and (3) a postsynthesis period of about 8 hours.

The X-ray studies of Thoday (1954) indicate that chromosome reduplication in *Vicia faba* root-tip cells takes place during the same period of interphase as the DNA synthesis. Thoday found that of the isolocus breaks induced during the presynthesis period, 40 to 80% showed no evidence of sister-chromatid union, whereas of the isolocus breaks induced during the postsynthesis period, less than 10% were of the sister non-union type. Isolocus breaks with both proximal and distal sister non-union are believed to be of the chromosome type, i.e., induced in the chromosome before it is effectively split, whereas isolocus breaks with sister union in one or both of the fragments are believed to be of the chromatid type, i.e., induced when the chromosome is already differentiated into chromatids (Sax and Mather, 1939). Thus, the results of Thoday show that after irradiation with X-rays, the transition from chromosome to chromatid aberrations coincides with the period during which DNA is synthesized. Similar conclusions can be drawn from the results of Revell (1960), mentioned above. He obtained only chromatid aberrations during the last third of interphase, a period corresponding to the postsynthesis period. Most of the aberrations of the chromosome type were, on the other hand, induced during the first third of interphase, i.e., during the presynthesis period.

In *Tradescantia* microspores and pollen the transition from aberrations of the chromosome type to aberrations of the chromatid type also seems to coincide with the period of DNA synthesis (Taylor, 1953a; Moses and Taylor, 1955). The *Tradescantia* studies are even more convincing than the *Vicia* ones, since in the former material it is possible to choose the stage of development in such a way that only chromosome aberrations or only chromatid aberrations are obtained. However, according to Sax and King (1955), the two events do not coincide in meiotic cells.

It should be pointed out that workers using microspores of *Tradescantia* as experimental material claim that ionizing radiation induces chromatid aberrations (post-split changes) in early prophase and chromosome aberrations (pre-split changes) in interphase (Sax and Swanson, 1941). Thus, in this material the transition from pre-split changes to post-split changes would coincide in time not only with

DNA synthesis, but also with the transition from interphase to prophase. In *Vicia faba*, on the other hand, post-split aberrations are induced before visible prophase.

This apparent discrepancy between results obtained in *Tradescantia* and *Vicia* can be explained as a difference of definition. It appears from the descriptions and photographs of Beatty and Beatty (1953) that the stage defined by them as very early prophase corresponds to "late interphase" according to the terminology of authors working with *Vicia faba*. The analysis in *Tradescantia* is much facilitated by the fact that the mitoses are rather well synchronized in microspores from the same anther. In *Tradescantia* microspores, where the duration of the total mitotic cycle is a week or more, it is also easier to observe such changes in nuclear structure as precede a division than in *Vicia* root tips, where the total time is about 24 hours.

However, if we accept the idea that the transition from pre-split to post-split changes corresponds to the transition from interphase to prophase, it would mean that in *Vicia* root tips, the prophase is of longer duration than the interphase, since in this material chromatid aberrations can be induced during at least two-thirds of the approximately 20 hours during which the chromosomes are invisible (Revell, 1955b, 1960). The true interphase would be the presynthesis period G_1, whereas the postsynthesis period G_2 and most of the synthesis period S would be early stages of prophase.

Thoday (1954) has pointed out that the ability of ionizing radiation to produce structural chromosomal changes after the completion of the DNA synthesis shows that radiation-induced chromosome damage is not tied up with the metabolism of synthesis. For the same reason, the radiomimetic effect of *N*-methylated oxypurines cannot be due to an effect on the synthesis of DNA. It seems likely that chromosome-breaking agents with not-delayed effects act directly on the chromosomes, and not on chromosome precursors or on the processes involved in chromosome synthesis. The significance of DNA synthesis in the effects of these agents seems to be only that it is involved in the transition from chromosome to chromatid aberrations.

It appears to be quite another situation in the case of agents with delayed effects. No structural chromosomal changes can be observed the first 8 hours after treatments with these agents. Thus, they seem to be unable to produce chromosomal aberrations in cells where the DNA synthesis is completed. This indicates that agents with delayed effects act on chromosome precursors, rather than on the chromosome itself (Revell, 1953). Since the aberrations are always of the chromatid type, they are probably formed, or at least "initiated" (see page 48), in connection with the DNA synthesis and reduplication of the chromosome.

This is not difficult to understand, since the products formed by the chemical reaction between the chromosome precursors and the radiomimetic agents are expected to be incorporated into the chromosomes in connection with the reduplication process.

D. Localized Chromosome Breakage

It has appeared from the description of the effects of radiomimetic chemicals given in Section II that the distribution of aberrations induced by these agents, as a rule, seems to be nonrandom. The most extreme localization is found in the cases of ethoxycaffeine and maleic hydrazide. After treatments with these agents, almost all the resulting aberrations may be confined to a certain locus; this is the nucleolar constriction in the case of ethoxycaffeine, and a heterochromatic segment close to the centromere in the nucleolar arm of the M-chromosome, in the case of maleic hydrazide (see Fig. 6A and B).

The distribution of X-ray-induced isochromatid aberrations and chromatid interchanges appear to be nonrandom, too, but to a much lesser degree than is the distribution of aberrations induced by chemicals (Evans, 1960).

In the case of ethoxycaffeine, the possible reason for the localization has already been discussed (page 27). In other cases, the fact that interchanges are nonrandom and often occur between homologous loci in homologous chromosomes has been explained as being due to the fusing of heterochromatic segments, where the breaks are localized, in the interphase nucleus into chromocenters (McLeish, 1953). If we accept Revell's exchange hypothesis, the localization of all types of aberrations in heterochromatic segments, which is observed after treatments with agents with delayed effects, could also be explained as due to these segments being fused into chromocenters (Revell, 1953).

However, the chromocenter hypothesis does not quite satisfactorily account for the different degrees and types of localization obtained after treatments with different agents. Other factors, such as the reaction mechanism of the agent in question, its distribution within the nucleus, as well as the chemical composition and physiological activity of the affected segments, may also be involved in the production of localized chromosome breakage.

E. The Breakage-First Hypothesis and the Exchange Hypothesis

The two main hypotheses for the formation of chromosomal aberrations are the "breakage-first" hypothesis and Revell's "exchange hypothesis" (Revell, 1955a, 1959). Both of these hypotheses have been constructed mainly with the effect of ionizing radiation in mind.

According to the breakage-first hypothesis, the primary event is a

chromatid (or a chromosome) break. This occurs when an ionizing particle passes through, or in the immediate vicinity of, the chromosome, provided that a sufficient number of ionizations are produced within the sensitive volume. The ends in the breakage point may then either remain open or they may rejoin. What usually happens, according to the hypothesis, is that the ends rejoin with each other to restore the original configuration (restitution). The ends may also, however, rejoin with ends from other breaks, provided that the latter have occurred close enough in space and time. The results of such rejoining of ends from different breaks are unions of sister chromatids in an isochromatid break, and the various types of exchanges. Which of these alternatives will occur, is usually determined within 1 hour.

According to the exchange hypothesis, the primary event is not a break, but some other kind of disturbance in the chromosome. This disturbance decays with time, i.e., reverts to normal or to another state incapable of taking part in exchange formation. However, the primary event may be succeeded by another stage, which is called "exchange initiation" by Revell. This stable stage arises when two primary events are "close enough in space and time." In the exchange initiation stage, which can be of considerable duration, no genetic changes have yet occurred. Only during subsequent stages of chromosome development is the exchange-initiation stage transformed into a real chromatid exchange (Revell, 1959). According to Revell's hypothesis, all types of chromatid aberrations arise as a result of an exchange process. The so-called isochromatid break is one of four possible types of chromatid intrachanges and the chromatid break is an incomplete chromatid intrachange.

Revell had previously shown, in a qualitative manner, that all the different types of chromatid aberrations which occur after treatments with chromosome-breaking agents could have arisen as a result of chromatid intra- and interchange (Revell, 1955a). Recently, he has published data from an extensive, quantitative study which convincingly show that the different types of chromatid aberrations occur with frequencies expected on the basis of the exchange hypothesis (Revell, 1959). It may be added that facts such as the relationship between dose and effect, as well as the effects of dose rate and dose fractionation on the frequencies of X-ray-induced interchanges, do not conflict with Revell's exchange hypothesis in its present form.

Thus, Revell has presented very strong evidence in favor of his interpretation of chromatid aberrations. According to the opinion of the present author, one of the most attractive features of the exchange hypothesis is that it makes it much easier to understand why unreactive

chemicals, such as *N*-methylated oxypurines, are able to induce chromosomal aberrations.

Cytological studies have provided strong evidence in favor of a multistranded structure of the chromosome (for references, see Ris, 1957; Steffensen, 1960; Read, 1960). Each chromatid seems to contain at least 64 strands of DNA double helices. And the chromosome does not consist only of DNA, but also of different types of protein (e.g., Mirsky and Ris, 1947, 1951), ribonucleic acid (e.g., Brachet, 1942; Mirsky and Ris, 1947; Taylor, 1953b; Pelc and Howard, 1956), lipoids (e.g., Idelman, 1957; LaCour *et al.*, 1958), metals such as calcium (Steffensen and Bergeron, 1959), and various low-molecular compounds.

According to Wolff and Luippold (1955), breaks in covalent bonds are involved in chromosome breakage. If we accept this, it is not easy to see how radiations and chemicals can break such a complex structure as the chromosome appears to be. These difficulties exist in the case of ionizing radiation (see, for instance, Sparrow *et al.*, 1952; Read, 1960), but they are particularly pronounced in the case of the radiomimetic chemicals.

The chromosome-breaking effect becomes a little more understandable if it is assumed that the chromosome has a particulate structure with weaker bonds between the structural units (Marquardt, 1950; Ambrose and Gopal-Ayengar, 1953; Mazia, 1954). The breaks would always occur between the structural units, which should be linked together by hydrogen bonds (Ambrose and Gopal-Ayengar, 1953) or by bridges of divalent ions (Mazia, 1954; Steffenson, 1957). However, if we accept the idea that radiomimetic agents produce breaks, e.g., by reacting with such molecular groups in the chromosomes as are responsible for the bonds between the structural units (Marquardt, 1950), the question immediately arises: how is it possible that broken ends which have been changed by chemical reactions, some 10 or 20 minutes later are able to rejoin with other, similarly affected ends?

If we accept the exchange hypothesis, all these difficulties are avoided. In this case the primary event is not a break, but some considerably less drastic disturbance, which becomes transformed and magnified into an exchange only later. The chromosome has a known capacity to exchange hereditary material. Normally, the exchange process takes place under cellular control in the pachytene stage of meiosis. It is possible to suppose that agents which induce chromosomal aberrations do so by creating a situation in the mitotic cell which leads to a similar kind of exchange process. Such exchanges must, of course, be abnormal and therefore often asymmetrical, sometimes incomplete, and mostly between

heterologous chromosomes. The similarities between the meiotic crossing-over process and the exchange processes initiated by chromosome-breaking agents have also been pointed out by other authors, e.g., Marquardt (1950) and Östergren and Wakonig (1954).

In addition to making the chromosome-breaking effects of chemicals and radiations more understandable, the exchange hypothesis also provides an explanation for the fact that aberrations tend to be localized in heterochromatin. Since the heterochromatic chromosome segments fuse in the interphase nucleus into chromocenters, exchanges between these parts would be expected to occur much more easily than between other parts of the chromosomes. In this connection the recent findings of Evans (1960) are of considerable interest. Evans showed that the distribution of X-ray-induced aberrations within chromosomes is in part correlated with the distribution of heterochromatin, and he suggests "that the increased aberration frequency at or near heterochromatic regions is a consequence of the fusion of heterochromatin in the interphase nucleus into chromocentres." Since the distribution of a type of aberration such as an isochromatid break should be affected by the spatial arrangement of the chromosomes in the interphase nucleus if it had arisen as a result of an exchange, but not necessarily if it had arisen as a result of a break followed by sister union, Evans' findings seem to support the exchange hypothesis.

Revell (1959) has correctly pointed out that the term chromatid break has been used in the past as a common name for a number of changes, most of which are not real breaks at all, but merely achromatic gaps in a continuous chromosome. He proposes that the comparatively few real chromatid breaks which occur, arise as a result of incomplete chromatid intrachange. Since the so-called isochromatid breaks are also presumed to be a type of chromatid intrachange, one might ask whether breaks occur at all after treatments with chromosome-breaking agents, except as a result of incomplete exchanges. It is the opinion of the present author that there is at least one type of break which almost certainly does not arise by an exchange mechanism. The type of break referred to is the one found in connection with the extreme fragmentation of some or all chromosomes in the cell, a phenomenon which is sometimes seen after treatments with chemicals and radiations. This extreme fragmentation has been called chromosome "shattering" by Lovelace (1954), "pulverization" by D'Amato (1950), and "totaler Zusammenbruch" by Marquardt (1950). The fact that breaks may occasionally arise by other processes than chromatid exchange does not, of course, invalidate the exchange hypothesis.

It was pointed out in the introduction that, during the last few

years, chromosome breakage has been a subject of biochemistry rather than of descriptive cytology or biophysics, as it was fifteen years ago. The present review article has also been concerned mainly with the biochemical aspects of chromosome breakage. However, although a biochemical approach to the problem of chromosome breakage may give us much valuable information, it is becoming increasingly clear that this phenomenon belongs among those which cannot really be understood until the gaps in our knowledge of their "submolecular" or "electronic" aspects (Szent-Györgyi, 1960) have been filled. Thus, it is felt that a successful approach to the problem would require not only biochemistry but also biophysics in its modern form, specifically, quantum mechanics of living material.

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References

Albuquerque, R. M., and Serra, J. A., 1951. Nucleolar composition and the nucleolar zones of the chromosomes. *Portugaliae Acta Biol. Sér. A.* **3,** 187–193.

Alper, T., 1956. The modification of damage caused by primary ionization of biological targets. *Radiation Research* **5,** 573–586.

Alper, T., and Howard-Flanders, P., 1956. Role of oxygen in modifying the radiosensitivity of *E. coli* B. *Nature* **178,** 978–979.

Ambrose, E. J., and Gopal-Ayengar, A. R., 1953. Molecular orientation and chromosome breakage. *In* "Symposium on Chromosome Breakage." *Heredity* **6,** Suppl., pp. 277–292.

Armstrong, J. A., 1956. Histochemical differentiation of nucleic acids by means of induced fluorescence. *Exptl. Cell Research* **11,** 640–643.

Auerbach, C., 1951. Some recent results with chemical mutagens. *Hereditas* **37,** 1–16.

Bauch, R., 1949. Selektive Speicherung von Trypaflavin durch die Nukleoproteide der Chromosomen. *Biol. Zentr.* **68,** 113–118.

Baud, C. A., 1948. A cytochemical study of the perinuclear lipidic layer in the liver cell. *Nature* **161,** 559.

Beatty, A. V., and Beatty, J. W., 1957. Chromosome breakage and rejoining in *Tradescantia* microspores. *Am. J. Botany* **44,** 778–783.

Beatty, A. V., and Beatty, J. W., 1959. Metabolic inhibitors and chromosome rejoining. *Am. J. Botany* **46,** 317–323.

Beatty, A. V., and Beatty, J. W., 1960. Postirradiative effects on chromosomal aberrations in *Tradescantia* microspores. *Genetics* **45,** 331–344.

Beatty, A. V., Beatty, J. W., and Collins, C., 1956. Effects of various intensities of X radiation on chromosomal aberrations. *Am. J. Botany* **43,** 328–332.

Beatty, J. W., and Beatty, A. V., 1953. Duration of the stages in microspore development and in the first microspore division of *Tradescantia paludosa*. *Am. J. Botany* **40,** 593–596.

Beers, R. F., Hendley, D. D., and Steiner, R. F., 1958. Inhibition and activation of polynucleotide phosphorylase through the formation of complexes between acridine orange and polynucleotides. *Nature* **182,** 242–244.

Biesele, J. J., Berger, R. E., Clarke, M., and Weiss, L., 1952. Effects of purines and other chemotherapeutic agents on nuclear structure and function. *In* "The Chemistry and Physiology of the Nucleus." *Exptl. Cell Research, Suppl.* **2,** pp. 279–300.

Blum, H. F., 1941. "Photodynamic Action and Diseases Caused by Light" (Am. Chem. Soc. Monograph Ser. 85), 309 pp. Reinhold, New York.

Booth, J., and Boyland, E., 1953. The reaction of the carcinogenic dibenzcarbazoles and dibenzacridines with purines and nucleic acid. *Biochim. et Biophys. Acta* **12,** 75–87.

Brachet, J., 1942. La localisation des acides pentosenucléiques dans les tissus animaux et les oeufs d'Amphibiens en voie de développement. *Arch. biol. (Liège)* **53,** 207–257.

Callan, H. G., and Tomlin, S. G., 1950. Experimental studies on amphibian oocyte nuclei. I. Investigation of the structure of the nuclear membrane by means of the electron microscope. *Proc. Roy. Soc.* **B137,** 367–378.

Clare, N. T., 1956. Photodynamic action and its pathological effects. *In* "Radiation Biology" (A. Hollaender, ed.), Vol. III, pp. 693–723. McGraw-Hill, New York.

Cohn, N. S., 1958. An analysis of the rejoining of X-ray-induced broken ends of chromosomes in the root tips of *Allium cepa*. *Genetics* **43,** 362–373.

Crouse, H. V., 1954. X-ray breakage of lily chromosomes at first meiotic metaphase. *Science* **119,** 485–487.

D'Amato, F., 1950. The chromosome breaking activity of chemicals as studied by the *Allium cepa* test. *Pubbl. staz. zool. Napoli* **22,** Suppl., 158–170.

Darlington, C. D., and Koller, P. C., 1947. The chemical breakage of chromosomes. *Heredity* **1,** 187–221.

Darlington, C. D., and McLeish, J., 1951. Action of maleic hydrazide on the cell. *Nature* **167,** 407–408.

DeBruyn, P. P. H., Farr, R. S., Banks, H., and Morthland, F. W., 1953. *In vivo* and *in vitro* affinity of diaminoacridines for nucleoproteins. *Exptl. Cell Research* **4,** 174–180.

Deeley, E. M., Davies, H. G., and Chayen, J., 1957. The DNA content of cells in the root of *Vicia faba*. *Exptl. Cell Research* **12,** 582–591.

Deschner, E. E., and Gray, L. H., 1959. Influence of oxygen tension on X-ray-induced chromosomal damage in Ehrlich ascites tumor cells irradiated *in vitro* and *in vivo*. *Radiation Research* **11,** 115–146.

Dewey, D. L., 1960. Effect of oxygen and nitric oxide on the radiosensitivity of human cells in tissue culture. *Nature* **186,** 780–782.

Evans, H. J., 1960. The frequency and distribution of interchange and isochromatid aberrations induced by the irradiation of diploid and tetraploid cells. *In* "Symposium on the Effects of Ionizing Radiation on Seeds and their Significance for Crop Improvement." Karlsruhe, Aug. 8–12, 1960. In press.

Evans, H. J., and Neary, G. J., 1959. The influence of oxygen on the sensitivity of *Tradescantia* pollen tube chromosomes to X-rays. *Radiation Research* **11,** 636–641.

Evans, H. J., Neary, G. J., and Tonkinson, S. M., 1957. The use of colchicine as an indicator of mitotic rate in broad bean root meristems. *J. Genet.* **55,** 487–502.

Fabergé, A. C., 1940. An experiment on chromosome fragmentation in *Tradescantia* by X-rays. *J. Genet.* **39,** 229–248.

Ford, C. E., 1949. Chromosome breakage in nitrogen mustard treated *Vicia faba* root tip cells. *Proc. Intern. Congr. Genet., 8th Congr. Stockholm* (*Hereditas,* Suppl. Vol.), pp. 570–571.

Fredga, K., and Nyman, P. O., 1961. Study of the penetration of 8-ethoxycaffeine into roots of *Vicia faba. Exptl. Cell Research* **22,** 146–150.

Giles, N. H., 1954. Radiation-induced chromosome aberrations in *Tradescantia. In* "Radiation Biology" (A. Hollaender, ed.), Vol. I, pp. 713–761. McGraw-Hill, New York.

Giles, N. H., and Beatty, A. V., 1950. The effect of X-irradiation in oxygen and in hydrogen at normal and positive pressures on chromosome aberration frequency in *Tradescantia* microspores. *Science* **112,** 643–645.

Giles, N. H., and Riley, H. P., 1949. The effect of oxygen on the frequency of X-ray induced chromosomal rearrangements in *Tradescantia* microspores. *Proc. Natl. Acad. Sci. US.* **35,** 640–646.

Giles, N. H., and Riley, H. P., 1950. Studies on the mechanism of the oxygen effect on the radiosensitivity of *Tradescantia* chromosomes. *Proc. Natl. Acad. Sci. US.* **36,** 337–344.

Gordon, S. A., and Surrey, K., 1958. A biochemical basis for the far-red potentiation of X-ray induced chromosomal breaks. *Radiation Research* **9,** 121.

Gordon, S. A., and Surrey, K., 1960. Red and far-red action on oxidative phosphorylation. *Radiation Research* **12,** 325–339.

Graf, G. E., 1957. Chromosome breakage induced by X-rays, maleic hydrazide, and its derivatives in relation to knob number in maize. *J. Heredity* **48,** 155–159.

Gray, L. H., 1953. The initiation and development of cellular damage by ionizing radiations. *Brit. J. Radiol.* **26,** 609–618.

Gray, L. H., Green, F. O., and Hawes, C. A., 1958. Effect of nitric oxide on the radiosensitivity of tumour cells. *Nature* **182,** 952–953.

Hackett, D. P., 1959. Respiratory mechanism in higher plants. *Ann. Rev. Plant Physiol.* **10,** 113–146.

Howard, A., and Pelc, S. R., 1953. Synthesis of desoxyribonucleic acid in normal and irradiated cells and its relation to chromosome breakage. *In* "Symposium on Chromosome Breakage." *Heredity* **6,** Suppl., 261–273.

Howard-Flanders, P., 1957. The effect of nitric oxide on the radiosensitivity of bacteria. *Nature* **180,** 1191–1192.

Howard-Flanders, P., 1958. Physical and chemical mechanisms in the injury of cells by ionizing radiations. *Advances in Biol. and Med. Phys.* **6,** 553–603.

Howard-Flanders, P., 1959. Primary physical and chemical processes in radiobiology. *In* "Radiation Biology and Cancer," pp. 29–40. Univ. of Texas, Austin, Texas.

Howard-Flanders, P., and Alper, T., 1957. The sensitivity of microorganisms to irradiation under controlled gas conditions. *Radiation Research* **7,** 518–540.

Howard-Flanders, P., and Jockey, P., 1960. Similarities in the effects of oxygen and nitric oxide on the rate of inactivation of vegetative bacteria by X-rays. *Radiation Research* **13,** 466–478.

Hughes, C., and Spragg, S. P., 1958. The inhibition of mitosis by the reaction of maleic hydrazide with sulphydryl groups. *Biochem. J.* **70,** 205–212.

Idelman, S., 1957. Existence d'un complexe lipides-nucléoprotéines à groupements sulfhydrilés au niveau du chromosome. *Compt. rend. acad. sci.* **244,** 1827–1828.

Kalckar, H. M., 1954. The mechanisms of transglycosidation. *In* "The Mechanism of Enzyme Action" (W. D. McElroy and B. Glass, eds.) pp. 675–728. The Johns Hopkins Press, Baltimore, Maryland.

Kihlman, B. A., 1951. The permeability of the nuclear envelope and the mode of action of purine derivatives on chromosomes. *Symbolae Botan. Upsalienses* **11** (2), 1–40.

Kihlman, B. A., 1952. Induction of chromosome changes with purine derivatives. *Symbolae Botan. Upsalienses* **11**(4), 1–96.

Kihlman, B. A., 1955a. Chromosome breakage in *Allium* by 8-ethoxycaffeine and X-rays. *Exptl. Cell Research* **8,** 345–368.

Kihlman, B. A., 1955b. Studies on the effect of oxygen on chromosome breakage induced by 8-ethoxycaffeine. *Exptl. Cell Research* **8,** 404–407.

Kihlman, B. A., 1955c. Oxygen and the production of chromosome aberrations by chemicals and X-rays. *Hereditas* **41,** 384–404.

Kihlman, B. A., 1956. Factors affecting the production of chromosome aberrations by chemicals. *J. Biophys. Biochem. Cytol.* **2,** 543–555.

Kihlman, B. A., 1957. Experimentally induced chromosome aberrations in plants. I. The production of chromosome aberrations by cyanide and other heavy metal complexing agents. *J. Biophys. Biochem. Cytol.* **3,** 363–380.

Kihlman, B. A., 1958a. The effect of oxygen, nitric oxide, and respiratory inhibitors on the production of chromosome aberrations by X-rays. *Exptl. Cell Research* **14,** 639–642.

Kihlman, B. A., 1958b. Respiration and radiosensitivity of broad bean roots. *Nature* **182,** 730–731.

Kihlman, B. A., 1959a. Induction of structural chromosome changes by visible light. *Nature* **183,** 976–978.

Kihlman, B. A., 1959b. On the radiomimetic effects of cupferron and potassium cyanide. *J. Biophys. Biochem. Cytol.* **5,** 351–353.

Kihlman, B. A., 1959c. The effect of respiratory inhibitors and chelating agents on the frequencies of chromosomal aberrations produced by X-rays in *Vicia. J. Biophys. Biochem. Cytol.* **5,** 479–490.

Kihlman, B. A., 1959d. Effect of nitric oxide on the production of chromosomal aberrations by X-rays. *Exptl. Cell Research* **17,** 588–590.

Kihlman, B. A., 1959e. Studies on the production of chromosomal aberrations by visible light: the effects of cupferron, nitric oxide, and wavelength. *Exptl. Cell. Research* **17,** 590–593.

Kihlman, B. A., 1960. The radiomimetic effect of N-nitroso-N-methylurethan in *Vicia faba. Exptl. Cell Research* **20,** 657–659.

Kihlman, B. A., 1961a. Cytological effects of phenylnitrosamines. I. The production of structural chromosome changes in the presence of light and acridine orange. *Radiation Botany.* In press.

Kihlman, B. A., 1961b. Cytological effects of phenylnitrosamines. II. Radiomimetic effects. *Radiation Botany.* In press.

Kihlman, B. A., 1961c. Cytological effects of phenylnitrosamines. III. The effect on X-ray sensitivity at low oxygen tensions. *Radiation Botany.* In press.

Kihlman, B. A., and Levan, A., 1951. Localized chromosome breakage in *Vicia faba. Hereditas* **37,** 382–388.

Kihlman, B. A., and Overgaard-Hansen, K., 1955. Inhibition of muscle phosphorylase by methylated oxypurines. *Exptl. Cell Research* **8,** 252–255.

King, E. D., Schneiderman, H. A., and Sax, K., 1952. The effects of carbon monoxide

and oxygen on the frequency of X-ray induced chromosome aberrations in *Tradescantia*. *Proc. Natl. Acad. Sci. U.S.* **38,** 34–43.

Kirby-Smith, J. S., and Craig, D. L., 1957. The induction of chromosome aberrations in *Tradescantia* by ultraviolet radiation. *Genetics* **42,** 176–187.

Koch, A. L., 1956. The metabolism of methylpurines by *Escherichia coli*. I. Tracer studies. *J. Biol. Chem.* **219,** 181–188.

Kotval, J. P., and Gray, L. H., 1947. Structural changes produced in microspores of *Tradescantia* by α-radiation. *J. Genet.* **48,** 135–154.

LaCour, L. F., and Rutishauser, A., 1954. X-ray breakage experiments with endosperm. I. Sub-chromatid breakage. *Chromosoma* **6,** 696–709.

LaCour, L. F., Chayen, J., and Gahan, P. S., 1958. Evidence for lipid material in chromosomes. *Exptl. Cell Research* **14,** 469–485.

Lane, G. R., 1955. Chromosome breakage by diepoxide and by X-rays. *In* "Proceedings of the Radiobiology Symposium, Liège, 1954" (Z. M. Bacq and P. Alexander, eds.), pp. 265–274, Butterworths, London.

Lea, D. E., 1946. "Actions of Radiations on Living Cells." 402 pp. Cambridge Univ. Press, London and New York.

Levan, A., 1949. The influence on chromosomes and mitosis of chemicals, as studied by the *Allium* test. *Proc. Intern. Congr. Genet., 8th Congr. Stockholm* (*Hereditas,* Suppl. Vol.), pp. 325–337.

Levan, A., and Lotfy, T., 1950. Spontaneous chromosome fragmentation in seedlings of *Vicia faba*. *Hereditas* **36,** 470–482.

Lilly, L. J., 1958. Effects of cyanide and ionizing radiation on the roots of *Vicia faba*. *Exptl. Cell Research* **14,** 257–267.

Lilly, L. J., and Thoday, J. M., 1956. Effects of cyanide on the roots of *Vicia faba*. *Nature* **177,** 338–339.

Lovelace, R., 1954. Chromosome shattering by ultraviolet radiation. *Proc. Natl. Acad. Sci. U.S.* **40,** 1129–1135.

Loveless, A., 1951. Qualitative aspects of the chemistry and biology of radiomimetic (mutagenic) substances. *Nature* **167,** 338–342.

Loveless, A., 1953. Chemical and biochemical problems arising from the study of chromosome breakage by alkylating agents and heterocyclic compounds. *In* "Symposium on Chromosome Breakage." *Heredity* **6,** Suppl., 293–298.

Loveless, A., and Revell, S., 1949. New evidence on the mode of action of "mitotic poisons." *Nature* **164,** 938–944.

McLeish, J., 1953. The action of maleic hydrazide in *Vicia*. *In* "Symposium on Chromosome Breakage." *Heredity* **6,** Suppl., 125–147.

McLeish, J., 1954. The consequences of localized chromosome breakage. *Heredity* **8,** 385–407.

Marquardt, H., 1950. Neuere Auffassungen über einige Probleme aus der Pathologie der Kernteilung. *Naturwissenschaften* **37,** 416–424, 433–438.

Mazia, D., 1954. The particulate organization of the chromosome. *Proc. Natl. Acad. Sci. U.S.* **40,** 521–527.

Michaelis, A., and Rieger, R., 1960. Einige experimentelle Ergebnisse zur Wirkung von Myleran auf die chromosomen von *Vicia faba* L. *Der Züchter* **30,** 150–163.

Michaelis, A., Ramshorn, K., and Rieger, R., 1959. Äthylalkohol—radiomimetisches Agens bei *Vicia faba* L. *Naturwissenschaften* **46,** 381–382.

Mirsky, A. E., and Ris, H., 1947. Chemical composition of isolated chromosomes. *J. Gen. Physiol.* **31,** 7–18.

Mirsky, A. E., and Ris, H., 1951. The composition and structure of isolated chromosomes. *J. Gen. Physiol.* **34,** 475–492.

Moh, C. C., and Withrow, R. B., 1959a. Nonionizing radiant energy as an agent in altering the incidence of X-ray-induced chromatid aberrations. II. Reversal of the far-red potentiating effect in *Vicia* by red radiant energy. *Radiation Research* **10,** 13–19.

Moh, C. C., and Withrow, R. B., 1959b. Nonionizing radiant energy as an agent in altering the incidence of X-ray-induced chromatid aberrations. III. Action spectrum of far-red potentiation. *Radiation Research* **11,** 18–23.

Moses, M. J., and Taylor, J. H., 1955. Desoxypentose nucleic acid synthesis during microsporogenesis in *Tradescantia*. *Exptl. Cell Research* **9,** 474–488.

Moutschen-Dahmen, J., and Moutschen-Dahmen, M., 1958. Sur l'évolution des lésions causées par la 8-ethoxycaféine chez *Hordeum sativum* et chez *Vicia faba*. *Hereditas* **44,** 18–36.

Muir, R. M., and Hansch, C., 1953. On the mechanism of action of growth regulators. *Plant Physiol.* **28,** 218–232.

Naylor, A. W., and Davis, E. A., 1951. Respiration response of root tips to maleic hydrazide. *Bull. Torrey Botan. Club* **78,** 73–79.

Neish, W. J. P., 1948. On the solubilization of aromatic amines by purines. *Rec. trav. chim.* **67,** 361–373.

Novick, A., 1956. Mutagens and antimutagens. *In* "Mutation." *Brookhaven Symposia in Biol.* **8,** (*BNL 350(C-22)*) 201–214.

Östergren, G., and Wakonig, T., 1954. True or apparent sub-chromatid breakage and the induction of labile states in cytological chromosome loci. *Botan. Notiser* **107,** 357–375.

Pelc, S. R., and Howard, A., 1956. Metabolic activity of salivary gland chromosomes in *Diptera*. *Exptl. Cell Research* **10,** 549–552.

Powers, E. L., Kaleta, B. F., and Webb, R. B., 1959. Nitric oxide protection against radiation damage. *Radiation Research* **11,** 461.

Powers, E. L., Webb, R. B., and Kaleta, B. F., 1960. Oxygen and nitric oxide as modifiers of radiation injury in spores of *Bacillus megaterium*. *Proc. Natl. Acad. Sci. US.* **46,** 984–993.

Pullman, B., and Pullman, A., 1958. Electron-donor and -acceptor properties of biologically important purines, pyrimidines, pteridines, flavins, and aromatic amino acids. *Proc. Natl. Acad. Sci. US.* **44,** 1197–1202.

Read, J., 1960. Chromosome size, structure, and radiation damage. *In* "Symposium on the Effects of Ionizing Radiation on Seeds and their Significance for Crop Improvement." Karlsruhe, Aug. 8–12, 1960. In press.

Read, J., and Kihlman, B. A., 1956. Comparison of the effects of 8-ethoxycaffeine and X-rays on the cytology and growth of roots of *Vicia faba*. *Hereditas* **42,** 487–507.

Revell, S. H., 1953. Chromosome breakage by X-rays and radiomimetic substances in *Vicia*. *In* "Symposium on Chromosome Breakage." *Heredity* **6,** Suppl., 107–124.

Revell, S. H., 1955a. A new hypothesis for "chromatid" changes. *In* "Proceedings of the Radiobiology Symposium, Liège, 1954" (Z. M. Bacq and P. Alexander, eds.), pp. 243–253. Butterworths, London.

Revell, S. H., 1955b. The exchange hypothesis for chromatid aberrations induced by X-rays. *Brit. Empire Cancer Campaign, Ann. Rept.* **33,** 48–49.

Revell, S. H., 1959. The accurate estimation of chromatid breakage, and its rele-

vance to a new interpretation of chromatid aberrations induced by ionizing radiations. *Proc. Royal Soc.* **B150,** 563–589.

Revell, S. H., 1960. An attempt at continuous metaphase estimation of chromatid and chromosome aberration frequencies in broad bean root-meristem cells in the period 2 to 23 hours after 50 r of X-rays. *In* "Symposium on the Effects of Ionizing Radiation on Seeds and their Significance for Crop Improvement." Karlsruhe, Aug. 8–12, 1960. In press.

Rieger, R., and Michaelis, A., 1958. Cytologische und stoffwechsel-physiologische Untersuchungen am aktiven Meristem der Wurzelspitze von *Vicia jaba. L.* I. Der Einfluss der Unterwasser-Quellung der Samen auf die chromosomale Aberrationsrate. *Chromosoma* **9,** 238–257.

Rieger, R., and Michaelis, A., 1960a. Über die radiomimetische Wirkung von Äthylalkohol bei *Vicia faba. In* "Chemische Mutagenese." Erwin-Baur-Gedächtnisvorlesungen I. *Abhandl. deut. Akad. Wiss. Berlin. Kl. Med.* **1,** 54–65.

Rieger, R., and Michaelis, A., 1960b. Der Einfluss von Stoffwechselinhibitoren auf die radiomimetische Wirkung von Äthylalkohol. *Monatsber. deut. Akad. Wiss. Berlin* **2,** 290–297.

Riley, H. P., Giles, N. H., and Beatty, A. V., 1952. The effect of oxygen on the induction of chromatid aberrations in *Tradescantia* microspores by X-irradiation. *Am. J. Botany* **39,** 592–597.

Ris, H., 1957. Chromosome structure. *In* "The Chemical Basis of Heredity" (W. D. McElroy and B. Glass, eds.), pp. 23–62. The Johns Hopkins Press, Baltimore, Maryland.

Ross, W. C. J., 1953. The chemistry of cytotoxic alkylating agents. *Advances in Cancer Research* **1,** 397–449.

Ross, W. C. J., 1958. *In vitro* reactions of biological alkylating agents. *Ann. N.Y. Acad. Sci.* **68,** 669–681.

Sax, K., 1939. The time factor in X-ray production of chromosome aberrations. *Proc. Natl. Acad. Sci. US.* **25,** 225–233.

Sax, K., 1940. An analysis of X-ray induced chromosomal aberrations in *Tradescantia. Genetics* **25,** 41–68.

Sax, K., 1941. Types and frequencies of chromosomal aberrations induced by X-rays. *Cold Spring Harbor Symposia Quant. Biol.* **9,** 93–101.

Sax, K., and King, E. D., 1955. An X-ray analysis of chromosome duplication. *Proc. Natl. Acad. Sci. US.* **41,** 150–155.

Sax, K., and Mather, K., 1939. An X-ray analysis of progressive chromosome splitting. *J. Genet.* **37,** 483–490.

Sax, K., and Swanson, C. P., 1941. Differential sensitivity of cells to X-rays. *Am. J. Botany* **28,** 52–59.

Schenck, G. O., 1955. Apparate für Lichtreaktionen und ihre Anwendung in der präparativen Photochemie. *Dechema Monograph.* **24,** 105–145.

Schenck, G. O., Gollnick, K., and Neumüller, O. A., 1957. Darstellung von Steroid-Hydroperoxyden mittels phototoxischer Photosensibilisatoren. *Ann. Chem. Liebigs* **603,** 46–59.

Schenck, G. O., Meder, W., and Pape, M., 1959. Formation of radicals by ultraviolet and ionizing radiations and their reactions in plastics. *Proc. 2nd Intern. Conf. Peaceful Uses Atomic Energy, Geneva,* pp. 352–363.

Smith, H. H., and Lotfy, T., 1955. Effects of beta-propiolactone and ceepryn on chromosomes of *Vicia* and *Allium. Am. J. Botany* **42,** 750–758.

Smith, H. H., and Srb, A. M., 1951. Induction of mutations with β-propiolactone. *Science* **114,** 490–492.

Sparrman, B., Ehrenberg, L., and Ehrenberg, A., 1959. Scavenging of free radicals and radiation protection by nitric oxide in plant seeds. *Acta Chem. Scand.* **13,** 199–200.

Sparrow, A. H., Moses, M. J., and Dubow, R. J., 1952. Relationships between ionizing radiation, chromosome breakage and certain other nuclear disturbances. *In* "The Chemistry and Physiology of the Nucleus." *Exptl. Cell Research, Suppl.* **2,** pp. 245–262.

Stacey, K. A., Cobb, M., Cousens, S. F., and Alexander, P., 1958. The reactions of the "radiomimetic" alkylating agents with macromolecules *in vitro*. *Ann. N.Y. Acad. Sci.* **68,** 682–701.

Steffensen, D., 1957. Effects of various cation imbalances on the frequency of X-ray-induced chromosomal aberrations in *Tradescantia*. *Genetics* **42,** 239–252.

Steffensen, D., 1960. A comparative view of the chromosome. *In* "Structure and Function of Genetic Elements." *Brookhaven Symposia in Biol.* **12,** 103–118.

Steffensen, D., and Bergeron, J. A., 1959. Autoradiographs of pollen tube nuclei with calcium-45. *J. Biophys. Biochem. Cytol.* **6,** 339–342.

Swanson, C. P., 1940. A comparison of chromosomal aberrations induced by X-ray and ultra-violet radiation. *Proc. Natl. Acad. Sci. US.* **26,** 366–373.

Swanson, C. P., 1942. The effects of ultraviolet and X-ray treatment on the pollen tube chromosomes of *Tradescantia*. *Genetics* **27,** 491–503.

Swanson, C. P., 1947. X-ray and ultraviolet studies on pollen tube chromosomes. II. The quadripartite structure of the prophase chromosomes of *Tradescantia*. *Proc. Natl. Acad. Sci. US.* **33,** 229–232.

Swanson, C. P., 1949. Further studies on the effect of infrared radiation on X-ray-induced chromatid aberrations in *Tradescantia*. *Proc. Natl. Acad. Sci. US.* **35,** 237–244.

Swanson, C. P., and Merz, T., 1959. Factors influencing the effect of β-propiolactone on chromosomes of *Vicia faba*. *Science* **129,** 1364–1365.

Szent-Györgyi, A., 1960. Submolecular biology. *In* "Bioenergetics." *Radiation Research, Suppl.* **2,** pp. 4–18.

Taylor, J. H., 1953a. Autoradiographic detection of incorporation of P^{32} into chromosomes during meiosis and mitosis. *Exptl. Cell Research* **4,** 164–173.

Taylor, J. H., 1953b. Intracellular localization of labeled nucleic acid determined with autoradiographs. *Science* **118,** 555–557.

Thoday, J. M., 1951. The effect of ionizing radiations on the broad bean root. IX. Chromosome breakage and the lethality of ionizing radiations to the root meristem. *Brit. J. Radiol.* **24,** 572–576, 622–628.

Thoday, J. M., 1954. Radiation-induced chromosome breakage, desoxyribosenucleic acid synthesis and the mitotic cycle in root-meristem cells of *Vicia faba*. *New Phytologist* **53,** 511–516.

Thoday, J. M., and Read, J., 1947. Effect of oxygen on the frequency of chromosome aberrations produced by X-rays. *Nature* **160,** 608.

Thoday, J. M., and Read, J., 1949. Effect of oxygen on the frequency of chromosome aberrations produced by alpha-rays. *Nature* **163,** 133–134.

Wagner, R. P., Haddox, C. H., Fuerst, R., and Stone, W. S., 1950. The effect of irradiated medium, cyanide and peroxide on the mutation rate in *Neurospora*. *Genetics* **35,** 237–248.

Weil-Malherbe, H., 1946. The solubilization of polycyclic aromatic hydrocarbons by purines. *Biochem J.* **40,** 351–363.

Withrow, R. B., and Moh, C. C., 1957. Nonionizing radiant energy as an agent in altering the incidence of X-ray-induced chromatid aberrations. I. Effects of far-red and infrared radiant energy on *Tradescantia* and *Vicia*. *Radiation Research* **6,** 491–500.

Wolff, S., 1954. Delay of chromosome rejoining in *Vicia faba* induced by irradiation. *Nature* **173,** 501–502.

Wolff, S., 1960a. Radiation studies on the nature of chromosome breakage. *Am. Naturalist* **94,** 85–93.

Wolff, S., 1960b. On the apparent synergistic effect of far-red and X-rays in the production of intergenic mutations (chromosome aberrations). *Third Intern. Congr. Photobiol., Copenhagen.* (The Finsen Memorial Congress). Summaries of Papers, p. 87.

Wolff, S., and Atwood, K. C., 1954. Independent X-ray effects on chromosome breakage and reunion. *Proc. Natl. Acad. Sci. US.* **40,** 187–192.

Wolff, S., and Luippold, H. E., 1955. Metabolism and chromosome-break rejoining. *Science* **122,** 231–232.

Wolff, S., and Luippold, H. E., 1956a. The biochemical aspects of chromosome rejoining. *In* "Progress in Radiobiology" (J. S. Mitchell, B. E. Holmes, and C. L. Smith, eds.), pp. 217–221. Oliver and Boyd, Edinburgh.

Wolff, S., and Luippold, H. E., 1956b. The production of two chemically different types of chromosomal breaks by ionizing radiations. *Proc. Natl. Acad. Sci. US.* **42,** 510–514.

Wolff, S., and Luippold, H. E., 1958. Modification of chromosomal aberration yield by postirradiation treatment. *Genetics* **43,** 493–501.

Wyss, O., Clark, J. B., Haas, F., and Stone, W. S., 1948. The role of peroxide in the biological effects of irradiated broth. *J. Bacteriol.* **56,** 51–57.

Yamamoto, N., 1958. Photodynamic inactivation of bacteriophage and its inhibition. *J. Bacteriol.* **75,** 443–448.

Yost, H. T., 1951. The frequency of X-ray induced chromosome aberrations in *Tradescantia* as modified by near infrared radiation. *Genetics* **36,** 176–184.

Yost, H. T., and Robson, H. H., 1959. Studies on the effects of irradiation of cellular particulates. III. The effect of combined radiation treatments on phosphorylation. *Biol. Bull.* **116,** 498–506.

THE GENETICS OF TRANSFORMATION

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	Page
I. Introduction	62
A. Historical Review	62
B. Extent of the Phenomenon of Transformation	65
C. Previous Reviews of the Field	71
II. Chemical Nature of the Transforming Agent	72
A. DNA as an Essential and Sufficient Constituent of the Transforming Agent	72
B. Current Views on the Molecular Structure of DNA	76
C. Reactivity of DNA	79
D. Biosynthesis of DNA	93
III. Competence	94
IV. Penetration	100
A. Does DNA of High Molecular Weight Penetrate?	100
B. Does DNA Pass through Localized "Holes" in the Bacterial Surface or Is Penetration an Enzymatically Catalyzed Process Occurring at Specific Receptor Sites?	106
V. Genetic Integration and Phenotypic Expression	111
A. Reverse Transformations and the Existence of "Allelic" Transforming Agents	111
B. Genetic Heterogeneity of Transforming DNA and the Existence of Determinants Transferred Independently of Each Other	112
C. Linkage of Determinants Affecting Different Characters	114
D. Linkage of Determinants Affecting the Same Character	117
E. Intramolecular Recombination and Genetic Integration	123
F. Phenotypic Expression of the Infecting Determinant	129
VI. Mechanism of Recombination Occurring in Transformation	131
A. Copy-Choice vs. Breakage-Reunion	131
B. Comparison of the Mechanisms of Recombination Involved in Transformation, Transduction, and Conjugation	140
VII. Mechanism of the Heterocatalytic Function of the Transforming Agent	142
VIII. Interspecific Transfer of Bacterial Genes and Bacterial Evolution	147
References	151

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I. Introduction

A. Historical Review

Thirty-three years have passed since the important report was made by the English bacteriologist Griffith (1928) of a specific genetic change inducible in the pneumococcus. Interested in conditions that would favor the transformation of avirulent pneumococci into virulent ones, Griffith tried the subcutaneous injection into mice of living, avirulent organisms simultaneously with virulent organisms that had been previously killed by heat. It had already been known that the virulence of pneumococci depended upon the secretion around their outer walls of a capsule composed of polysaccharide. The chemical and antigenic specificity of the capsular polysaccharide was a genetic character of the strain or type of pneumococcus, and there were many distinct types of virulent pneumococci known. Moreover, many unencapsulated, avirulent organisms had been found to arise, apparently by mutation, in virulent strains both *in vitro* and *in vivo*. Griffith found that his injected animals succumbed to pneumonia, and that at autopsy living, encapsulated pneumococci were recoverable. The significant finding was that the type of virulent strain recovered did not correspond to the type of virulent strain from which the living, unencapsulated strain used in the experiment had been derived. It corresponded, however, to the type of virulent strain from which the heat-killed vaccine had been prepared. The interesting hypothesis presented itself that some substance emanating from the heat-killed pneumococci had converted the living, avirulent organisms into virulent ones having the capacity of synthesizing the type of capsular polysaccharide characteristic of the strain from which the heat-killed bacteria were prepared. In the current language of microbial genetics, the hypothesis would be said to postulate the genetic transformation of a recipient cell or organism by a specific agent produced by a donor cell or organism and having the properties of an infectious, genetic unit. The genetic properties of the infectious transforming agent are implied in the fact that the transformed bacteria proceed to synthesize, concomitantly with their own reproduction, more of the same type of agent responsible for their transformation.

Since specific hereditary changes induced by nonliving agents represented a novel and truly important discovery, it was imperative to exclude alternative explanations. The existence of a few viable organisms in the vaccine was ruled out by the fact that, not only was the vaccine alone incapable of resulting in any pneumococcal infection of the treated animal (Griffith, 1928), but the type transformation could

occur *in vitro* (Dawson and Sia, 1931) and could be induced by a cell-free extract of the donor organisms (Alloway, 1933).

At this point the investigation of pneumococcal transformations was concerned largely with the chemical identification of the substance in the donor extract that was capable of inducing genetic transformations. A careful study, lasting over ten years, by Avery and his co-workers at the Rockefeller Institute culminated in the now classic report (Avery *et al.*, 1944) assigning to deoxyribonucleic acid (DNA) the property of possessing and transmitting genetic information. This conclusion was all the more remarkable because no direct evidence had yet been obtained for the function of the nucleic acids, although their universal distribution in living cells and the almost exclusive localization of the deoxyribonucleic acids in the chromosomes were established, and had suggested an important role for this class of substance (Leslie, 1955). Among the significant properties of the transforming agent shown by Avery and his collaborators (Avery *et al.*, 1944; McCarty and Avery, 1946a; McCarty, 1946b) was its insensitivity to proteolytic enzymes and protein-denaturing agents, to ribonuclease [the enzyme that specifically depolymerizes ribonucleic acid (RNA)], to antibodies and other substances capable of binding pneumococcal proteins and polysaccharides, although it was rapidly rendered biologically ineffective by deoxyribonuclease DNase), the enzyme capable of specifically depolymerizing DNA.

From 1945 until the present date the study of genetic transformations has proceeded along two main lines, which, it should be added, have merged from time to time with fortunate results. The first line was primarily chemical, and concerned not only confirmation of the identification of deoxyribonucleic acid as the transforming substance, but also some of the chemical and physical properties of this substance, such as its molecular weight and structure, the effect of various chemical and physical factors on its structure and transforming activity, the relation between its structure and its genetic function. The results of this line of investigation will be reviewed in the next section (II). The other principal line of investigation dealt with the mechanism of transformation, which soon revealed that the process of genetically transforming bacteria was complex and consisted of several, readily definable stages. Consequently, these stages will be considered separately in the sections to follow (III–VII).

While these chemical and genetic studies were taking place, several other notable discoveries were being made in bacteria, having special relevance to the general problem of transformation. These discoveries had to do with other modes of transfer of genetic information from one bacterium to another. In 1946 Lederberg and Tatum reported a

process of genetic transfer in *Escherichia coli*, which resembled sexual conjugation in higher organisms. This process has been considerably elucidated since that time as a result of the work of Lederberg and his co-workers (Lederberg *et al.*, 1951; Lederberg, 1955), Jacob and Wollman (1958) and Hayes (Hayes, 1953; Wollman *et al.*, 1956). In brief, this mode of genetic transfer is characterized by a unidirectional transmission of genetic material requiring contact between the donor (Hfr or F+) and recipient (F—) strains and involving a considerable portion of the donor genome, generally several genes being transferred (although not necessarily integrated subsequently) into the recipient organism. This type of genetic transfer is prevented if the donor and recipient bacteria are separated by a filter which allows only viruses or smaller particles to pass (Davis, 1950). Photographs using phase-contrast and electron microscopy have been obtained showing conjugating pairs of morphologically marked donor and recipient bacteria (Anderson *et al.*, 1957; Lederberg, 1956). The requirement for physical contact distinguishes this mode of genetic transfer in bacteria, which is generally referred to as conjugation. The feature of conjugation that relates it to transformation is the fact that the F— ex-conjugant produces, among its progeny, bacteria possessing the genetic characters of the F+ donor strain.

Later, a third type of genetic transfer was discovered by Zinder and Lederberg (1952) in the *Salmonellae*. In this type, referred to as transduction, a small portion of the genetic material of the donor bacterium, similar in amount to that transferred in DNA-mediated transformations (see Section V,B), is transferred to the recipient bacterium by means of a virus that had previously reproduced in the donor and had subsequently infected the recipient. Since the bacterial virus (or bacteriophage or phage) serving as a vector need not necessarily be virulent, that is, cause death of the infected recipient, the consequences of simultaneous infection by genetic material of the donor may often be observed. These consequences are similar to those occurring in conjugation and transformation in that progeny of the infected bacterium possess genetic characters of the donor strain. While being carried by the viral vector, the genetic material of the donor bacterium is insensitive to DNase, presumably being protected like the phage DNA itself by the proteins of the mature phage coat. There is considerable evidence to indicate, however, that both in conjugation and transduction DNA is the material transferred from donor to recipient (see Section VI,B). While this fact correlates the mechanisms underlying transformation, transduction, and conjugation, there are sufficient differences between the modes of transfer involved in these three processes to provide well-defined operational

criteria for distinguishing them. They are (1) conjugation: transfer requires donor-recipient contact; insensitive to DNase; (2) transduction: transfer mediated by virus passing from former host (donor) to recipient; insensitive to DNase; (3) transformation: transfer is sensitive to DNase. A more detailed comparison of the mechanism of recombination occurring in these modes of transfer will be made in Section VI.

B. Extent of the Phenomenon of Transformation

1. *In Terms of the Variety of Bacterial Species in Which It Is Known to Occur*

It was found that the phenomenon of transformation is not limited to the pneumococci. In recent years, conditions were found in which transformations would occur regularly in several species of the genera *Hemophilus* and *Neisseria* (Alexander and Leidy, 1953; Alexander and Redman, 1953). The amount of research that has already been conducted on the phenomenon of transformation in these species indicates that the conditions for obtaining precise and reproducible results are well understood for them. A few workers have also been able to carry out transformations in several species (or genera) of *Streptococcus,* some of which are apparently closely related to the pneumococcus (Bracco *et al.,* 1957; Pakula *et al.,* 1958a).

Boivin and his colleagues (Boivin, 1947; Boivin *et al.,* 1945, 1946) studied in considerable detail the occurrence in *Escherichia coli* of transformations analogous to those previously demonstrated in pneumococcus. In the case reported by Boivin, the highly polymerized DNA fraction of an encapsulated colibacterium (the donor strain) was capable of inducing in an unencapsulated (recipient) strain the hereditary ability to synthesize a specific capsular polysaccharide identical with the one produced by the donor strain. Like the pneumococcal transforming agent, the agent capable of transforming capsule types in *E. coli* was also highly sensitive to DNase. Unfortunately, however, the susceptibility to DNA transforming agents (probably similar to the state of competence to be discussed below) varied widely from one strain of *E. coli* to another. The strain in which transformations could be regularly produced was lost some time after Boivin's death in 1949. This fact is all the more unfortunate because, despite numerous attempts to find transformations in the enteric bacteria (*Escherichia-Salmonella-Shigella* group), their occurrence has been observed only fleetingly and has never been regularly reproduced (reported in *Shigella* by Weil and Binder, 1947; in *Salmonella* by Demerec and his co-workers, 1955, 1958 and by Kanazir and Subotic, referred to in Thomas, 1957). It would be

TABLE 1
Bacterial Species in Which Genetic Transformations Have Been Reported

Species	Genetic characters involved *	Reference †
I. Species for which workers could provide reproducible conditions for transformation.		
Diplococcus pneumoniae (or *Streptococcus pneumoniae*) ‡	Synthesis of type-specific capsular polysaccharide	Griffith (1928)
Streptococcus viridans §	Streptomycin resistance	Bracco *et al.* (1957)
Streptococcus sbe §	Streptomycin resistance	Pakula *et al.* (1958a,b)
Streptococcus, serological group H, hemolytic strain *Challis*	Streptomycin resistance	Pakula *et al.* (1958a,b)
Hemophilus influenzae	Synthesis of type-specific capsular polysaccharide	Alexander and Leidy (1951)
Hemophilus parainfluenzae	Streptomycin resistance	Leidy *et al.* (1956)
Hemophilus suis	Streptomycin resistance	Leidy *et al.* (1956)
Neisseria meningitidis	Synthesis of type-specific capsular polysaccharide	Alexander and Redman (1953)
Neisseria sicca	Streptomycin resistance	Catlin (1960a)
Escherichia coli ‖	Synthesis of type-specific capsular polysaccharide	Boivin *et al.* (1945)
	Synthesis of two enzymes involved in galactose utilization	Kaiser and Hogness (1960)
Agrobacterium tumefaciens	Ability to induce crown-gall tumors in tomato	Klein and Klein (1953)
Agrobacterium radiobacter	Ability to induce crown-gall tumors in tomato	Klein and Klein (1953)
Agrobacterium rubi	Ability to induce crown-gall tumors in tomato	Klein and Klein (1953)
Xanthomonas phaseoli	Polysaccharide production (colony morphology)	Corey and Starr (1957a,b)
	Streptomycin resistance	Corey and Starr (1957c)
Bacillus subtilis	Ability to synthesize indole; ability to synthesize anthranilic acid; ability to synthesize nicotinic acid	Spizizen (1958)
Rhizobium sp.	Ability to form nodules on alfalfa	Balassa (1955, 1956)
Staphylococcus aureus	Streptomycin resistance	Imshenetskii *et al.* (1959)

TABLE 1 (*Continued*)

Species	Genetic characters involved *	Reference †
II. Species in which apparently reproducible conditions for obtaining transformation were not found.		
Shigella paradysenteriae	Synthesis of type-specific antigens	Weil and Binder (1947)
Salmonella typhimurium	Ability to synthesize tryptophan; streptomycin resistance	Demerec *et al.* (1955, 1958)
Salmonella sp.	Ability to synthesize proline	Kanazir and Subotic, referred to in Thomas (1957)

* Unless otherwise indicated, the genetic character first shown to be susceptible of transformation is indicated. If other characters have been transformed in this species, they are indicated in Table 2.

† The first report of transformation in the species is always given; other reports are sometimes added.

‡ The taxonomic position of the so-called pneumococcus is not agreed upon. Although in the English system of classification the pneumococcus is included in the genus *Streptococcus,* in the American system, recorded in "Bergey's Manual of Determinative Bacteriology" (7th ed., Williams and Wilkins, Baltimore, Maryland, 1957), this organism is provided a genus of its own, *Diplococcus.*

§ In addition, streptococcal strains capable of serving as effective DNA donors, although refractory to autologous or heterologous DNA, are reported in the reference cited.

|| Boivin's strain of *E. coli,* in which capsular-type transformations could be regularly produced, has been lost. Transformation reported by Kaiser and Hogness is in strain K12, and has the unique feature of requiring a bacteriophage "helper" (see text, Section I,B,1).

especially valuable to be able to obtain transformable strains of enteric bacteria since transduction and conjugation occur in these organisms and a detailed genetic comparison of the three processes in the same strain would be extremely useful.

Recently, Kaiser and Hogness (1960) have reported the successful genetic transformation of *Escherichia coli* strain K12. The requirements for transformation, however, are somewhat unique. The character transformed is the ability to synthesize at least two enzymes (galactokinase and galactose-1-phosphate uridyl transferase) involved in the utilization of galactose. Synthetic ability (gal+) is transferred to deficient strains (gal—) by means of DNA extracted from defective bacteriophage (λ dg) known to carry, as part of its genome, the bacterial genes necessary for galactose utilization in the gal+ strain. While the specificity of this transfer of genetic information has been shown clearly

to reside in the DNA extracted from λ dg bacteriophage, another essential component of the transformation system is "helper" bacteriophage.[1] The "helper" phage are nondefective λ, but successful infection by the latter is not essential since one may use as recipients bacteria that are already lysogenic for λ and, hence, are immune to superinfection. The recipient bacteria to be transformed are simply exposed to λ a short period of time before (or simultaneously with) the addition of the purified transforming DNA. So far, only genes known to be present in the λ dg genome are known to be transferred by λ dg DNA and, as a matter of fact, all of the phage genome (about 1/100 that of the bacterial genome, in terms of the amount of DNA) appears to be transferred every time the gal+ character is transformed in a given recipient bacterium. Although the dependence of transformation on a nonspecific bacteriophage makes this system quite different from that of conventional systems, it nevertheless must be regarded as an example of transformation, in that the criterion of the dependence of the transferred specificity on DNase-sensitive DNA is fulfilled.

Although not a great deal of work has yet been done with them, a few other species of bacteria have been reported to undergo DNA-mediated transformations. In 1953 the Kleins described the transfer in *Agrobacterium* strains of the genetic ability to cause crown-gall tumors in plants. Avirulent strains were made genetically virulent by exposure to DNA extracted from virulent strains of *Agrobacterium*. Probably because of the difficulty of the assay procedures (Klein and Klein, 1956), these investigations have not been followed up. The use of genetic markers other than virulence in plants (see below) may prove useful in this regard. Corey and Starr (1957a,b,c) have described in some detail the genetic transformation of strains of *Xanthomonas*. In this case, two different genetic characters were studied, polysaccharide production and resistance to streptomycin. In terms of the specificity of the transforming DNA employed and its sensitivity to DNase, the conditions for transformation appear to be comparable to those observed in pneumococcus and *Hemophilus*. More recently, the important finding was made by Spizizen (1958) that transformations could be effected in *Bacillus subtilis*. This species can grow and be transformed in a simple,

[1] A previous instance of a report that bacteriophage may help in the successful infection by transforming DNA is that of Brown *et al.* (1955). In this case, nonmotile strains of *Bacillus anthracis* were presumably transformed by a cell-free, DNase-sensitive, DNA-containing lysate from a motile variant. However, the transforming activity could not be separated by centrifugation from a bacteriophage known to be present in the lysate. Later reports (Sterne and Proom, 1957), however, have cast doubt on the validity of this finding.

chemically defined medium, a property which, until very recently, was not shared by pneumococci or *Hemophilus* species. Moreover, several well-studied biochemical mutants and lysogeny (the property of carrying a symbiotic bacteriophage, a potential transducing agent) are known in this species. Indeed, transformation of *Bacillus subtilis* was quickly confirmed and the investigations were extended in other laboratories (Schaeffer and Ionesco, 1959; Ephrati-Elizur and Zamenhof, 1959). Until her recent untimely death Balassa had been investigating the transformation of several species of *Rhizobium,* which are also capable of growth in a simple chemically defined medium; among the characters transformed have been the ability to infect and produce nitrogen-fixing nodules in leguminous roots (Balassa, 1954, 1955, 1956, 1957a), streptomycin resistance (Balassa, 1957a,b), streptomycin dependence (personal communication), and cysteine independence (Balassa, 1960).

Several other reports have been made in the past of the genetic transformation of bacteria by means of culture filtrates or cell-free extracts from mutant strains. In some of these cases, suitable controls were lacking for the demonstration of either the specificity of the transforming agent or of its sensitivity to DNase. These cases (for review, see Austrian, 1952b) are, therefore, not included in Table 1, which summarizes the variety of bacterial species and genera that have been demonstrated to be susceptible to DNA transforming agents.

2. *In Terms of the Variety of Genetic Characters Capable of Being Transformed*

Although the first character genetically altered by a DNA transforming agent was that of specific polysaccharide synthesis, the process of transformation does not appear to be limited in any way to a certain class or category of hereditary characters. Whatever be the species of bacterium in which transformation is studied, one generally finds that any character that can be conveniently investigated is susceptible to hereditary transformation by the appropriate DNA agent. What guides the investigator as to the character used in transformation reactions is simply the availability of techniques for selectively screening the transformed bacteria from the untransformed recipient population. Particularly when the frequency of transformation is relatively low (less than 1% of the treated population), the utilization of a selective screening technique becomes important. For this reason, investigators interested in the mechanism of transformation have studied changes in characters that are readily screened in selective media. Such characters as drug or antibiotic resistance, or independence of specific growth substances in the medium, lend themselves particularly well to such studies.

TABLE 2
Variety of Hereditary Characters Transformable in Bacteria

Character transformed	Species	Reference *
I. Capsular polysaccharide synthesis		
Six different types †	*D. pneumoniae*	Avery *et al.* (1944) McCarty and Avery 1946b); Austrian (1952a)
Six different types †	*H. influenzae*	Alexander and Leidy (1951)
Two types †	*N. meningitidis*	Alexander and Redman (1953)
One type	*E. coli*	Boivin *et al.* (1945)
One type	*X. phaseoli*	Corey and Starr (1957a,b)
II. Filamentous type of growth	*D. pneumoniae*	Taylor (1949a); Austrian (1953a, b)
III. Specific protein antigens		
M protein	*D. pneumoniae*	Austrian and MacLeod (1949a,b)
IV. Drug and antibiotic resistance		
Penicillin: 3 levels	*D. pneumoniae*	Hotchkiss (1951)
Streptomycin: several levels	*D. pneumoniae*	Hotchkiss (1951); Schaeffer (1956b); Bryan (1961)
Streptomycin: high level	*Strep. viridans* ‡	Bracco *et al.* (1957); Pakula *et al.* (1958a)
Streptomycin: high level	*H. influenzae*	Alexander and Leidy (1953)
Streptomycin: high level	*N. meningitidis*	Catlin (1960b)
Streptomycin: high level	*X. phaseoli*	Corey and Starr (1957c)
Streptomycin	*Rhizobium* sp.	Balassa (1957a,b)
Sulfanilamide: several levels	*D. pneumoniae*	Hotchkiss and Marmur (1954); Hotchkiss and Evans (1957, 1958)
Optochin	*D. pneumoniae*	Lerman and Tolmach (1959)
Erythromycin: several levels	*D. pneumoniae*	Green (1959); Ravin and Iyer (1961)
Erythromycin	*N. meningitidis*	Catlin (1960b)
Bryamycin	*D. pneumoniae*	Marmur and Lane (1960)
Canavanine	*D. pneumoniae*	Ephrussi-Taylor (1957)
Amethopterin	*D. pneumoniae*	Drew (1957); Sirotnak *et al.* (1960a)
Aminopterin	*D. pneumoniae*	Ephrussi-Taylor (1958)
8-Azaguanine	*D. pneumoniae*	Sirotnak *et al.* (1960b)
Cathomycin	*H. influenzae*	Goodgal and Herriott (1957b)
V. Antibiotic dependence		
Streptomycin	*N. meningitidis*	Catlin (1960b)

TABLE 2 (*Continued*)

Character transformed	Species	Reference *
VI. Synthesis of specific enzymes		
Mannitol dehydrogenase	*D. pneumoniae*	Hotchkiss and Marmur (1954)
Salicin fermentation enzyme	*D. pneumoniae*	Austrian and Colowick (1953)
Maltase	*D. pneumoniae*	Lacks and Hotchkiss (1960)
Lactic acid oxidase	*D. pneumoniae*	Ephrussi-Taylor (1954); Udaka *et al.* (1959)
Sucrase	*B. subtilis*	Spizizen (1959)
β-Galactosidase	*B. subtilis*	Spizizen (1959)
Enzyme necessary for synthesis of cysteine	*Rhizobium* sp.	Balassa (1960)
Enzyme necessary for synthesis of anthranilic acid §	*B. subtilis*	Spizizen (1958)
Enzyme necessary for synthesis of indole §	*B. subtilis*	Spizizen (1958)
Enzyme necessary for synthesis of nicotinic acid	*B. subtilis*	Spizizen (1958)
VII. Sporulation	*B. subtilis*	Schaeffer *et al.* (1959)
VIII. Other characters		
Ability to infect plants	*Agrobacterium* sp.	Klein and Klein (1953)
	Rhizobium sp.	Balassa (1955)
Intermediate encapsulated types	*D. pneumoniae*	MacLeod and Krauss (1947); Taylor (1949b); Ravin (1959a)
Mixed or binary encapsulation	*H. influenzae*	Leidy *et al.* (1953)
	D. pneumoniae	Austrian *et al.* (1958)
Abnormal capsular type	*D. pneumoniae*	Beiser and Hotchkiss (1954)

* In general, the reference cited is the first one reporting on transformation of the specified character in the species in question.

† The transformation of each type is effected by a specific transforming agent.

‡ Transformations of streptomycin resistance in other streptococcal species are reported in the second reference cited.

§ Two separate steps in the synthesis of tryptophan are involved, the one prior to the formation of anthranilic acid, the other between anthranilic acid and indole.

Table 2 summarizes the variety of characters susceptible of transformation by DNA agents, and indicates that transformation can indeed affect any part of the genetic repertoire of the recipient organism.

C. Previous Reviews of the Field

Genetic transformations have been treated in several reviews in the past. The attention of the reader is called, in particular, to Austrian

(1952b), Ephrussi-Taylor (1955), Hotchkiss (1955), and Thomas (1957). The review by Hotchkiss is especially noteworthy for the wealth of evidence it brings to bear on the question of DNA as the bearer of genetic information in transformation and bacteriophage infection. The reviews by Ephrussi-Taylor and Thomas concern themselves largely with the mechanism of transformation; in Thomas' review, the relation of the macromolecular structure of DNA to the transformation process is especially considered.

II. Chemical Nature of the Transforming Agent

A. DNA as an Essential and Sufficient Constituent of the Transforming Agent

One of the most significant aspects of the phenomenon of transformation is the fact that the material involved in the transfer of hereditary information is molecular in dimensions and is readily accessible to physical and chemical treatment. This property of the transforming substance is helpful in clarifying the molecular basis of genetic transmission in organisms, for it allows, among other things, the investigation of the structural features of the molecular species in question and of the relation of these features to the specific heterocatalytic and autocatalytic functions it plays in the metabolic economy of the organism.

While progress along these lines has begun only recently, a sufficient amount of information has been obtained to warrant its review in the section (II,B) to follow. The task of the present section will be to assess the evidence in favor of deoxyribonucleic acid as the genetic vector involved in bacterial transformations.

The classic work in this regard is that of Avery, MacLeod, and McCarty (1944), who showed the remarkable similarities of the substance responsible for pneumococcal type transformations to purified deoxyribonucleic acid from calf thymus. The carbon, hydrogen, nitrogen, and phosphorus ratios indicated, in particular, that protein impurities could not constitute a significant fraction of the transforming material. The biuret and Millon tests for protein were negative; the orcinol test for ribonucleic acid was weakly positive, but no more so than is obtained with purified calf thymus DNA. Treatment with crystalline trypsin, chymotrypsin, or ribonuclease caused no loss of activity of the transforming material. On the other hand, enzyme preparations from various animal sources having the ability to depolymerize fish and mammalian DNA rapidly inactivated the transforming material. Enzymes lacking such DNase activity failed to inactivate the transforming material.

Destruction by heat of the DNase activity in certain enzyme preparations also destroyed their power to inactivate the transforming material. Furthermore, the temperature at which DNase activity was lost closely paralleled that at which the ability to destroy the transforming factor was lost.

An important finding was that, in the course of purifying the transforming factor, the ability to combine with pneumococcal antisera progressively diminished, so that there could not have been more than 5% of a serologically active protein or more than 0.05% of type-specific polysaccharide present in the most purified preparation. The conclusion of Avery *et al.* (1944) that ". . . the transforming substance in purified state exhibits little or no serological reactivity . . . in striking contrast to its biological specificity in inducing pneumococcal transformation" will be reviewed in the next section in the light of more recent evidence.

Finally, these authors found that their highly purified transforming material (which was reactive at concentrations of one part in 6×10^9) behaved like a homogeneous substance in electrophoresis and in the analytical ultracentrifuge (the molecules being apparently uniform in size and very asymmetric). The authors estimated the molecular weight of the transforming factor to be of the order of 5×10^5, which, probably because of the admitted assumptions of the authors as to certain physical characteristics of the molecules they were dealing with, is at least an order of magnitude lower than that obtained in more recent measurements (see Section II,B). Like nucleic acids in general (RNA as well as DNA), the absorption of ultraviolet light by the purified transforming material was at a maximum at 2600 A and at a minimum at 2300 A.

As a whole, the work of Avery *et al.* (1944) constituted impressive evidence that deoxyribonucleic acid was the substance capable of inducing genetic transformations in pneumococcus. However, the assignment of biological specificity to the nucleic acids was still a novelty in 1944, the proteins and polysaccharides being the only classes of biological macromolecules which had been demonstrated to possess specificity up to that time. Thus, it was especially important to prove the postulated role of DNA as a genetic material capable of infecting cells, of being exactly replicated, and of controlling a specific metabolic function. In particular, arguments were raised (Mirsky, 1947) that, while the studies of Avery and his collaborators demonstrated that DNA was essential to the activity of the transforming material, it was far from proved that DNA was the material bearing the specificity transferred during genetic transformation. A very small amount of protein, undetectable by the crude tests for protein that were available, and pro-

tected by DNA in some fashion from protein-denaturing and proteolytic agents, could account for the specificity of the transforming preparation.

McCarty (1946a) purified the enzyme deoxyribonuclease from beef pancreas, and demonstrated its specificity in depolymerizing deoxyribonucleic acids. McCarty and Avery (1946a) tested this purified enzyme on the pneumococcal transforming substance, and demonstrated the high sensitivity of the transforming activity to the enzyme. These findings added further evidence that DNA was at least essential for the activity of the transforming material. Was it, indeed, sufficient for this activity? An unequivocal, definitive answer is extremely difficult, if not impossible to provide, for it requires proof that no substance whatsoever, other than DNA, is present in highly purified transforming preparations. At best, one can only improve methods for detecting substances other than DNA and gradually lower the maximum estimate of foreign material possibly present.

Hotchkiss (1948, 1952) has particularly addressed himself to this problem. He showed that, during the course of purifying the transforming material, the purine and pyrimidine base composition approached closer and closer to that of purified calf thymus DNA. In particular, the nitrogen to phosphorus ratios of the pneumococcal transforming agent and of calf thymus DNA are identical. This could not be the case if, as previously pointed out by Avery *et al.* (1944) there were substantial contamination of the transforming substance by protein. The most striking result, however, was the gradual disappearance of α-amino acids from hydrolyzates of the transforming material as it was progressively purified. Indeed, the only α-amino acid produced by acid hydrolysis was glycine (Hotchkiss, 1952), and the rate at which this amino acid was liberated by hydrolysis exactly paralleled that at which glycine is liberated by the degradation of adenine (a slow, linear process) rather than that of the hydrolysis of a protein or peptide (an initially rapid, then progressively diminishing process) (Hotchkiss, 1948). All of the glycine liberated by acid hydrolysis, moreover, could be quantitatively accounted for as that liberated from the amount of adenine present in the transforming material (Hotchkiss, 1952). On the basis of these findings, Hotchkiss could estimate that, if any protein were present, it could not constitute more than 0.02% of purified transforming preparations. Since these preparations were active in highly dilute solutions, the presence of a genetically active contaminant became remote.

The substance capable of transforming *Hemophilus influenzae* has also been subjected to stringent purification methods (Zamenhof *et al.*, 1952). By electrophoresis and further deproteinization, these workers

could separate the DNA from the bulk of the other macromolecular constituents without any loss of transforming activity. DNA preparations purified in this way possessed activity at concentrations less than 0.0004 μg per milliliter, although they could contain no more than 1% RNA, 0.3% serologically active polysaccharide, and 2.5% protein.

More recent studies, even if they have not pushed the chemical analysis of transforming preparations any further, have nevertheless provided additional corroboratory evidence. Zamenhof and his colleagues (1953) have followed in parallel the loss of viscosity of the DNA in transforming preparations of *H. influenzae* and the loss of biological activity of these preparations, as a result of treatment by heat, acid, or alkali, and other chemical conditions. In every case, biological activity and loss of viscosity (an indication of the native state of DNA) dropped under precisely the same conditions. Similarly, Thomas (1954, 1957) has found that under certain environmental conditions the ultraviolet spectrum of DNA undergoes irreversible changes, constituting a very sensitive test of the denaturation of DNA, and under these conditions the activity of pneumococcal transforming preparations is rapidly lost. Urea, which neither denatures DNA (Thomas, 1954) nor diminishes the activity of pneumococcal transforming preparations, does appear to affect the aggregation of DNA molecules and causes changes in the kinetics of X-ray inactivation of pneumococcal transforming material, as could be expected of alterations in average aggregate sizes (Ephrussi-Taylor, 1957; Thomas, 1957). Finally, if DNA were solely a protecting substance for some other component of the transforming preparation to which the specificity of the transformation reaction were due, it would not be expected that any DNA would necessarily be incorporated by the transformed bacteria. On the contrary, several investigators have shown, with P^{32}-labeled *Hemophilus* (Goodgal and Herriott, 1957a) and pneumococcus preparations (Lerman and Tolmach, 1957; Fox, 1957), that DNA-P^{32} does penetrate the transformed bacteria, that it is localized in the DNA fraction of these bacteria, and that the amount of P^{32} irreversibly incorporated into these bacteria is directly proportional to the number of transformed bacteria.

In sum, the weight of evidence is in favor of the conclusion that DNA is the essential and sufficient substance capable of inducing genetic transformations in bacteria. The burden of proof appears to be on those who would continue to claim that some substance other than DNA is the genetically specific substance in transformation reactions.

This conclusion is in harmony, furthermore, with the results of chemical studies of bacteriophage infection. Hershey and Chase (1952) have shown, by their elegant experiments utilizing coliphage marked

with P^{32}, which is restricted to the DNA component of the virus, and with S^{35}, which is restricted to the protein component, that practically all of the viral protein remains outside of the infected bacterium and that most of the viral DNA penetrates the host. This finding means that DNA rather than protein is responsible for the directed specific synthesis of bacteriophage in infected bacteria. Meselson and Weigle (1961) were able to show, by studying the distribution in a density gradient of bacteriophage descending from parents marked with N^{15} and C^{13}, that the replication of bacteriophage DNA is semi-conservative (see Section II,B), which is to say that a certain stability resides in the DNA molecule during DNA synthesis. Diffuse exchange between atoms of the DNA molecule and atoms of other substances in the local environment apparently does not occur. The stability of DNA is supported by several other types of investigations, including the manner of replication of DNA in multiplying bacteria (Meselson and Stahl, 1958) and algae (Sueoka, 1960), and the manner of replication of chromosomes (Taylor *et al.*, 1957).

The same kind of stability of DNA is implied in the correlation between the DNA content of tissues and their ploidy (Swift, 1953). A fairly exact correspondence exists between the degree of ploidy of a cell and its content of DNA, such that haploid cells contain, in general, half the DNA content of diploid cells, and that with each doubling of the number of chromosomes over the diploid number there is a doubling of the amount of DNA. Such a constant correlation between ploidy and DNA content does not appear to exist for any other class of macromolecule in the cell. The significance of this stability of DNA in actively metabolizing and reproducing cells lies in the expectation that, if the genetic information of a cell is contained in the particular structure and configuration of a certain class of macromolecule, this structure and configuration would need to be preserved and would be jeopardized by an active metabolic turnover of the atoms or larger subunits of which these molecules were composed.

B. Current Views on the Molecular Structure of DNA

The chemical and physical evidence on which our knowledge of DNA structure rests may be summarized as follows:

1. The DNA molecule is a polymer of nucleotide units. The nucleotide consists of three essential parts: a purine or pyrimidine base linked to a 5-carbon sugar, deoxyribose, which is linked in turn to a phosphate radical. Since the sugar and phosphate moieties are similar from one nucleotide to another, differences between nucleotides consist in differ-

ent bases. Only two purines are found in DNA, adenine and guanine; the number of known pyrimidines in DNA is only slightly higher, there being two common ones, thymine and cytosine, and two analogs of cytosine found less frequently, 5-methylcytosine and 5-hydroxymethylcytosine. In the polynucleotide polymer, nucleotides are joined to each other in the fashion depicted in Fig. 1, leaving the bases to stick out from the sugar-phosphate backbone of the molecule.

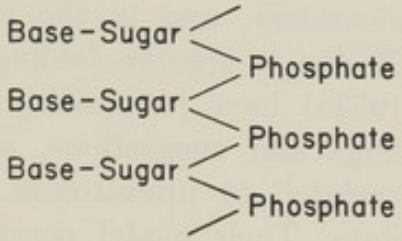


FIG. 1. Diagrammatic structure of a single chain of DNA.

2. Solutions of DNA are highly viscous and birefringent, which conditions are attributable to the orientation and asymmetry of the individual molecules. Long fibers having a tendency to form parallel-oriented aggregates, would be consistent with these physical properties.

3. The molecular weight of DNA appears to be quite high, ca. 10^7, indicating an order of magnitude of 10^4 nucleotides per molecule. Molecular weight determinations have been obtained in part by direct physical measurements (light scattering, Reichmann *et al.*, 1954; G. L. Brown *et al.*, 1955; sedimentation and diffusion, Goodgal and Herriott, 1957a); and in part by applying the target theory to data on the inactivation of transforming DNA by various agents. The various determinations fall fairly close to each other, and the results obtained with transforming preparations will be considered further in the following section. There is evidence (G. L. Brown *et al.*, 1955; Marmur and Doty, 1959) that the molecular weight of DNA, constant for a given species, nevertheless is greater in those species in which the DNA has a higher content of guanine and cytosine relative to adenine and thymine.

4. The proportion of bases in DNA is constant for a given species, but varies considerably from one species to another. Whatever be the particular base composition of a given DNA, one feature is always observed: the proportions of adenine to thymine and of guanine to cytosine are equal (Chargaff, 1955).

5. X-ray diffraction measurements of purified DNA are consistent with a bipartite, helical structure for the molecule consisting of two chains coiled around the long axis of the helix having a diameter of

20 A (Wilkins *et al.*, 1953; Hamilton *et al.*, 1959). Electron micrographs of pure DNA preparations do indeed show fibers with a diameter of this magnitude (Hall, 1956).

On the basis of this information, it has been proposed that the specificity of the DNA molecule resides in the particular proportion of bases contained in it, in the particular sequence of nucleotides in the polymer (there being obviously an astronomically large number of sequences possible with even four fundamental nucleotides, given a chain length of 10^4 nucleotides), and in the particular over-all configuration of the molecule due to specific folding along its length.

Watson and Crick (1953a) have proposed a specific model incorporating all of these findings and suggestions, and accounting for the periodic characteristics, related to interatomic distances, revealed by X-ray crystallographic data. Their model consists of two polynucleotidic chains coiled plectonemically around a common axis and linked to each other by hydrogen bonds between the nucleotide bases facing each other at regular intervals (see Fig. 4 of Watson and Crick, 1953c). The important element in the proposed structure is that the bonding between bases on the two chains of the molecule is highly specific, so that adenine can pair only with thymine and guanine only with cytosine (or 5-methylcytosine or 5-hydroxymethylcytosine). Thus, the two chains in the molecule are complementary, the structure of one of the chains specifying the sequence of bases along the other. No restriction is imposed, however, on the possible sequence of bases that may occur along a given chain. These latter features will account obviously for point 4, mentioned above. One of the seductive aspects of the Watson-Crick model is that it suggests a mechanism for DNA replication and mutation (Watson and Crick, 1953b,c). If the two plectonemically coiled strands of the molecule can become unwound from each other in some way (which presents an enormous problem in itself), replication may be thought to consist of the alignment of the specified nucleotides along each separate chain, acting as a template, with subsequent linkage between the nucleotides of the newly formed chain so as to result in two double-stranded helices. Each new helix consists of complementary chains, one of which is an old chain (of the parental molecule) and the other of which is a chain formed *de novo* from the nucleotide building blocks. Such a mechanism of replication is a semiconservative one, in which half of each new molecule has remained intact. The other possible ways of regarding DNA replication are as conservative or dispersive (see Fig. 2; also Delbrück and Stent, 1957). The semiconservative mode of replication accords best with the chemical

findings, described above, of DNA synthesis in microorganisms and of chromosome replication in higher organisms.

Mutation, on the other hand, is regarded as due to a rare tautomeric shift in one (or more) of the bases along one of the chains of the DNA molecule, such that, at the time of replication, a different base is specified at that locus (or loci) than would be normally. Hence, a "mutant" complementary strand is formed which thereafter will cause the reproduction of a "mutant" molecule (Watson and Crick, 1953c).

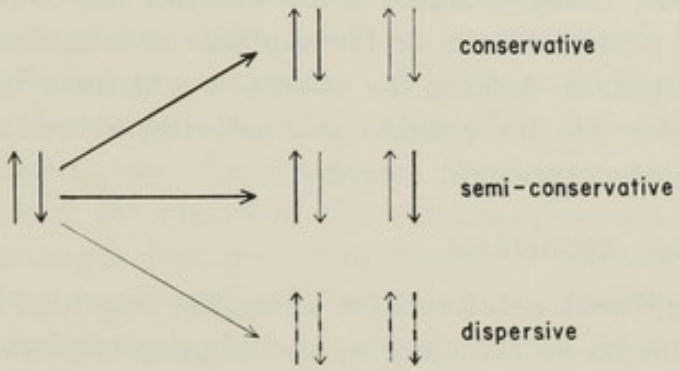


FIG. 2. Possible modes of DNA replication. Arrows pointing in opposite directions indicate complementary nature of the two strands of the DNA molecule. Heavy type represents parental molecule; lighter type represents newly formed material.

Nevertheless, there are several difficulties attending the explanatory value of this model. One of these difficulties has already been mentioned, namely, that of the "unwinding" of the complementary strands of the DNA molecule prior to replication (see Levinthal and Crane, 1956). Others come from considerations of other known properties of genetic material, including the transforming agent. These will be discussed further below, but for the present it will suffice to indicate that synapsis of homologous DNA molecules (regarded as an essential step in the transformation process) or of homologous chromosomes (which can be cytologically demonstrated) as well as "crossing-over" (the effective exchange of homologous regions between chromosomes) will need explanation by any model of DNA structure.

C. Reactivity of DNA

It has been implied in preceding sections that DNA is a very stable substance, particularly in its replication. This conclusion is nevertheless consistent with experimental findings that many different kinds of agents, chemical and physical, are capable of altering the structure of the DNA molecule. Some of these alterations have proved useful in studying the behavior of the transforming agent. If known modifications

in the structure of the DNA-transforming agent can be provoked under certain conditions, and if these modifications result in derangement of a specific step in the transformation process (see Sections III–VII), the relation between DNA structure and the process of transformation can be better understood.

In many of these studies, effects are sought not only upon the structure of DNA but also upon its biological activity. To date the only known method of measuring the activity of DNA is in bacterial transformations. Thus, many workers have utilized the DNA-transforming preparations of pneumococcus or *Hemophilus* as a material for physicochemical investigation. Among the effects sought have been the relation between molecular size (or weight) and activity, as well as the induction of mutations in structure and activity.

1. *Intermolecular Attractions*

There are sufficient reactive sites along the length of the DNA molecule, both in the bases attached to the sugar-phosphate backbone and in the phosphate moieties themselves, to allow a considerable amount of chemical binding. One of the most common types of binding is that which occurs between DNA molecules themselves, leading to the formation of molecular aggregates (Butler and James, 1951; Ephrussi-Taylor, 1957). The concentration of bivalent cations in the medium in which the DNA molecules are dispersed plays a significant role in the extent of aggregation, Ca^{++} and Mg^{++} ions apparently forming bridges between molecules (Thomas, 1957). In any event, bivalent cations combine with DNA, modify its ultraviolet spectrum (Cavalieri, 1952), protect it from denaturation at concentrations much lower than those at which monovalent cations are effective (Thomas, 1954), and maximize the extent of aggregate formation (Doty *et al.*, 1960).

DNA molecules can not only form linkages or aggregates with each other, but can also bind basic proteins. It is interesting in this regard that DNA is generally found in the chromosome in the form of a nucleoprotein, generally as a nucleohistone. Under certain conditions, mixture of pneumococcal DNA with ribonuclease results in a large reduction of transforming activity. This action is apparently not due to any action of ribonuclease on the recipient cells, for the effect depends largely on the concentration of DNA employed, more so than on the concentration of RNase. Nor does the action appear to be a result of enzymatic action on DNA, for it is temperature-independent while the activity of the enzyme is not (Thomas, 1960). DNA preparations containing a protein residue show marked heterogeneity in sedimentation properties. Treatment of these DNA preparations with chymotrypsin reduces the fraction

having high sedimentation coefficients. An interpretation is that this fraction is due to protein-DNA aggregates (Butler *et al.*, 1957).

2. *Depolymerization by DNase*

There are a variety of deoxyribonucleases from various sources and their mode of action in cleaving DNA appears to be different (for review, see Schmidt, 1955). The best-studied DNase, however, is that obtained from mammalian cells, especially pancreatic tissue. The available evidence indicates that the enzyme is highly specific for deoxypolynucleotides, and is without effect notably on ribonucleic acids or ribopolynucleotides. Prolonged treatment of DNA with this enzyme yields a variety of acid-soluble nucleotides, including tri-, di-, and mononucleotides, without any formation of inorganic phosphate. These results conform with the idea that the enzyme splits the DNA molecule at the phosphate-sugar linkages. The degree of polymerization of the molecule is without appreciable influence on the kinetics of enzymatic cleavage. It is interesting that bivalent cations (especially Mg^{++} or Mn^{++}) are essential activators of the enzyme.

Although attempts have been made in several laboratories, no mutations have been induced in transforming preparations following brief treatment with DNase. Furthermore, no fractionation of DNA-transforming agents has been reported as a result of DNase action on preparations known to contain many different genetic factors (see Section V,B below). Lerman and Tolmach (1957, 1959) have shown that the kinetics of inactivation of pneumococcal DNA by DNase is essentially that of a one-hit process, there being no shoulder in the inactivation curve obtained by plotting surviving DNA activity as a function of length of exposure to enzyme. Furthermore, as they found with several other inactivating agents, destruction of genetic activity of the DNA molecule occurs much more rapidly than loss of its ability to be taken up irreversibly by the host cell, indicating that inactive DNA can be taken up.

One of the interesting questions raised concerning DNase is the role it plays, or rather fails to play, in transformation reactions. There is some evidence (see Austrian, 1952b) that bacteria secreting considerable amounts of this enzyme cannot be transformed because of the digestion of the transforming substance. Indeed, it is common practice (starting with McCarty and Avery, 1946b) to add sodium citrate to the medium in which pneumococcal cells are lysed in order to bind magnesium ions essential for the action of DNase. Be that as it may, an important question still remains: what prevents endocellular DNase from destroying the penetrating DNA? Apparently, endocellular DNase, which can

be detected in many bacterial cells following special methods of cell treatment or extraction, is effectively blocked from acting upon either endogenous or exogenous DNA. Such blocking may exist only during a certain part of the generation cycle of the bacterium. The same problem exists in the infection of bacteria by bacteriophage. Here too the infecting DNA is free to alter the metabolic processes of the host, which may culminate in lysis of the host, although DNase is known to be present in the cells, for it is unmasked under certain conditions of treatment (for reviews, see Hotchkiss, 1955 and Thomas, 1957).

3. *Effects of Radiation*

DNA is highly sensitive to radiation. Transforming activity of pneumococcal or *Hemophilus* preparations has been reduced by means of either exposure to ultraviolet light (Green, 1959; Lerman and Tolmach, 1959), to X-rays (Ephrussi-Taylor and Latarjet, 1955; DeFilippes and Guild, 1959; Lerman and Tolmach, 1959), to electron, proton, and deuteron bombardment (Fluke *et al.*, 1952; Marmur and Fluke, 1955; Guild and DeFilippes, 1957), or to the decay of P^{32} atoms incorporated in the DNA molecules (Ephrussi-Taylor, personal communication).

In general, irrespective of the kind of radiation used, the inactivation of transforming DNA is of a complex sort, consisting of an initial rapid inactivation followed, at higher doses, by a more gradual rate of destruction. Most workers (Green, 1959; Lerman and Tolmach, 1959; Guild and DeFilippes, 1957) agree, however, that the ultimate rate of destruction is definitely exponential. The general form of the inactivation curve is shown in Fig. 3.

The kinetics of inactivation by radiation has been variously interpreted. To account for his findings with ultraviolet light, Green (1959) utilized the interpretation proposed by Doermann *et al.* (1955) for the effects of irradiating bacteriophage. According to this interpretation, the molecule of transforming DNA is longer than the segment of it occupied by the particular genetic marker being examined. Certain discrete damages occurring anywhere along the length of the molecule may act as "lethal" determinants in the sense that the molecule bearing them cannot be replicated in the recipient bacterium. It is the production of such damages that is assumed to cause the "initial slope" of inactivation. At low doses of radiation, more than one "lethal" hit per molecule of transforming DNA is improbable, and recombination of the genetic marker in question away from the damaged region of the transforming molecule would effectively "rescue" it and allow transformation to occur. At higher doses, where multiple "lethal" hits are more probable, the probability of "rescue" by recombination diminishes. The "ultimate

slope" of inactivation is governed, therefore, by the size of the genetic marker itself, and, indeed, is a measure of this "target" size. Lerman and Tolmach (1959), finding similar complexity in the ultraviolet inactivation of pneumococcal DNA, do not invoke, however, the same interpretation. By different reasoning, they also arrive, nevertheless, at the conclusion that only a small part of the DNA molecule is necessary and sufficient for the retention of genetic activity.

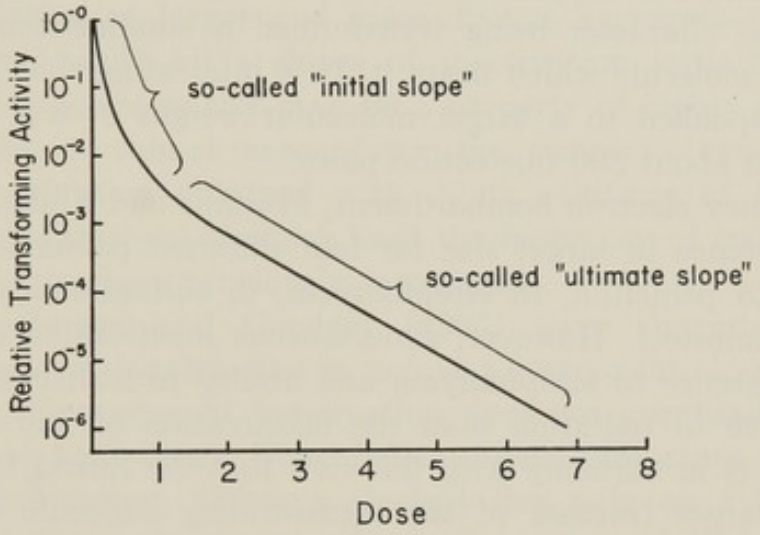


FIG. 3. A typical dose-survival curve for transforming DNA inactivated by ionizing or ultraviolet irradiation. As can be seen from the intervals on the ordinate and abscissa axes, the plot is semi-logarithmic. The relative transforming activity is the activity surviving irradiation expressed as the per cent of initial activity. The dose is expressed in arbitrary units.

Guild and DeFilippes (1957), using several kinds of cyclotron particles for bombarding pneumococcal DNA, and Lerman and Tolmach (1959), using X-rays, found the same two components of inactivation. However, Guild and DeFilippes (1957) found that the "initial slope" varies with conditions and is difficult to reproduce. The "ultimate slope," on the other hand, was independent of several treatments of the DNA including ultrasonics (the significance of the latter to be discussed below). In view of Latarjet and Ephrussi-Taylor's results with disaggregation of DNA solutions by urea (see Latarjet, 1956 and Ephrussi-Taylor, 1957), it is significant that Guild and DeFilippes could show that neither component of the inactivation curve reflected an *a priori* distribution of particle sizes or aggregates in the transforming preparation. This conclusion is based on resuspending, redrying, and rebombarding samples that had received doses sufficient to produce "ultimate slope" inactivation. The result was a similar two-component curve of inactivation, in which, however, the initial activity corresponded to that

surviving the original bombardment. Guild and DeFilippes interpret the "initial slope" as due to the formation of aggregates of DNA molecules on drying in salt (a step taken prior to bombardment), some of which redisperse on suspension (a step taken prior to testing for surviving activity) unless irradiated. In this regard, Setlow and Doyle (1954) did observe gel formation upon irradiation of dry DNA with ultraviolet light and with deuterons. Like Green, and Lerman and Tolmach, however, Guild and DeFilippes assume that the genetic marker involved in the particular character being transformed is smaller than that of the whole DNA molecule which bears it. It is interesting that the "ultimate slope" corresponded to a target molecular weight of 3×10^5, which is equivalent to about 500 nucleotide pairs.

Using 2-mev electron bombardment, Marmur and Fluke (1955) could find no difference in target size for four different pneumococcal factors (resistance to penicillin, to streptomycin, to sulfanilamide, and ability to utilize mannitol). However, simultaneous inactivation of two linked factors (resistance to streptomycin and ability to utilize mannitol) was more sensitive to radiation than the inactivation of any single factor. This finding is in harmony with the view that the linked factors occupy together a larger fraction of the transforming molecule bearing them than either one alone. In this connection, Green (1959) found that the "ultimate slope" of inactivation of the streptomycin-resistance marker he studied depended on the presence in the transforming DNA of a factor, a so-called "depressor," which was apparently linked to the streptomycin-resistance determinant and was capable of interfering with its genetic integration into the host genome. Lerman and Tolmach (1959) found two pneumococcus markers (affecting resistance to optochin and to streptomycin, respectively) which possessed markedly different sensitivities to ultraviolet light. An important fact regarding this discrimination between the two markers by ultraviolet light is that other inactivating agents, such as heat, DNase, X-rays, or nitrogen mustards, failed to make this discrimination, indicating a difference in the mechanism of inactivation by ultraviolet light. Zamenhof *et al.* (1956, 1957) also have evidence of different sensitivities of *Hemophilus* markers to ultraviolet light.

Latarjet and Ephrussi-Taylor (see Latarjet, 1956 and Ephrussi-Taylor, 1957) have recently provided evidence that the state of aggregation of concentrated DNA solutions affects the form of the initial portion of the inactivation curve. Urea in 5 *M* concentration is capable of affecting certain solutions so as to convert its inactivation from a multi-hit type to the single-hit type shown in Fig. 3. Two different states of aggregation are believed to occur under different conditions. A

physicochemical interpretation is given in Thomas (1957), according to which aggregates may either

occur in parallel [≡] or in a linear fashion [~~~].

In any event, these authors believe that the initial slopes of inactivation produced by radiation generally reflect the state of aggregate heterogeneity in the transforming DNA solution, the more heterogeneous solution consisting of larger and more diverse aggregate-sizes yielding more complex ("multi-hit") slopes of inactivation initially. However, while aggregate heterogeneity may be a property of highly concentrated DNA solutions, it cannot account for the complex (two-component) curves of inactivation obtained with dilute solutions of transforming DNA, the concentrations of which limit the frequency of transformation, as observed by workers previously mentioned.

Recently, Rupert and Goodgal (1960) have suggested that the "ultimate" slope of inactivation is not at all exponential; that is, on a semi-log plot, the curve of inactivation does not eventually assume a straight line at high doses. They believe their data more closely fit a power-law inactivation, fitting a straight line only on a log-log plot. However, the data of other workers (Lerman and Tolmach, 1959; Tolmach, personal communication) fail to fit a power-law equation. The question of the kinetics of inactivation of transforming DNA by radiation is obviously extremely important for an eventual interpretation of the damage done to the molecular structure of DNA.

Lerman and Tolmach (1959) have studied the effects of ultraviolet irradiation on two separate steps of transformation, namely, penetration into the host cell and genetic integration into the host genome (see Sections IV and V below). The capacity to penetrate was far less affected by the irradiation than the ability to be integrated. It is clear from this result that DNA factors inactivated by radiation do enter the recipient bacterium.

An interesting property of ultraviolet-damaged transforming DNA is its capacity to be reactivated by visible light. Since the now classic work of Kelner (1949), it has been known that the lethal and mutagenic action of ultraviolet light on a variety of microorganisms can be reversed by brief exposure to visible light following ultraviolet irradiation. The photoreactivating wavelengths appear to be those absorbed by nucleic acids, but, since it has been found (von Borstel and Wolff, 1955) that nuclear damage by ultraviolet light is photoreversible whereas cytoplasmic damage is not, it appeared that DNA rather than RNA is principally involved in photoreactivation. This view is supported by the

discovery (Goodgal *et al.*, 1957; Rupert *et al.*, 1958) that the transforming DNA of *Hemophilus,* damaged by ultraviolet radiation *in vitro,* can be photoreactivated by visible light. In order for such photoreactivation to occur, a cell-free extract from a microorganism such as *Escherichia coli* must be present. This fact is interesting in that, although *E. coli* resembles most other bacteria in being photoreactivatable, neither *Hemophilus* nor pneumococcus, which lack certain heme-containing enzymes, can be photoreactivated following ultraviolet damage. The essential factors in the *E. coli* extract are a dialyzable, heat-stable and nondialyzable, heat-labile component; the active agent in baker's yeast, however, consists of a nondialyzable component only (Rupert, 1960). The available evidence (Rupert, 1960) is consistent with the hypothesis that an enzyme system present in presumably many microorganisms but absent in *Hemophilus* and pneumococcus, reacting specifically with DNA and requiring visible light for its action, is responsible for reversal of ultraviolet-induced damages in DNA. It should be pointed out that, as in photoreactivation of lethal and mutagenic effects in intact microorganisms, photoreactivation of ultraviolet-damaged DNA is incomplete, in that not all of the original activity of the irradiated DNA can be restored.

Pakula *et al.* (1960) have found that photoreactivation of ultraviolet-inactivated DNA of pneumococcus cannot be easily demonstrated because the *E. coli* extracts containing the essential enzyme system also contain a factor that strongly inhibits the transformation process. This factor appeared to be *E. coli* DNA itself, which competes more strongly for penetration into pneumococcus than into *Hemophilus* (see Section IV). In any event, no way was found for destroying this inhibiting factor without destroying the photoreactivating enzyme system in the *E. coli* extract. More extensive purification of the *E. coli* enzyme or judicious use of DNase should be able to clarify this point.

4. *Effects of Heat*

It has been known for some time that transforming DNA preparations, unlike most proteins, are quite stable to heat. Zamenhof *et al.* (1953) provided the first quantitative evidence with *Hemophilus* DNA that activity is retained upon heating to temperatures as high as 80°C for one hour. At the same temperature at which activity begins to fall (ca. 85°C), the viscosity of the preparation, a criterion of the native state of DNA, begins to fall also. Lerman and Tolmach (1959) have confirmed these results with pneumococcal DNA. They also obtained evidence that, depending on the temperature employed, heat-inactivated DNA may be irreversibly incorporated into a recipient cell although

incapable of transforming the recipient. Lacks and Hotchkiss (1960) have evidence indicating, moreover, that under certain conditions of heat inactivation, a DNA molecule may be partially denatured without affecting its ability to penetrate and to transform.

Doty and his collaborators (for review, see Doty *et al.*, 1959) have employed techniques of exposing DNA to carefully controlled temperatures for various lengths of time in order to study the effect of heat on denaturation. In various ways they were able to confirm the findings of Zamenhof *et al.*, (1953) and to show that heat denaturation does minimal damage to the chemical structure of the DNA molecule.

In a brilliant series of experiments Doty, Marmur, and their colleagues (Marmur and Lane, 1960; Doty *et al.*, 1960) were able to demonstrate the chemical nature of heat inactivation of DNA. After noting that heating, even at temperatures (120°C) that should have been high enough to denature all of the guanine-cytosine regions of the molecules, failed to destroy all of the original activity of pneumococcal transforming preparations, these authors considered the possibility that heat inactivation was reversible and consisted in the separation of the complementary strands of the DNA molecule postulated in the aforementioned Watson-Crick model. If strands separated by heat could reunite to form their original configurations, the reversibility of heat denaturation could be explained. Indeed, this hypothesis has been verified in numerous ways. First of all, Marmur and Lane (1960) investigated the effect of the rate of cooling (following the initial heating) on the extent of biological inactivation of pneumococcal transforming DNA. It was found that the slower the rate of cooling the greater the restoration of the original activity. Although not all of the original activity could be regained no matter how slow the cooling, the per cent restored varied, from one sample to another, from 15 to 50%. The concentration of DNA had a very important influence on the restoration of activity in slow-cooled preparations, the lower the concentration the smaller the restoration. Increasing the ionic strength of the solution, which would be expected to favor restoration by counteracting the negatively charged phosphate groups of the separated strands, also had a pronounced favorable effect. Finally, it was shown that the restoration which occurred was specific, in the sense that the addition, prior to heating and cooling, of homologous wild type DNA to a preparation containing a mutant streptomycin-resistance marker, increased the amount of streptomycin-resistance activity restored (similar to increasing the concentration of the mutant DNA) although the addition of heterologous DNA produced no such increase. The heterologous DNA came from a variety of sources, including *Salmonella*, *Micrococcus*,

Streptococcus, and calf thymus. Furthermore, if genetically marked DNA were first denatured and then slow-cooled in the presence of homologous DNA in the native state, no increase in biological activity was noted.

The conclusion that the complementary strands of the DNA molecule are separated by heating and reunite to a variable extent in subsequent cooling, was given support by physicochemical studies (Doty *et al.,* 1960). Molecules denatured by heat and rapidly cooled possessed half the molecular weight of the original DNA molecules and appeared to be single-stranded in electron micrographs. Molecules denatured by heat but renatured by slow cooling not only possessed as much as 50% of their original activity (possibly because only 50% of the molecules in the slow-cooled preparations were effectively reunited in their original configuration). The dependence of the renaturation process on molecular concentration was that to be expected from an equilibrium between two independent strands and a bimolecular complex. The densities of denatured and renatured DNA molecules also conformed to expectation on the basis of strand separation in denaturation and reunion in subsequent renaturation.

The specificity of strand reunion in the renaturation process is supported not only by the findings of Marmur and Lane (1960) but also by density-gradient experiments using N^{14}- and N^{15}-labeled DNA from *Escherichia coli.* If such DNAs are mixed prior to heating and slow-cooling, molecules are formed having the density expected of "hybrids." Such hybrid molecules do not form unless the two components of the mixture are denatured and renatured at the same time. Such hybrid molecules furthermore fail to occur if the two components of the mixture are from unrelated species of bacteria. In one interesting case, that of *E. coli* and *Shigella dysenteriae,* two rather closely related bacterial species possessing similar base compositions in their respective DNAs, evidence of hybrid formation was found. Similarity in base composition, however, is apparently not sufficient to permit hybrid formation. The DNA of a strain of *Streptococcus* failed to produce renatured molecules with pneumococcal DNA, although the base compositions of their respective DNAs are very similar.

In view of the evidence for strand separation and reunion, and for the specificity of reunion, the authors postulated that the formation of hybrid molecules requires that the reuniting complementary strands possess structures specifying each other over a large part of their respective lengths. That such specification is not required over the entire length of the respective strands was indicated by the fact (Marmur and Lane, 1960) that the addition of homologous wild type DNA to a

mutant DNA during the denaturation and renaturation processes allows restoration of transforming activity to the latter, in the same way as does an addition of mutant DNA. This hypothesis led to the prediction that hybrid molecules possessing one mutant marker on one strand and a different mutant marker on the complementary strand could be produced by mixing two mutant DNA molecules, each marked (i.e., mutated) at a different locus along its length. This prediction was quickly verified by Herriott (1961), who employed two linked mutations, resistance to cathomycin and to streptomycin, respectively, in *Hemophilus influenzae*. Linked mutations, as will be discussed in Section V,C, are borne on the same molecule of DNA. In Herriott's experiment, the two DNAs used came, respectively, from a cathomycin-resistant, streptomycin-sensitive strain and from a cathomycin-sensitive, streptomycin-resistant strain. Heating the two DNAs followed by slow-cooling resulted in the formation of molecules possessing the ability to transfer both streptomycin resistance and cathomycin resistance into a bacterium sensitive to both antibiotics. This fact was shown by the number of double transformants (i.e., both streptomycin- and cathomycin-resistant) varying linearly with the concentration of the heated mixture, while it varied as the square root of the concentration of the unheated mixture.

These results not only support the molecular model proposed by Watson and Crick, which predicts that either complement of the DNA molecule would be sufficient for the transfer of genetic information contained in that molecule, but they also indicate ways of studying how a bipartite molecule, such as proposed by Watson and Crick, can participate in synapsis (exact pairing) and recombination (production of molecules combining the structures of two homologous molecules differing somewhat in structure). Such problems will be reconsidered in Section VI.

5. *Effects of Molecular Shearing*

Two ways have been found of breaking the DNA molecule without separating the complementary strands. By either way the size, in terms of molecular weight, is reduced while maintaining the complementary base pairing and the bipartite nature of the fragments.

One method involves shearing by spraying at high pressures through an atomizer (Cavalieri, 1957). This results not only in a reduction in the average molecular weight of the molecules in solution, but a considerable reduction in the heterogeneity of molecular weights. Another method consists in exposing the DNA solution to ultrasonic oscillations (Litt *et al.*, 1958). The striking result is that, although reduced, trans-

forming activity remains in these solutions of fragmented molecules. This finding gives further weight to the view that the genetic marker being transferred in a given transformation reaction occupies only a fraction of the total length of the DNA molecule on which it is borne. Since the principal effect of the reduced molecular size is diminished capacity to penetrate the host cell (surprising as that may seem on first thought), these results will be discussed further in Section IV.

6. *Effects of Chemical Agents*

DNA in solution may react with a number of chemical agents. In most cases, a reaction resulting in either breaking of hydrogen bonds (in part believed to be essential for the base pairing between complementary strands) or deamination or oxidation leads to a loss of transforming activity. Zamenhof *et al.*, (1953) have shown that loss of transforming activity parallels very closely loss of viscosity of the DNA solution.

Chemical conditions (Zamenhof *et al.*, 1953; Zamenhof, 1956, 1957) that inactivate transforming DNA include high concentrations of either hydrogen or hydroxyl ions (pH below 5 and above 10), deamination by NO_2^- (which, unlike most other reagents, inactivates without causing viscosity changes) or formaldehyde, oxidation by ferrous ion in the presence of hydrogen peroxide, as well as other substances (McCarty, 1945). Conditions of high or low pH apparently cause the breaking of hydrogen bonds necessary for the integrity of the bipartite DNA molecule. The mechanism of oxidative inactivation is not known. Deamination apparently occurs to the free amino groups in the purine and pyrimidine bases in the DNA molecule.

Nitrous acid (NO_2^-) is especially interesting because Gierer and Mundry (1958) have reported the induction of mutations in tobacco mosaic virus (a small plant virus containing ribonucleic acid and protein) by exposing it to nitrous acid. The mutagenic effect is believed due to the deamination of as little as a single base in the RNA molecule. Consequently, Litman and Ephrussi-Taylor (1959) demonstrated that nitrous acid not only inactivates, but also is very effective in inducing mutations in pneumococcal DNA *in vitro*. In the latter regard, it was distinct from ultraviolet radiation which, while inactivating transforming DNA, was not mutagenic. The mutagenic action of nitrous acid was quantitatively different for different genetic markers. In any case, the number of induced mutations was strikingly high, being of the order of 10% of the total theoretically possible.

Formaldehyde, reported to be a mutagen (Rapaport, 1946; Englesberg, 1952), inactivates and reduces the viscosity of DNA only when used in fairly high concentrations (Zamenhof *et al.*, 1953). Nitrogen

mustards, also known to be mutagenic (Auerbach and Robson, 1944), are effective in inactivating transforming DNA (Lerman and Tolmach, 1959).

Since the discovery by Weygand *et al.* (1952) and by Zamenhof and Griboff (1954) that 5-bromouracil will replace thymine in the DNA of a bacterium grown in the presence of this halogenated pyrimidine, efforts have been made to determine the functional and mutagenic consequences of this base replacement in the DNA molecule. Litman and Pardee (1956) noted that the frequency of mutations is increased as a result of the replacement of 5-bromouracil for thymine in the DNA of an *E. coli* bacteriophage. However, it was also noted that the presence of the analog in a DNA molecule does not always cause mutation, for the analog is found in infectious, nonmutant phage progeny. Recently, Ephrati-Elizur and Zamenhof (1959) have studied the effect of the presence of 5-bromouracil in the transforming DNA of *Bacillus subtilis*. It was evident that DNA containing this analog may continue to possess transforming activity, and that the amount of residual activity depends on the particular genetic marker being followed. Using density gradient techniques, Szybalski *et al.* (1960) have shown that the specific fractions of *B. subtilis* DNA possessing an increased density as a consequence of the incorporation of 5-bromouracil were still capable of effecting transformation. As yet nothing is known of the possible mutagenic effect of this analog on transforming DNA.

7. *Fractionation of DNA*

As will be discussed in Section V,B, a DNA preparation from a given bacterium is genetically heterogeneous in the sense that different molecules are contained in it capable of transferring different pieces of genetic information. It is expected that such demonstrable genetic heterogeneity is the result of different species of DNA molecules, differing from each other in structure, probably in the sequence of bases along the length of the respective molecules. Indeed, a DNA preparation from a given cell-type does appear to be heterogeneous in respect to a number of chemical and physical properties. There is to be found, for example, a distribution of molecular sizes (Cavalieri, 1957) as well as a distribution of salt solubilities (Brown and Watson, 1953; Chargaff *et al.*, 1953; Lucy and Butler, 1954; Bendich *et al.*, 1956) and possibly a distribution of base ratios (Brown and Martin, 1955).

It has been disappointing, however, that no method of separating fractions of different salt solubility or sedimentation coefficient has succeeded in separating different genetic markers in a multiply marked DNA preparation. Two reports of such success (Lerman, 1955; Bendich *et al.*, 1956) have been nullified by alternative interpretations (Lerman,

1956; Brown and Ephrussi-Taylor, discussed in Ephrussi-Taylor, 1955); in general, the amount of a given marker as a function of the total amount of native (undenatured) DNA present does not appear to vary from one fraction to another.

One of the problems facing the successful chemical fractionation of DNA into its genetic components is the extreme homogeneity of a pure DNA preparation in respect to base composition. While DNAs from different species vary widely in their proportion of adenine and guanine to cytosine and thymine (G. L. Brown *et al.*, 1955; Rolfe and Meselson, 1959; Sueoka *et al.*, 1959), the homogeneity in respect to this proportion is very high in the DNA from a given species (Rolfe and Meselson, 1959; Suoeka *et al.*, 1959), such that the standard deviation of the guanine and cytosine content in the DNA of a given bacterial species is less than a tenth of the range over which the mean guanine and cytosine content varies among the DNAs of different bacterial species. Even though the homogeneity in base content is high among the species of DNA molecules from a given cell or organism, there is still considerable opportunity for molecular differences based on different base sequences rather than on different base proportions. How one will be able to separate DNA molecules on the basis of their differential base sequences must be left to further chemical investigation.

8. *Antigenicity of DNA*

Since the work of Avery *et al.* (1944), DNA has been regarded as antigenically inactive. If the converse were true, however, there would be some new hope for fractionating DNA molecules possessing different genetic activity.

In recent years Plescia, Braun, and their co-workers (see Phillips *et al.*, 1957) have reinvestigated this question. Despite many technical difficulties, they have amassed evidence (Braun, Plescia, Palczuk, and Pootjes, personal communication) that antibodies specific for DNA can be prepared. Their evidence is based on the facts that such antibodies are present in antisera obtained by the injection of preparations rich in DNA, are absent in antisera obtained by the injection of preparations lacking DNA or treated with DNase, and that the precipitation by such antibodies of the reacting antigens is prevented by the prior treatment of the latter by DNase. One of the problems is that the antigen preparations inducing the formation of these antibodies in animals are impure, and, indeed, appear to require the presence of protein carriers. The evidence that the specificity of the antigen lies with DNA, and not protein, is based on the findings that: (1) specific inactivation of pneumococcal transformation occurs following exposure to antisera directed against DNA-rich preparations from pneumococci extracted

with 0.5% phenol, and (2) transforming DNA of *B. subtilis* is recoverable from washed precipitates after exposure of this DNA to antisera directed against DNA-protein from *B. subtilis*.

Since the discovery of antigenicity in DNA is of profound importance for further studies of the chemical and genetic properties of this class of macromolecule, further details and progress are awaited.[2]

D. Biosynthesis of DNA

The pathways by which DNA is synthesized in the living cell are important for understanding how the chemical and genetic specificity of the molecule is replicated. As a matter of fact, the biosynthesis of DNA may be directly involved in mutation and recombination. For example, if a mutation is an error in the replication of a particular species of DNA molecule, this error is presumed to occur during the act of synthesis. If genetic recombination occurs only when a DNA replica is copied from two alternative templates, the molecule containing the recombined structures is produced during synthesis. Finally, as will be discussed below in Section VI,A, if the genetic integration of the transforming agent is a process of recombination and if recombination occurs by the kind of copy-choice mechanism just described, integration would depend upon active DNA synthesis. Obviously, knowledge of the biochemical pathways of DNA synthesis is important to our understanding of several genetic phenomena.

It is impossible, however, within the confines of this review to consider the body of experimental data bearing on the mechanisms of DNA synthesis. A comprehensive review of this subject is provided by Brown and Roll (1955). It would be an oversight, nevertheless, if the recent work of Kornberg and his collaborators (Kornberg, 1957; Bessman *et al.*, 1958; Adler *et al.*, 1958) were not mentioned in this regard. It is now clear that a purified enzyme system, extracted from bacteria, is capable of polymerizing simple deoxynucleotides into a DNA chain. Such polymerization, however, depends upon the presence of a small amount of DNA "primer" in the reaction mixture, and upon the presence of Mg^{++} as well as the presence of all four deoxynucleoside triphosphates (those of thymine, adenine, guanine, and cytosine). An important question is whether the particular composition of the DNA molecule produced depends on the nature of the primer. It is to be hoped that in the near future DNA with transforming activity can be synthesized *in vitro* using a small amount of transforming DNA as a primer.

[2] Since sending this review to press, the author has learned of recent investigations (Levine *et al.*, 1960) providing strong evidence for the antigenicity of bacteriophage DNA. These studies further show that heat-denatured, presumably single-stranded, DNA is antigenically more active than either native or renatured DNA.

III. Competence

In the species of bacteria in which genetic transformations have been found to occur, it is clear that certain internal and external conditions must prevail in order for the bacteria to become transformed when exposed to transforming DNA. Indeed, it is by the definition of these conditions that one may hope that the phenomenon of transformation may be extended to other species of microorganisms and possibly to the cells of higher plants and animals as well.

The hereditary properties of certain species of bacteria or cells may make them refractory to transformation by DNA. If a strain secretes an active, exocellular DNase which, moreover, depolymerizes homologous as well as heterologous DNA, it may be impossible to transform such a strain unless the exocellular enzyme can be inactivated without inactivating the transforming DNA. Not much is known of the importance of this condition in actual cases studied (see Austrian, 1952b), but it is one that needs to be taken into account when exploring the possibility of transforming new strains. Another hereditary character that definitely has an influence is the nature of the cell coat. The elaboration of a thick, gummy, or mucoid capsule around the cell membrane or wall may inhibit the penetration of transforming DNA into the cell. In the case of pneumococcal strains differing quantitatively in the amount of polysaccharide capsule they secrete, it has been shown that transformability is related inversely to the amount of capsule secreted (Ravin, 1957).

McCarty *et al.* (1946) began the investigation of biochemical conditions contributing to the transformability of the pneumococcus. Certain chemical factors in the medium, other than the transforming DNA itself, appeared to be essential for successful transformation. One of these is inorganic pyrophosphate, although its role is unknown. In any event, the requirement for pyrophosphate probably depends on the nature of the medium, for it may be safely omitted from most media used today for transformation (Alexander and Leidy, 1951; Ravin, 1954). On the other hand, if *B. subtilis* cells growing in synthetic medium are treated with an inorganic polyphosphate prior to exposure to transforming DNA, the rate of appearance of prototrophic transformants is stimulated (Kohiyama and Saito, 1960). This effect is probably similar to that originally observed by McCarty, Taylor, and Avery (1946). The known chelating properties of the polyphosphates suggest that they stimulate transformation by complexing ions which may inhibit effective contact of transforming DNA with the recipient cells.

Another environmental factor regarded important by early workers was the presence of antibodies capable of agglutinating the recipient bacteria (Avery *et al.*, 1944). Antibodies per se were obviously not necessary, since other physical means of holding the recipients *in situ*, such as increasing the viscosity of the medium through the addition of agar, could replace antibodies (McCarty *et al.*, 1946; Ravin, 1954). Indeed, prevention of the dispersion of the recipient cells was found to aid one class of genetic transformations specifically, namely, those involving the acquisition of the ability to synthesize capsular polysaccharide (Ravin, 1956). Since "clumping" the recipient cells was without effect when brought about up to the time the cells reacted with the DNA, and was effective only during the period after the cells had been infected irreversibly by DNA (Ravin, 1956), and because other conditions (such as shaking) which disperse the already infected cells inhibit transformation (Hotchkiss, 1957), it is possible that these conditions of clumping provide suitable environmental conditions that favor selectively the growth of the encapsulated transformants.

Still another environmental factor that favored pneumococcal transformations was a substance, other than agglutinating antibodies, found in serum (Avery *et al.*, 1944). This substance was later identified as albumin (Hotchkiss and Ephrussi-Taylor, 1951). The role of albumin in promoting transformations is still not clear. Thomas (1955, 1960) has shown that albumin promotes the development of, and possibly the maintenance of, the physiological state of competence to be described shortly; i.e., it makes pneumococci more capable of making an effective contact with DNA although it has little, if any, effect on the genetic consequences of that contact. However, while the presence of albumin is stimulatory, it is not essential, for transformations of pneumococci (Odaka and Watanabe, 1959) and of *Hemophilus* (Alexander and Leidy, 1953) may occur in its absence. An interesting finding (Leidy, Hahn, and Alexander, personal communication) is that, whereas albumin does not ordinarily have to be added to promote competence of *Hemophilus influenzae* grown in complex media, albumin increases the rate at which competence is developed in chemically defined media containing potassium phosphate, aspartic acid, and calcium and magnesium ions. Thus, the role of albumin in promoting competence is not clear, but in view of recent results, it is suggestive that albumin contains, complexed reversibly to it, trace amounts of substances that stimulate the physiological development of competence. The fact that in complex media containing proteins the addition of albumin is not necessary can be explained by supposing that these other proteins may serve the same role as albumin. Interestingly enough, Eagle (1960) has recently ob-

served that albumin serves to promote the growth of animal cells *in vitro* by releasing in trace amounts substances reversibly bound to it. In addition, Spizizen (1959) has found that albumin will replace the small amount of tryptophan required for growth and transformation of a tryptophan-requiring strain by DNA from the homologous tryptophan-independent strain of *B. subtilis*.

Reference has just been made to a physiological state of competence that must be developed in recipient bacteria if they are to undergo transformation. The demonstration of this state was first made by McCarty *et al.* (1946) in pneumococci, but has subsequently been made in *Hemophilus* (Schaeffer, 1956a) and in *Bacillus subtilis* (Spizizen, 1959). Originally it was shown that DNA did not need to be present in the transforming medium at the time the recipient cells were inoculated into it. These cells had to multiply for a certain number of generations, after which time transforming DNA had to be present. The actual period of time in which the cells were competent, i.e., able to make an effective contact with the DNA, was fairly short. This could be shown in the following way: if transforming DNA were added at various times to the culture in which the inoculated recipient organisms were growing, a time (X) after inoculation was observed when DNA addition failed to produce transformants; similarly, if the cells were inoculated into medium containing DNA, and DNase were added at various times afterwards, a time ($X +$ less than 1 hour) was observed after which the enzyme failed to prevent transformations. It was also apparent from these results that the competent cells need to react with transforming DNA for only a short period of time in order eventually to produce transformed progeny. The actual duration of this reaction will be referred to again below (Section IV,B).

Thomas (1955) has studied the development of competence in some detail. He used as a genetic marker in his experiments with pneumococci one that conferred resistance to streptomycin, and which, as Hotchkiss (1954) had previously shown, was phenotypically expressed in the "infected" bacteria 90 minutes after contact with the DNA containing this marker. If streptomycin-sensitive recipients are inoculated into an appropriate medium containing albumin but no DNA, and then diluted at various times thereafter into fresh medium containing transforming DNA (DNase always being added 20–30 minutes after the DNA), it is found that the number of streptomycin-resistant transformants produced at 90 minutes rises at first as a function of the time during which the bacteria had been growing in the albumin-containing medium, and after reaching a peak, this number drops as rapidly. Thus, a "peak" of competence is observed. Dilution acts to forestall the development of

competence, without affecting the bacteria that have already become competent. The "peak" of competence is retarded if albumin is provided late during the initial growth period (prior to dilution into fresh DNA-containing medium), but most notably the time at which the peak appears is a function of the density of the inoculum transferred into the albumin-containing medium in which competence arises. The lower the initial bacterial density, the greater the amount of time required for competence to arise. With low initial densities, moreover, it is sometimes possible to observe "waves" of competence, i.e., competence appearing, disappearing, then reappearing in the growing population. Thomas (1955) calculated that the average length of time that the individual bacterium remains competent is in the order of 15 minutes, which is approximately half of the time required for the population to double in the medium employed.

The rate at which competence disappears from a population as a result of dilution into fresh medium depends on the temperature of the medium in which the dilution takes place, the rate decreasing with decreasing temperature (Thomas, 1955). The Q_{10} is in the neighborhood of 1.7. Indeed, several investigators starting with Fox and Hotchkiss (1957) now preserve batches of competent bacteria for transformation experiments by freezing them in 10% glycerol.

The work of Hotchkiss (1954) is also in conformity with the idea that competence is a physiological property that arises during a fraction of the generation period of the bacterial organism. Moreover, his evidence indicates that the period of competence corresponds to a particular period of the bacterial division process, since, if this were indeed the case, any environmental influence that induces division synchrony should increase the "peaks" of the waves of competence arising in the population. Brief chilling, by removing the bacteria from 37°C, bringing them rapidly (within 2 minutes) to 25°C for 15 minutes, and then returning them to 37°C, not only induces division synchrony but also synchronizes them with respect to transformability. The number of competent cells is also very high at the end of the cooling period, higher than that found in the control maintained at 37°C. These findings are explained by supposing that cells that were competent prior to cooling remain so while others acquire their competence during the cooling period.

What proportion of the recipient population is competent during a "peak" period? This figure is not easy to determine directly, for the measure one takes is that of the frequency of transformed progeny eventually arising from the organisms having made effective contacts with transforming DNA. Generally, one finds that only a small fraction of

the progeny descending from the treated population is transformed (Hotchkiss, 1954; Ravin, 1956), and even under the most favorable conditions the frequency of transformants rarely rises above 10%. One might argue (Alexander and Leidy, 1951) that only a fraction of the population is genetically capable of becoming competent to react with DNA, and that this fraction limits, and indeed determines the maximal frequency of transformations observable. That the maximal frequency of transformants is not determined by a genetic incapacity of the treated population to react with DNA is shown by a number of lines of evidence. Ravin (1954) showed that every pneumococcus inoculated into a semisolid medium containing albumin and transforming DNA gives rise to a clone of about 10^5 cells in which at least some transformants are observed. The actual proportion of transformants per clone is low and varies from one clone to another; it also depends upon the concentration of DNA in the medium, but at high concentrations of DNA, every clone contains some transformed cells. Thus, every pneumococcus has the hereditary capacity to react with DNA. At low concentrations of DNA, some clones fail to contain transformed cells, but if bacteria from these clones are isolated and re-exposed to transforming DNA, they will react with DNA as well as untransformed bacteria isolated from clones in which transformations did occur (Ravin, unpublished). Finally, Schaeffer (1957a) has used inhibiting DNA (see Section IV) to determine if the maximal frequency of transformants in a given *Hemophilus* population is determined by the frequency of competent cells that reacted with the transforming DNA. It is possible, for example, that in populations exhibiting a "peak" of competence, all of the bacteria are competent, but that the encounter between a competent bacterium and a molecule of transforming DNA has only a low probability (say, in the order of 10^{-1} to 10^{-2}) of eventually giving rise to transformed progeny. After all, events within the recipient, transpiring after contact, may also limit the frequency of transformation. The minimal concentration of inhibiting DNA which will produce a detectable lowering of the transformant frequency, under conditions where the "multiplicity of infection" is low (less than 1 molecule per 10 bacteria), is expected to be quite different in the two cases: (a) where only a fraction of the cells in a "peak" culture are competent to react with DNA, and (b) where all the cells are competent, but only a fraction of the cells contacted by DNA gives rise to transformed progeny. The results of Schaeffer's experiments were consistent with the latter hypothesis, that 100% of the bacteria are competent.

It is of considerable interest to define the biochemical conditions under which competence is favored in the bacterium. In the first place,

an understanding of the biochemical basis of competence could lead to the discovery of a wider variety of cells and organisms (including cells of higher organisms, cultured *in vitro*) capable of being transformed by DNA. Secondly, definition of the biochemical requirements for competence also could simplify the medium in which transformations are carried out. For a long time the study of genetic transformations has been impeded by the fact that the two genera of bacteria in which transformations would occur reproducibly were *Diplococcus* and *Hemophilus*, both of which require fairly complex, undefined media for growth. This fact made impossible the utilization of biochemically marked mutants lacking the capacity to carry out some specific biochemical step necessary for growth in a simple medium. In part, this problem has been overcome by the discovery of transformable species of bacteria, such as *B. subtilis*, which can grow in synthetic media (Spizizen, 1958). Studies have also been carried out to determine the basic requirements for the development of competence in pneumococcus and *Hemophilus* and to prepare chemically defined media in which competence would arise.

Fox and Hotchkiss (1957) were able to use frozen cultures of competent pneumococcal cells to determine some of the factors necessary for the loss and reacquisition of competence. Upon prolonged storage at −20°C, or following melting and subsequent incubation at 37°C, bacteria lose their competence. The reacquisition of this state can occur within 20 minutes, and depends not only upon the presence of serum factor best supplied by purified serum albumin, but also of calcium ions at a concentration of 10^{-3} to 10^{-4} *M*. The reacquisition of competence appears also to require protein synthesis, for it requires the presence of a mixture of amino acids, is facilitated by the presence of glucose as an energy source, and is completely inhibited by chloramphenicol, a relatively specific inhibitor of protein synthesis.

Rappaport and Guild (1959) developed a defined medium for the growth of pneumococcus. It consisted of salts, a mixture of amino acids, a mixture of vitamins, and two energy sources (glucose and sodium pyruvate). Mutants which required certain specific metabolites (pyrimidines and specific amino acids) for growth were obtained by ultraviolet irradiation followed by penicillin screening (Davis, 1948; Lederberg and Zinder, 1948). Unfortunately, however, after repeated transfer in this medium pneumococci appear to lose their ability to react with transforming DNA. This is possibly related to the fact that the generation time in this medium is rather low, from 100 to 200 minutes.

Hemophilus appears to be somewhat more suitable in this regard. Talmadge and Herriott (1960) have developed a synthetic medium for

the growth of *Hemophilus,* consisting of salts, glucose, acetate, a mixture of vitamins, a mixture of amino acids, a mixture of nucleotides, hemin, buffers, and surface-active agents. The generation time is about 60 minutes (about twice that in complex medium), but transformations were observed in cells grown in this medium. The frequency of transformation was, however, low, usually less than 1% that obtained with cells grown in complex medium. Cells grown in complex medium lose their competence on repeated washing with buffered saline. An 80% recovery of competence was observed when such cells were resuspended in the synthetic medium, indicating that factors essential for competence are among the constituents. Leidy, Hahn, and Alexander (personal communication) also report the development of competence in three *Hemophilus* species using a potassium phosphate-buffered saline solution (pH 7.4) containing 1% aspartate and calcium and magnesium ions. As mentioned above, in this synthetic medium albumin is not essential but increases the rate at which competence is developed.

IV. Penetration

A. Does DNA of High Molecular Weight Penetrate?

It is clear that recipient bacteria must be physiologically competent to react with DNA in such a way as to give rise eventually to genetically transformed progeny. What transpires when a competent bacterium makes contact with DNA? How many molecules of transforming DNA must make contact with a competent bacterium to cause it to produce transformed progeny? Is contact with one molecule sufficient? Regardless of how many molecules of transforming DNA are required, does the DNA get into the bacterium at all? Or does some low-molecular-weight product resulting from the reaction between bacterium and DNA actually do the penetrating and the eventual transfoming? Finally, regardless of the nature of the penetrating entity, if anything penetrates at all, what becomes of it in the host bacterium? Obviously, the genetic information residing in its structure must be transferred in two ways: (a) it must be integrated into the genetic constitution of either the host cell itself, or into one of its descendants, such that a portion of the clone descending from the "infected" host bacterium is replicating the transferred genetic information in synchrony with its own reproduction; (b) it must modify the metabolic machinery of either the host cell, or a portion of its descendant clone, such that a new phenotype is expressed corresponding to the one possessed by the donor strain. The first function of the transforming agent may be termed autocatalytic, and it will be considered in detail in Section V and VI. The

second function of the transforming agent may be termed heterocatalytic, and it will be discussed in Sections V and VII.

In this section the initial questions set forth above will be dealt with. These questions concern the earliest steps in the transformation process, namely, those responsible for bringing the transforming agent into the cell and near the site where its autocatalytic and heterocatalytic functions can be performed.

It has been amply demonstrated that one unit of transforming activity, presumably one molecule of DNA, is sufficient to make effective contact with a recipient bacterium in order to result in a progeny containing genetically transformed organisms. Ravin (1954) inoculated recipient pneumococci into a semisolid medium containing serum albumin and DNA capable of transforming the capsule character. Each pneumococcus was held *in situ* as it reproduced and gave rise to a clone of descendants. These could become competent and react with the transforming DNA. After a suitable period of time, the clones were individually isolated and examined for the presence of transformed, encapsulated cells. The proportion of the isolated clones containing transformants proved to be a function of the concentration of DNA in the medium. At sufficiently high concentrations, all of the clones contained some transformed bacteria; at low concentrations, the frequency of clones containing transformed bacteria was directly proportional to the DNA concentration. This finding is consistent with the notion that a single molecule of transforming DNA making an effective contact with a competent bacterium can cause it to give rise to transformed progeny. The same conclusion is arrived at by treating a series of replicate cultures with increasing dilutions of DNA (Stocker *et al.*, 1953; Ravin, 1960b).

Subsequently, by the use of DNA containing genetic markers for drug and antibiotic resistance, the linear relationship between DNA concentration and transformant frequency could be demonstrated directly at the cellular level (Alexander *et al.*, 1954; Thomas, 1955; Hotchkiss, 1957; Ravin, 1957). With such markers the effort of "screening" the transformed bacteria is greatly reduced because the untransformed background population, which represents the vast majority of bacteria in the treated culture, can be eliminated by the particular selective agent employed (streptomycin, or penicillin, or sulfanilamide, etc.). If one exposes samples of a given population of bacteria in which competence has been developed, to various concentrations of transforming DNA for a brief period of time (15 minutes or less), and then determines in each sample the number of transformed (say, streptomycin-resistant) bacteria at such time afterwards, when the newly

acquired character has had an opportunity to express itself, one obtains the type of titration curve shown in Fig. 4. At saturating concentrations of DNA, the proportion of transformed bacteria to the total number of bacteria (N_t/N_0) is always less than 1. The actual proportion depends in part on the proportion of cells that were competent in the reaction mixture, but it also depends on two other conditions, namely, the quality of the DNA preparation used, and the average number of generations

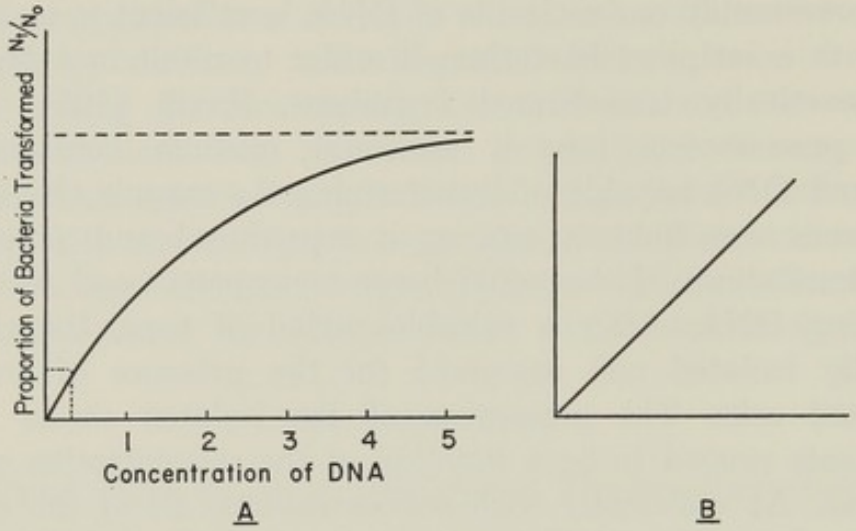


FIG. 4. A typical titration curve obtained by plotting the proportion of bacteria transformed as a function of DNA concentration. Time of exposure to DNA is held constant and may be 15 minutes or less. B represents an enlargement of the region enclosed by dots in A. N_t = number transformed bacteria; N_0 = number reacting bacteria. The plateau value of N_t/N_0 shown in A is always less than 1, and is in part determined by the proportion of bacteria that are competent in the reacting culture.

that elapse prior to genetic integration of the "infecting" DNA (Hotchkiss, 1956, 1957). Both of these conditions will be discussed further below. Nevertheless, the important conclusion may be reached that one unit of transforming activity is sufficient to launch the transformation process in a given recipient bacterium. This unit is presumed to be a molecule of DNA, or that part of the molecule containing the specific genetic information being transferred.

It does not take very long for a competent bacterium to make effective contact with a unit of transforming activity. This is shown by mixing various samples of a competent culture with a given amount of transforming DNA, and then adding DNase to these samples at different times. If DNase is present at the time DNA is added, no transformations are observed; but if as few as 10 seconds elapse between the times DNA and DNase are added, an irreversible step is activated in the reacting bacteria, such that transformed progeny will eventually

ensue from them (Hotchkiss, 1954). Since the DNA molecule is so large, it is justifiable to ask whether the entire molecule of DNA, or only a part of it, enters the recipient organism. Indeed, it may be asked whether the transformation process is triggered by a reaction occurring at the surface of the bacterium in which no material actually enters the organism. While difficult to conceive, for one would have to imagine a DNA species being synthesized within the cell[3] in accordance with a model held on the outside of the cell, the question is nevertheless fair to present. Fortunately, evidence has been provided from a number of lines of investigation to permit us to make an unequivocal decision. In the first place, material from the transforming DNA does enter the recipient bacterium. This fact has been demonstrated by studies in which DNA was labeled with radioactive phosphorus (P^{32}), obtained by growing the donor strain in a medium containing the radioactive label (Lerman and Tolmach, 1957; Goodgal and Herriott, 1957a; Fox, 1957). P^{32} is taken up by the recipient bacteria, and some of it can be removed by washing or by treatment with DNase. The amount of P^{32} remaining, i.e., that which is fixed to the bacteria in such a way that DNase can no longer affect it, is directly proportional to the number of transformants produced. Thus, there is a direct correlation between the amount of DNA-P^{32} taken up irreversibly and the frequency of transformations. Furthermore, the P^{32} label is found specifically in the DNA fraction of the recipient organisms, as immediate lysis of the recipients proves (Goodgal and Herriott, 1957a). Fox (1960) was able to recover more than 50% of the P^{32} label in DNA reisolated from the recipient bacteria. Thus, if the P^{32} ever exists in the form of a low-molecular-weight compound, the existence of the latter can only be highly transitory.

There is further evidence, however, that the material that gains entrance to the recipient is in the form of highly polymerized, native DNA. If a polynucleotide of specific length, smaller in size than the native DNA molecule of which it was a part but larger than the individual mononucleotides, were the material gaining entrance, one would expect that the inactivation of transforming DNA by DNase would be "multi-hit" in nature, that is, it would require several enzymatic ruptures per molecule to destroy the integrity of the polynucleotide carrying the specific genetic information to be transferred. Yet the inactivation of transforming DNA by DNase shows no indication of a "multi-hit" process (Lerman and Tolmach, 1957, 1959). Both incorporability and genetic activity are reduced apparently when only one cleav-

[3] The DNA in the bacterial cell is located centrally in, usually, a single, Feulgen-positive body (see Hayes, 1960).

age per molecule is produced, although the latter is considerably more sensitive to enzymatic attack. Furthermore, transformation may be inhibited by adding a nontransforming DNA to the mixture of reacting bacteria and transforming DNA (Hotchkiss, 1954, 1957). The nontransforming DNA may be of the form of homologous, "unmarked" DNA or DNA from a foreign source, such as a different species of bacterium or calf thymus DNA. The inhibition is competitive in nature, the frequency of transformation being unaffected when the total concentration of DNA in the mixture is insufficient to saturate the competent bacteria; but the "plateau" frequency of transformants obtained at saturating concentrations of DNA is diminished to the extent that the proportion of nontransforming DNA is increased (Hotchkiss, 1957). Indeed, there is good reason to believe that the competing DNA enters the recipient bacterium, although it may not result in any observed genetic transformation. Using labeled DNA, Goodgal and Herriott (1957a) found that recipient bacteria take up P^{32} from homologous, "unmarked" DNA as well as they take it up from transforming, "marked" DNA. Fox (1957) has shown that the amount of P^{32} taken up per observed transformant varies with the state and quality of that preparation. With the most effective, fresh preparations, about 1.4 times the DNA content of the cell is incorporated when one cell is transformed for a given property. Older, less active preparations show more DNA incorporated per bacterium transformed, thus indicating that genetically inactive DNA can also be taken up by the recipient cell. Similarly, Lerman and Tolmach (1959) have shown that the P^{32} label of transforming DNA inactivated in its genetic properties by any one of a number of physical and chemical agents can nevertheless be incorporated irreversibly by the recipient organism. Finally, as will be discussed in Section VIII, Schaeffer (1957a,b) came to similar conclusion with regard to the uptake by *Hemophilus* strains of P^{32} label from transforming DNA of the same or different species: the same number of DNA molecules is incorporated regardless of the source, although transforming DNA of heterospecific origin has a lower probability of inducing any genetic change.

Thus, native DNA incapable of inducing a genetic transformation inhibits transformation by native, transforming DNA by competing for entry into the recipient organism. DNA in its highly polymerized form appears to be essential for the initial reaction with the competent bacterium that will eventually ensue in its transformation. This conclusion is substantiated by two further types of evidence. Two ways have been used for shearing native DNA molecules so that their average length (molecular weight) is reduced but their double helical structure is

maintained; ultrasonic vibration and spraying through an atomizer. By the former method, Litt *et al.* (1958) were able to show that the transforming activity of the DNA molecule depends on its molecular weight, small changes in the latter being accompanied by loss of activity. By the latter method, and using P^{32}-labeled transforming DNA, Rosenberg *et al.* (1959) were able to show that the greatest contribution made to loss of activity by molecular shearing is diminution in ability to penetrate the bacterium ("penetration" being used here in the sense of including all of the initial reactions from effective contact or absorption up to, but not including genetic integration of the marker in question). There is about a 75% diminution in penetrability when as little as a 20% reduction of the number-average molecular weight has occurred. Some of the loss of transforming activity, however, could be interpreted as due to damage of the genetic "marker" itself, within the molecule of DNA bearing it, such as to reduce its probability of integration into a bacterial genome. The sensitivity to this latter type of damage varied from one "marker" to another. This could be interpreted as indicating different polynucleotidic lengths for different markers, the longer ones being the most sensitive, an interpretation which accords with many other results obtained with inactivating agents discussed above.

All of the results lead to the conclusion that, while the length of the DNA molecule that must eventually be integrated to effect a genetic transformation is considerably smaller than that of the DNA molecule as a whole, the size of the active unit necessary for effective contact with the recipient bacterium is that of the molecule as a whole. It might be argued that, while the entire DNA molecule is necessary for effective contact (a contact necessary for absorption of genetic information into the cell), a result of that contact is the splitting-off of an information-carrying fragment or fragments which enter the recipient. The evidence contradictory to this view is that of Goodgal and Herriott (1957a) and of Fox and Hotchkiss (1960) that, upon absorption, the P^{32} label of transforming DNA is found specifically in the DNA fraction of the recipient. The latter authors found that they could recover over 50% of the input P^{32} in the DNA reisolated from DNase-treated recipient cells 15 to 40 minutes after exposure to transforming DNA. Moreover, the ratio of the biological activity of the newly introduced marker to that of a resident marker remained constant during subsequent growth of the transformed culture. Thus, fairly early after exposing recipients to transforming DNA (certainly by the time of the second division following penetration of the DNA) the introduced marker begins to increase at precisely the same rate as does the resident DNA of the transformed recipients. However, subsequent work by Fox (1960)

showed that, when recipient cells are exposed for very brief periods of time (less than 15 minutes) to transforming DNA, and then DNA reisolated from them 5 minutes after termination of DNA fixation, not all of the original activity is recovered. The fraction of this activity recovered, however, increases with increasing time of exposure to DNA (so that with 15 minutes or more of exposure all is recovered), and also increases with increasing time of growth of the recipients following their brief exposure (15 minutes) to transforming DNA (so that in less than one generation all is recovered). Evidently, transforming DNA immediately after fixation does not possess biological activity, but this activity reappears before one division occurs and eventually increases at a rate identical to that of resident DNA and to that of the bacterial population as a whole. This finding would, of course, be made if absorbed DNA fragments were rapidly reintegrated into DNA molecules—or if native DNA, after penetration, were fragmented within the cell and the fragments used in subsequent DNA synthesis. Another possibility is, of course, that the loss of activity of the introduced marker subsequent to fixation is due to its transient but firm binding to some surface receptor or other constituent of the cell, from which assocation it is not released upon lysing and extracting the cell's contents. Ephrussi-Taylor (1960c) has been able to show that the latter possibility is, indeed, the correct explanation of the phenomenon. Using transforming DNA labeled with N^{15} and P^{32}, she found that DNA firmly bound to cellular structures could be released by ultracentrifugation. Indeed, by density gradient techniques it was found that the recovered DNA had the molecular weight of native DNA. The normal release of transforming DNA from the binding-sites appears to require metabolic activity, for if cells exposed to DNA are kept from multiplying by lack of glucose, no transforming activity is found in lysates prepared 20 minutes later. Nevertheless, ultracentrifugation can "unmask" all of the original activity, which is found specifically in fractions corresponding to the molecular weight of native DNA. Therefore, there seems to be no reason to invoke the notion that anything less than a native, high-molecular-weight DNA molecule enters the recipient bacterium. What may be the fate of this molecule in events subsequent to penetration is another matter, and will be left for further discussion below.

B. Does DNA Pass through Localized "Holes" in the Bacterial Surface or Is Penetration an Enzymatically Catalyzed Process Occurring at Specific Receptor Sites?

The nature of the penetration is not without interest, even to the geneticist, for if only special cell surfaces, arising under special condi-

tions, will allow DNA to penetrate them, this information is important to know, particularly if DNA-mediated transformations are to be extended to other material than bacteria.

Two somewhat different hypotheses have been proposed to account for penetration. Each is based on an assumption of the underlying features of bacterial competence. One hypothesis, to be termed the "localized protoplast" hypothesis for reasons about to be explained, regards the competent bacterium as one having attained in its processes of growth and division a partially "naked" surface. The "open" regions may be, for example, where the division septum is forming, which may be temporarily free of cell wall material. Since such regions would appear periodically during the growth of a clone dividing synchronously, the periodicity of competence would be explained. The other hypothesis, to be termed the "enzymatic receptor" hypothesis, regards the competent bacterium as one having synthesized on its surface protein enzymes with the specific catalytic ability to bind DNA. Since the synthesis of these hypothetical receptor sites may be assumed to be geared in some fashion to the metabolic processes of growth and reproduction, the periodicity of competence can be equally well explained by this hypothesis.

The "localized protoplast" hypothesis is based on some of the known properties of bacterial protoplasts. These are viable forms of bacteria in which cell wall synthesis is blocked (Lederberg and St. Clair, 1958; Prestidge and Pardee, 1957), and consequently multiplication is defective. Complete protoplasts may be produced by the use of a number of agents, including lysozyme and penicillin. They usually assume swollen, spherical shapes, and are highly sensitive to osmotic changes although fairly stable in hypertonic sucrose media containing Mg^{++}. Unless cell wall synthesis is genetically blocked, the protoplast condition can be reversed. In the protoplast condition, moreover, the bacterium is capable of considerable biosynthesis: nucleic acid and protein synthesis continue. Certain interesting parallels suggest themselves between the protoplast and the competent bacterium. First of all, the protoplast appears to have altered permeability characteristics. Spizizen (1957) and Fraser, Mahler, and their co-workers (Fraser *et al.*, 1957; Mahler and Fraser, 1959) have found that, while *E. coli* protoplasts cannot be infected by intact phage, they are infectable by disrupted phage preparations. The latter group of investigators (1959) found that the protoplast-infective agent contains both DNA and protein, but that unlike intact phage it is sensitive to destruction by both trypsin and DNase. Another parallel between the competent bacterium and the protoplast is that the latter is stabilized by serum albumin (Fraser *et al.*, 1957). It is altogether possible, of course, that there is no connection between the influence of

albumin on competence and on protoplast stability. Finally, it has been suggested that DNA alone may be able to penetrate protoplasts. Chargaff *et al.* (1957) have reported the transformation of protoplasts of a lysine-requiring *E. coli* strain by DNA from a lysine-independent strain. Certain important controls were lacking in their experiments, however, and the results have not since been confirmed.[4] Spizizen (1958) first reported the transformation of *B. subtilis* using germinating spores. Actively multiplying vegetative cells appeared, on the contrary, to be refractory to DNA-mediated transformations. This finding was significant in that there are certain resemblances between the germinating spore and the "localized protoplast." At that point on the spore case where germination is occurring, a "naked" protoplast, temporarily free of cell wall material, is issuing forth. However, subsequently, Schaeffer and Ionesco (1959) and Spizizen (1959) have found conditions in which vegetative cells could be transformed. Nevertheless, Spizizen (1959) is still of the opinion that competent vegetative cells are those at a stage of growth in which they are partially free of cell wall. If certain wall precursors, such as L-alanine or L-glutamic acid, are added to the growing culture of vegetative cells, transformation is inhibited.

One of the chief pieces of evidence, however, that the competent cell is a "localized protoplast" is that provided by Thomas (1955). By diluting competent pneumococci into a fresh medium containing transforming DNA, and then adding DNase at various times afterwards, he could follow the loss of sensitivity of the reacting cells to DNase as a function of time. Similarly, by diluting competent pneumococci into fresh medium not containing DNA, and then testing them for residual competence at various times afterwards by challenging them with transforming DNA, he could follow the loss of competence as a function of time. Carried out at two different temperatures (37° and 25°C), the experiments demonstrated that the irreversible fixation of DNA is accompanied by loss of competence. An explanation of these results is that competence involves a temporary permeability of the bacterium to large molecules, including DNA and DNase. As competence is lost, any transforming DNA that has penetrated the cell is sheltered from attack by exogenous DNase.

[4] After sending this review to press, however, the author learned of the claim (Wacker, 1959; Wacker and Laschet, 1960) that *E. coli* protoplasts could be transformed in respect to folic acid synthesis and streptomycin sensitivity by employing a modification of the procedure of Chargaff *et al.* (1957). From the few published details, it appears that no control was performed to check the specificity of the DNA. In view of the failure of other investigators (including Lederberg and St. Clair, 1958) to transform *E. coli* protoplasts, the issue must be regarded as unsettled.

In this regard, the work of Kaiser and Hogness (1960) is pertinent. The DNA of defective bacteriophages that transduce gal+ genes in *Escherichia coli* K12 is also capable of transforming these bacteria for the gal+ character. In this case, however, a "helping" bacteriophage is necessary, since the DNA alone cannot transform intact *E. coli*. Apparently, the "helper" provides the "hole" through which the DNA can pass. The reaction between the phage-infected bacteria and the transforming DNA is fairly slow (the maximum frequency of transformants being achieved only after 120 minutes of contact), and the efficiency of the process is low compared to the efficiency with which gal+ genes can be transduced by whole phage. Yet, the evidence, on the whole, indicates that bacteriophage tail protein provides the enzymes for producing a hole through which DNA penetrates. The penetration is fast if the DNA is not separated from the phage, slow if the DNA is in "free" form.

On the other hand, strong evidence has been provided by Fox and Hotchkiss (1957) that the development of competence involves the synthesis of enzymatically active receptor sites on the surface of the bacterium. Populations of pneumococci that had become competent were preserved in this condition in the frozen state. After thawing, such a competent population is transferred into a growth medium containing transforming DNA. DNA is removed by DNase at the end of the desired time of exposure, and the cells are transferred to 37°C for 2 hours to permit expression of the new character (streptomycin resistance in their case). It is found that, after a short induction period, the number of bacteria that will eventually produce transformed progeny increases linearly with time of exposure to DNA. The linear increase occurs for over an hour, during which time the bacteria have not yet begun to divide. The rate of this linear increase was found to be influenced by the temperature at which the initial reaction between competent cells and DNA occurred; 32°C was found to be optimal, rather than 37°, which is optimal for growth of the bacteria and expression of the newly acquired genetic determinant. Such temperature dependence is likely to be associated with a metabolic process of adsorption and penetration. Moreover, using the optimal temperature for effective contact between competent bacteria and transforming DNA, it could be shown that the linear rate of initiation of transformants depended upon the concentration of DNA and on the concentration of bacteria in ways exactly as predicted by a rapid reversible adsorption of the DNA to the bacteria followed by a slow irreversible incorporation:

$$\mathrm{B(s)} + \mathrm{DNA} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} [\mathrm{B(s)} \cdot \mathrm{DNA}]\ \text{complex} \xrightarrow{k_3} B(\mathrm{tr}),$$

where B(s) represents the concentration of bacterial adsorption sites, assumed to be directly proportional to the number of bacteria, and B(tr) represents the concentration of bacteria in which transformation has been irreversibly initiated. The authors were able to calculate, using a standard treatment of their data in terms of enzyme-substrate reactions, that about 30–75 adsorption sites existed per competent bacterium. The authors suggested that the phenomenon of competence was associated with the appearance and disappearance of such sites in a growing population of bacteria.

If frozen, competent cultures are thawed in simple saline solutions, completely inadequate for growth, they are found to be able to fix DNA. Apparently, the irreversible incorporation of DNA does not require growth, although the completion of the later stages of transformation (expression and replication of the newly acquired agent) does (Fox and Hotchkiss, 1957). However, the presence of Ca^{++} and albumin, important for the development of competence, promotes fixation. Further evidence that fixation of DNA is an enzymatically catalyzed process is the necessity to provide conditions for protein synthesis in order for competence to arise. Competence not only depends on Ca^{++} and albumin being present, but on the presence of a mixture of amino acids and glucose as an energy source. The development of competence is also blocked by chloramphenicol, which is known to be a rather specific inhibitor of protein synthesis.

Furthermore, if penetration of DNA were simply due to nonspecific permeability of competent bacteria, as Thomas (1955) has proposed, one would not expect such a strict dependence of penetration on molecular size, as Rosenberg *et al.* (1959), Lerman and Tolmach (1957, 1959), and others have shown. Incorporation of P^{32} label from transforming DNA should not be greatly affected if molecular length were reduced without disturbing its native, double-helical configuration. Yet the converse is true. These results, too, support the view of specificity in the penetration of DNA. Schaeffer (1958), in studying the ability of DNA of different sources to compete with DNA of *Hemophilus influenzae* to gain entry into recipient bacteria of this species, found some evidence for species specificity in DNA penetration. While these recipients could not discriminate between the DNAs from *H. influenzae, H. parainfluenzae,* and *H. suis,* they could discriminate against the DNAs from *H. ducreyi, H. pertussis, Brucella bronchiseptica,* and calf thymus.

In summary, it may be possible that both mechanisms, the development of a "localized protoplast" condition and the development of adsorbing sites, play a role in the ability of bacteria to react with

transforming DNA in a genetically consequential way. Irreversible incorporation of DNA may be carried out by enzymes at fixed receptor sites on the membrane of the bacterium. The facility to reach these sites may very well depend on their accessibility, which is lacking or nearly so, when a cell wall surrounds the membrane. If this were so, animal cells should make better material for the incorporation of DNA than either bacteria or plant cells, provided they have retained the capacity to produce DNA-adsorption sites on their membranes. One trouble with this unitary view, of course, is that there is little advantage to enzymatic receptors which are separated from their substrates. However, there are many examples of this type of phenomenon in cells, and it may very well be that enzymes are present if conditions that will enable them to meet their substrates have a fair probability of arising.

V. Genetic Integration and Phenotypic Expression

A. Reverse Transformations and the Existence of "Allelic" Transforming Agents

On the basis of the evidence already cited, it is fair to assume that it is DNA in its native state that enters a recipient bacterium, and that the penetration of one molecule is sufficient to result in a genetic transformation. It is of interest to determine the consequences of penetration by a single DNA molecule. Evidence to be discussed supports the view that the penetrating DNA molecule becomes intimately associated with an endogenous DNA molecule of the recipient bacterium, that this association involves pairing or synapsis between homologous regions, and that an interaction between exogenous and endogenous DNA molecules results in a recombinant form of DNA molecule, that is, one possessing part of the information of each "parental" DNA molecule. The recombinant form of DNA is integrated into the genomes of progeny descending from the originally "infected" recipient, in the sense that they now transmit a part of the hereditary information contained in the donor strain of bacteria. Fairly soon after penetration into the recipient bacterium the exogenous DNA may express its presence by modifying the phenotype of the "infected" cell. Whether phenotypic expression may occur prior to the recombination event just mentioned is not yet clear. The evidence for synapsis and recombination comes from the demonstration of allelism and linkage in transforming agents, which will be considered in turn.

That transformation does not involve a mere addition of genetic material to the genome of the host was first shown by Taylor (1949a). She showed, on the contrary, that the recipient cell contains a homolog

of the transforming agent, and that transformation consists in the replacement of the homolog by the transforming agent. Pneumococci that have the hereditary character of forming chains (fil+) can be converted into the more usual diplococci (fil—) by treating them with DNA from fil— bacteria. Such transformed fil— bacteria can be transformed back into fil+ forms by treating them with DNA from fil+ donors. In other words, a transformation can be reversed by transformation; the reverse transformation has sometimes been referred to as a reciprocal transformation. This finding proves that the recipient cell contains something corresponding to the infecting agent, but which determines an alternative form of the character in question. This finding has been confirmed, moreover, in other studies. By taking advantage of the linkage of the determinants affecting mannitol utilization and streptomycin resistance, Hotchkiss and Marmur (1954) were able to show that the transformation of streptomycin resistance, as well as that of mannitol ultilization, can be reversed. Ravin (1959a) has shown that pneumococci transformed to the encapsulated condition can be transformed back to the capsule-deficient condition. The nature of the reverse transformation is specific, moreover, in that while there are several kinds of capsule deficiencies, the one produced by reverse transformation corresponds to the kind possessed by the donor of the transforming agent.

These results lead to the conclusion that a transforming agent can exist in one of at least two alternative forms, each form being hereditary and determining an alternative form of some specific character. These alternative forms of transforming agents correspond obviously to the allelic forms of chromosomal determinants. With due appreciation of the problem of allelism in modern genetics (see Carlson, 1959a,b), the term "allele" will nevertheless be employed in the course of this review in the exclusive sense of alternatives; i.e., a haploid cell, such as the bacterium commonly dealt with, can possess one form of the genetic material or the other, but not both simultaneously. If, for example, two mutant forms of genetic material, brought together by recombination, can exist together in the same cell, they shall not be regarded here as alleles.

B. Genetic Heterogeneity of Transforming DNA and the Existence of Determinants Transferred Independently of Each Other

It is easy to demonstrate that transforming DNA, in terms of the genetic information it bears, is complex and resolvable into distinct elements. The manner in which this is done is to use a DNA from a

donor strain differing from the recipient in several hereditary characters as shown in the diagram.

recipient a− b− c− · · · × DNA a+ b+ c+ · · · →	singly-transformed	transformant a+ b− c− · · ·
		transformant a− b+ c− · · ·
		transformant a− b− c+ · · ·
	doubly-transformed	transformant a+ b+ c− · · ·
		transformant a− b+ c+ · · ·
		transformant a+ b− c+ · · ·
	triply-transformed	transformant a+ b+ c+ · · ·

In the diagram above, a, b, c . . . etc. represent different hereditary characters capable of being mutated independently of each other, and + and − represent two alternative forms of each character. In the diagram, a "triply-marked" DNA is shown to be reacting with a recipient strain. Rarely is more than one character transformed per bacterium. Thus, of the seven possible transformant types, the singly-transformed types occur most often, and the doubly- and triply-transformed types are exceptional. Clearly, there are separable agents for characters a, b, c . . . etc.

It can also be shown that certain separable agents are transferred independently of each other. Correcting for the percentage of cells in the population exposed to DNA that are not competent and, hence, do not react, one finds that the frequency of doubly-transformed cells is what would be expected if the transfer of the agents affecting the respective characters occurred independently of each other (Hotchkiss and Marmur, 1954):

$$\frac{\text{no. a+ b+ transformants}}{\text{assumed no. competent bacteria}} = \frac{\text{total no. a+ transformants}}{\text{assumed no. competent bacteria}} \times \frac{\text{total no. b+ transformants}}{\text{assumed no. competent bacteria}}$$

Somewhat fewer double transformants than expected are generally observed when the concentration of transforming DNA saturates the competent bacteria; under these conditions, competition between DNA molecules for entry into the same bacterium (Hotchkiss, 1954) lowers the frequency of transformation by two or more separate factors. Indeed, such deviations in the direction below expectation may be taken as a criterion of independent transfer.

Such evidence for independent transfer indicates either: (1) that the bacterial genome is divided up into DNA elements, existing as separate entities in the cell and liberated in their separate forms whenever a cell

is extracted of DNA; or (2) the process of extracting and purifying DNA breaks up an organelle consisting of an organized array of different DNA molecules into separate DNA molecules; or (3) the process of incorporation and/or integration of DNA by a recipient bacterium fragments the infecting material such that the resulting fragments are integrated at random, some being excluded; or (4) some combination of the preceding mechanisms obtains.

Neither cytological observations nor *a priori* genetic considerations support the first-mentioned mechanism (1). Like other bacteria, transformable strains possess discrete Feulgen-positive bodies (Robinow bodies or chromatinic structures) which contain apparently all of the DNA of the cell. The number of these bodies per bacterium may vary from one to four, or even more, depending on the stage of growth. In any event, there is a clonal regularity of transmission of these bodies synchronous with the division of the bacteria (for review, see Hayes, 1960). A diffuse, disordered distribution of DNA is not supported by the cytological evidence, nor does it seem likely on genetic grounds. Bacteria reveal the same genetic stability as do other organisms, and it is not easy to see how they could transmit hereditary factors in a regular fashion from generation to generation if these determinants were not assembled in some organelle (or organelles). This argument leaves us with mechanisms (2) and (3), or a combination of these two. The physicochemical heterogeneity within a DNA preparation from a given source (see Section II,C,7 above) indicates that (2) possibly obtains. However, as will be seen from considerations of linked factors, each molecule of DNA undoubtedly carries several pieces of genetic information, but not all of this information is necessarily integrated by a recipient genome. Thus, in a sense, a fragmenting and a fragment-selecting mechanism do operate within the recipient. On the one hand, therefore, there appear to be separate DNA molecules of different genetic specificity in the transforming preparation obtained from a given clone. Determinants borne by different molecules may be presumed to be transferred independently of each other. On the other hand, different pieces of genetic information reside in a given molecule of DNA, and these pieces can be separated from each other during a process of recombination within the recipient organism. Determinants borne by the same molecule of DNA are often transferred in a "linked" fashion (i.e., not independently), and whether any determinants on the same molecule may be transferred independently is not known.

C. Linkage of Determinants Affecting Different Characters

The first report that a pair of genetic markers present in a given transforming preparation were transferred together at a frequency

considerably higher than that expected for randomly transferred agents was that of Hotchkiss and Marmur (1954). The markers involved were in pneumococcus; one conferred resistance to streptomycin and the other conferred the ability to utilize mannitol as an energy source. When DNA containing both of these markers (*str-r, mann+*) was added for as short a period of time as 5 minutes to a streptomycin-sensitive, mannitol-nonutilizing (*str-s, mann—*) population, the number of double transformants (*str-r, mann+*) was often about one-fourth the total number of *mann+* transformants produced and about one-twentieth the number of *str-r* transformants produced. The number of double transformants produced, moreover, was often as high as fifteen times the number expected on the assumption that they arose by successive or simultaneous random transformations. If, on the other hand, a mixture is made of two DNAs, each containing one of the selective markers (DNA *str-s, mann+* plus DNA *str-r, mann—*) and this mixture added to an *str-s, mann—* population, the frequency of *str-r, mann+* transformants is what would be expected if the markers *str-r* and *mann+* in this case were borne by different molecules. Hence, no special susceptibility of the recipient cells to the *str-r* and *mann+* agents can be invoked. Furthermore, no selective aggregation of the *str-r* and *mann+* factors can be invoked because the DNA prepared from a mixed culture of *str-s, mann+* and *str-r, mann—* cells behaved like a simple mixture of DNA from *str-s, mann+* cells with DNA from *str-r, mann—* cells. Support of the hypothesis of linkage between the determinants for mannitol utilization and streptomycin resistance comes from the fact that if one transforms *str-r, mann—* cells with DNA containing the markers *str-s, mann+*, and then selects for *mann+* transformants, a high proportion of the latter are *str-s*. Similarly, if one transforms *str-s, mann+* cells with DNA containing the markers *str-r, mann—*, and then selects for *str-r* transformants, a high proportion of the latter are *mann—*. The proportions of double transformants obtained in this way are similar to those obtained when both selective markers are in the DNA preparation. The degree of linkage of *str* to *mann* was found to be independent of the degree of purification of the DNA preparation (Hotchkiss, 1956) and of the concentration of DNA used (Hotchkiss, unpublished). It was also independent of the origin of the donor strain; thus, the same degree of linkage was observed between *mann+* and *str-r* if the DNA came from a strain that had acquired its streptomycin-resistant character by spontaneous mutation or by transformation.

All of these findings (Hotchkiss and Marmur, 1954; Hotchkiss, 1956) lead to the conclusion that two different genetic determinants may be borne by the same molecule of DNA and that the positions they occupy

on this molecule are fixed, both in extracellular transforming DNA and in the DNA of a cell's genome. The degree of linkage (given by the proportion of double to single transformants) may be regarded as representing the distance between the "loci" of these determinants, for two possibilities exist to account for this proportion. One possibility is that the molecules bearing these markers are fragmented in the course of preparing the DNA, and that the closer the markers are to each other (assuming random fragmentation), the higher the recovery of fragments continuing to bear both markers and hence the higher the frequency of double transformants induced by the preparation. The other possibility is that there is homogeneity of the molecules bearing these two markers in a given DNA preparation, but that the possibility of irreversible incorporation and/or genetic integration of both markers by the recipient bacterium is a function of the distance between them on the molecule that bears them, the shorter the distance, the higher the probability. Since DNA preparations subjected to different extents and types of purification result in the same proportion of linked transfers (Hotchkiss, 1956), it seems unlikely that the former possibility obtains.

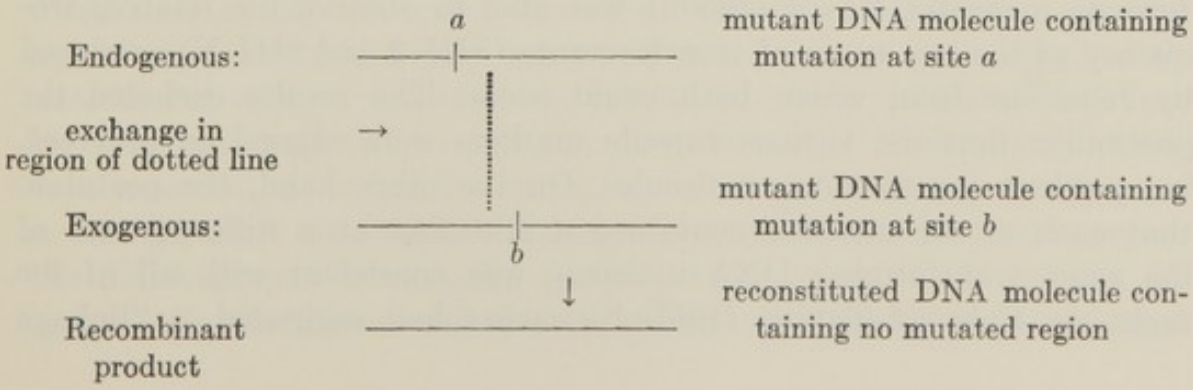
Subsequently, a number of instances have been found of linkage between genetic determinants in transforming DNA. Hotchkiss and Marmur (1954) reported a lesser degree of linkage between the *str-r* factor and a factor (*sulfa*+) conferring resistance to sulfanilamide, than was observed between *str-r* and *mann*+. In *Hemophilus* factors determining cathomycin resistance and streptomycin resistance are linked (Goodgal and Herriott, 1957b). Spizizen (1959) reported linkage of the factors determining indole independence and sucrose synthesis, as well as very close linkage between the factors for β-galactosidase synthesis and sucrose synthesis. Nester and Lederberg (1961) have also furnished strong evidence for linkage between the factor determining indole independence and one of the several known factors determining histidine independence in *B. subtilis*. All cases of reported linkage must, however, be supported by the same evidence as has been marshaled for the case of streptomycin resistance and mannitol utilization in pneumococcus: (1) linked transfer of factors when present in the same DNA preparation; independent transfer from a mixture of two DNA preparations, each contributing one of the factors; (2) degree of linkage independent of DNA concentration; (3) linked transfer regardless of the factorial combination (i.e., a+b− or a−b− or a−b+ or a+b+) insofar as this can be determined (for example, the transfer of a−b− would not be readily detectable if there were no means of selecting this class). Ravin (1956) reported the possible linkage of the Type III capsule determinant and a factor for streptomycin resistance. However, in subsequent work (Ravin, 1959a), the frequency of double relative to

single transformations was found to diminish rapidly as the DNA concentration was reduced, indicating that the incompetence of a large fraction of the treated population was the cause of the high frequency of double transformants at saturating DNA concentrations.

In the cases of linked factors cited above, the characters they determine do not appear to be in any way related biochemically. Furthermore, they undergo mutation independently of each other. Thus, there is good reason to suppose that factors affecting quite different biochemical reactions may be borne by the same molecule of DNA.

D. Linkage of Determinants Affecting the Same Character

Chronologically, the first evidence that a given molecule of DNA could bear more than one piece of genetic information was provided in 1951 by Ephrussi-Taylor. She obtained several mutants of independent origin that were defective in their ability to synthesize Type III capsular polysaccharide (Taylor, 1949b). When one of these mutants was exposed to the DNA extracted from another mutant, transformants restored in their ability to synthesize the capsule were observed (Ephrussi-Taylor, 1951a). (This finding was not observed when a mutant was exposed to DNA extracted from cells of the same mutant clone.) The evidence favored the view that the various capsule-deficient mutants had undergone mutations at different sites of the same molecule of transforming DNA, namely, the molecule bearing the information for Type III polysaccharide synthesis. When this molecule containing mutation *a*, for example, enters a recipient bacterium whose homologous DNA molecule bears mutation *b*, an interaction can occur between the endogenous and exogenous molecules leading to the restitution of a molecule containing neither mutation, and hence capable of directing capsule synthesis. The nature of the reaction was hypothesized to be something like "crossing-over" within complex gene loci (Ephrussi-Taylor, 1951a,b), in which the exogenous DNA first had to synapse in a fairly homologous point-to-point fashion with its endogenous homolog, as shown in the diagram.



Probably by the same kind of mechanism, the genome necessary for normal Type VIII (or Type II) synthesis can be reconstituted when a capsule-deficient mutant of Type VIII (or Type II) is treated with the DNA of an independently derived mutant of that Type (MacLeod and Krauss, 1956; Jackson *et al.*, 1959). A similar phenomenon, apparently having a similar basis, was reported by Leidy *et al.* (1953) in the acquisition of mixed capsule types in *Hemophilus*. Although not as much study was carried out in this case, the evidence indicated that genetic markers affecting different capsular antigens could be recombined onto a single DNA molecule.

Confirmation and extension of the recombination hypothesis was provided by Ravin (1954, 1959a, 1960b). One of the Type III capsule-deficient mutants (SIII-2) produced a sufficient amount of capsule to make it distinguishable by colonial morphology from other capsule-deficient mutants (R or SIII-1), but it was still morphologically distinct from the normal encapsulated Type III strain ("wild type" or SIII-N; Taylor, 1949b). From the SIII-2 mutant strain, highly deficient strains (R) could be obtained by secondary mutation (Ravin, 1959a). The DNA from SIII-2 cells could transform highly deficient mutants into transformants capable of producing greater quantities of capsular polysaccharide. The kind of transformants produced depended on the genetic origin of the recipient: recipients having arisen by mutation from wild type SIII-N yielded two transformant types, SIII-2 and SIII-N; recipients having arisen by mutation from SIII-2 cells yielded only SIII-2 cells. Furthermore, the DNA from SIII-N cells could also transform highly deficient mutants into transformants capable of producing greater quantities of capsule, and again the types of transformants depended on the genetic origin of the recipient: recipients having arisen by mutation from SIII-N yielded SIII-N transformants only; recipients having arisen from SIII-2 cells yielded both SIII-2 and SIII-N transformants.

By transferring small numbers of his briefly treated recipient populations into tubes such that the probability was small of inoculating into a given tube more than one cell contacted by a given species of transforming molecule, Ravin (1960b) was able to observe the relative frequency of the two types of transformants (SIII-2 and SIII-N) produced by reactions from which both could occur. The results excluded the possibility that the various capsule markers were carried on different, independent transforming molecules. On the other hand, the postulate that each of the mutants contained a mutation at a different site of the same transforming DNA molecule was consistent with all of the facts. As Ephrussi-Taylor (1951a,b) earlier had suggested, a "linkage

map" could be constructed based on the relative ease with which certain recombinant molecules could be constructed following certain "crosses" (given recipient strains tested with given DNA preparations). A double mutant R1, which contained an R marker and the SIII-2 marker, rarely produced SIII-N cells in a "cross" with SIII-1, although SIII-2 transformants were frequent. With another double mutant of this kind, R11, both SIII-2 and SIII-N transformants were frequently produced in a "cross" with SIII-1. These facts suggested the order of markers R1–SIII-1–SIII-2–R11. It was found that at least nine different mutations of the Type III capsule character were linked to the same molecule of transforming DNA. One mutation (R6R) appeared to involve a segment

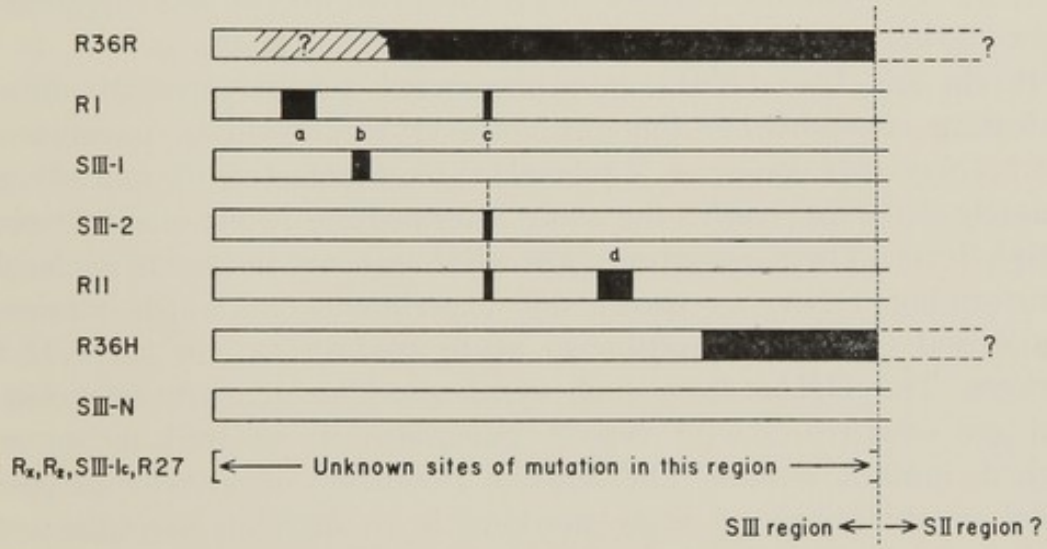


FIG. 5. The genetic complexity of a single molecule of DNA. A representation of the sites capable of undergoing mutation and resulting in impaired synthesis of Type III capsular polysaccharide in pneumococcus. (Taken from Ravin, 1960b.)

of the molecule large enough to include several other mutant sites, in that wild type capsule synthesizing ability could not be restored in crosses with this mutation. Such a mutation will be referred to as a multi-site mutation. An idea of the complexity of the molecule is indicated in Fig. 5.

Because of the relative difficulty of determining quantitatively the frequencies of capsule transformations, involving as they do a morphological character which does not permit the selective "screening" of the transformed classes, workers have turned to a study of mutations affecting drug and antibiotic resistance. Hotchkiss and Evans (1957, 1958) have reported an interesting case of a mutation, which apparently arose in a single step, conferring resistance to sulfanilamide in pneumococcus. The DNA prepared from the mutant strain, capable of resisting 800 μg/ml sulfanilamide, could transfer several discrete levels of resistance

to sensitive pneumococci. The levels of resistance included 800 μg/ml, which, however, was transferred least frequently; more frequently transferred were the abilities to resist 15, 20, 70, 80, 300, or 400 μg/ml of sulfanilamide. By isolating transformants of each type, extracting DNAs from them, and performing all possible "crosses," Hotchkiss and Evans were able to show that three separate factors, *a*, *d*, and *b*, had been present in the original mutant strain; that each factor was capable of conferring a specific level of resistance; and that the factors were "linked" but could recombine in all possible combinations to give the 7 levels of resistance observed. From the relative frequencies of the single, double, and triple transformations (i.e., involving the transfer of 1, 2, or 3 factors, respectively) the order *a-d-b* was suggested for these markers on the molecule of DNA bearing them.

Ravin and Iyer (1961) have discovered five independent mutations conferring resistance to the antibiotic erythromycin in pneumococcus. One marker (*r6*) confers a low level; three others (*r2*, *r3*, and *r5*), genotypically different, confer the same intermediate level; one (*r7*) confers a high level. These mutations are all borne on the same molecule of transforming DNA, as shown by experiments in which strains are synthesized by transformation so as to contain two or three of these markers. The DNAs from such synthesized strains are extracted and used on sensitive ("wild type") pneumococci, as well as on singly marked, mutant strains. The relative frequency of transfer of pairs or triplet combinations of these markers is so high as to indicate their linkage. Precise localization of these markers in relation to each other on the DNA molecule bearing them has not been completed. Interesting interactions appear to occur between certain combinations of these markers. Thus, *r2* and *r3* in combination confer about forty times as much resistance as either one alone. On the other hand, *r5* in combination with either *r2* or *r3* results in no increased level of resistance, whereas *r5* in combination with both *r2* and *r3* confers a lower level of resistance than that conferred by the combination of *r2* and *r3* alone.

Streptomycin resistance in pneumococcus, arising by a single step of spontaneous mutation, may result in any one of a wide range of levels of resistance (Schaeffer, 1956b; Bryan, 1961). Schaeffer (1956b) discovered four mutations conferring resistance to streptomycin; three of them appeared to be allelic, in the sense that transformation of one mutant strain by the DNA from another always resulted in the replacement of the endogenous marker by the exogenous one. The fourth mutation appeared to be complex, in that sensitive cells transformed by DNA from the strain carrying this mutation were always resistant to a lower level of streptomycin than that resisted by the donor strain

itself. To raise the level of resistance to that of the donor strain required a second round of treatment with the same DNA. Bryan (1961) isolated twenty-five independent mutations conferring streptomycin resistance in pneumococcus. The levels of resistance they conferred were spread over a wide range from 150 to 15,000 μg/ml. The DNA from any one of the mutants conferred the specific level of resistance possessed by the donor strain. Bryan found that two of the mutations could be combined by transformation in a single strain, and that these two markers exhibited linkage when the DNA from the doubly mutant strain was tested on a sensitive recipient. A third streptomycin-resistance marker, originally isolated by Ravin (1956), which conferred a higher level of resistance, was found by Rotheim (see Bryan, 1961) to be allelic to each of the aforementioned mutations, in that it replaced both of them. This is another instance of a multi-site mutation. Rotheim (personal communication) has continued the analysis of Bryan's mutants, as well as those of Schaeffer and Ravin. It is her conclusion that all the mutations that can be adequately studied (11 to date) are localizable on the same molecule of transforming DNA. This adds further evidence to the genetic complexity of a single DNA molecule.

Hotchkiss (1951, 1952) observed an interesting correlation between mutation and transformation to penicillin resistance in pneumococcus. The variety of independent mutations that arise by a single step from an originally sensitive strain all confer approximately the same level of resistance to penicillin. However, higher levels of resistance can be achieved by a series of steps in which secondary (tertiary, quaternary, etc.) mutations are selected. The DNA from a multiple mutant produced at the end of such a series of selections confers in one step only the low level of resistance characteristic of the initial mutant obtained in the series. Higher levels of resistance can be achieved by second, third, etc. rounds of transformation with this DNA. This finding indicates that apparently independent factors influence the level of penicillin resistance, and has been taken as support of Demerec's (1945, 1948) hypothesis that penicillin resistance is under the control of polygenes. However, Bryan (1961) studied in great detail a similar case affecting streptomycin resistance in pneumococcus. She found that in cultures of one of her resistant mutants, mutants capable of resisting a higher level regularly arose by spontaneous mutation. By appropriate transformation experiments, these secondary mutants were shown, however, to contain DNA containing two independent mutant markers: one was the mutation conferring the original low level of resistance, the other was an enhancing modifier, which conferred no resistance by itself, but raised the level of resistance conferred by the original mutation.

Thus, the evidence of Bryan (1961) and Rotheim (personal communication) supports the conclusion that all mutations conferring, per se, specific levels of streptomycin resistance are borne by the same species of DNA molecule, although independent modifiers may enhance the phenotypic activity of any or all of these mutations. It remains to be seen whether enhancing modifiers account for multi-step resistance in cases of resistance to other antibiotics.

Lacks and Hotchkiss (1960) have found eight linked mutations, each of which makes pneumococcus unable to synthesize the enzyme maltase. Their results indicate that the order of these factors on the molecule that bears them can be determined from the frequencies with which they recombine in "crosses" to yield wild type molecules. Certain mutations, however, appeared to occupy a segment of the molecule large enough to include several smaller mutant sites (i.e., behaved as multi-site mutations), in that "crosses" between one of these mutations failed to yield wild type recombinants with a particular group of other mutations. There was a good correlation between the size of the marker as determined genetically and the sensitivity to heat denaturation of the segment in the wild type molecule corresponding to that occupied by the marker (the smaller the segment, the less the sensitivity). One striking result, however, was the difference in frequencies of recombination obtained in reciprocal crosses. In a cross, say, between mutant x and mutant y, the frequency of wild type recombinations depended on whether mutation x was in the donor or in the recipient strain. Ephrussi-Taylor (1960b) has analyzed these results, and has proposed a specific model of recombination between DNA molecules according to which the relative lengths of two linked mutations, as well as the distance separating them, determines the frequency of recombination between them. Her model accounts for the absence of symmetry in the crosses between the maltase mutants.

Ephrati-Elizur, Srinavasan, and Zamenhof (1961) have recently been able to map in a linear order several linked mutations affecting histidine synthesis in *B. subtilis*. In many respects, their findings confirm the concept of the fine structure of transforming DNA obtained with pneumococcus.

Reversions of antibiotic-resistant mutants to antibiotic sensitivity are accompanied by corresponding changes in the transforming activity of their DNAs. Ravin (1959b) showed that several reversions in erythromycin-resistant strains of pneumococci were "back mutations," that is, involved the same segment of the transforming DNA molecule as that of the original mutation conferring resistance. This was shown in three ways: (a) by treating "wild type" sensitive strains with the

DNA of reverted strains, (b) by treating a reverted strain with the DNA of the "wild type" sensitive strain, and (c) by treating a reverted strain with the DNA of a different reverted strain. Since erythromycin resistance was not reconstituted, even at very low frequency (i.e., just above the spontaneous frequency of mutation to erythromycin resistance), by any of these methods, the reversions were regarded as "back mutations." Bryan (1961) observed a similar "back mutation" in one of her streptomycin-resistant strains.

A few comments should be made at this point regarding the nature of multi-site mutations. Several cases have been reported in pneumococcus (Ravin, 1960b; Lacks and Hotchkiss, 1960; Bryan, 1961) in which a given mutation extends over a considerable region of the transforming molecule such that it is allelic for a number of other mutations, recombinable with each other, that lie in this region. Such multi-site mutations have also been found in bacteria that undergo other types of genetic transfer than transformation, and in these cases multi-site mutations have been regarded as the equivalent of chromosomal deletions in higher organisms (Demerec, 1956). The question arises, however, as to what a deletion represents in a molecule of transforming DNA. Since the extent of purification does not appear to alter the ability of a transferred multi-site mutation (*str-r1*) to replace a complex of alleles (*str-r2-r3*) in the recipient genome (Rotheim, unpublished), the possibility is remote that a protein backbone is responsible for keeping intact the molecule of DNA possessing a physical deletion. Thus, if the *str-r1* multi-site mutation is a physical deletion of DNA, the molecule bearing this deletion should be shorter than its undeleted homolog. Since it is known (see Section IV above) that the main effect of reduced molecular weight is reduced ability to penetrate the recipient organism, one should expect reduced penetrability in the case of a multi-site mutation. Studies of the comparative penetrability of the apparently single-site *ery-r2* and the multi-site *str-r1* mutations reveal no difference between them (Green, 1959). Thus, one is led to the possibility that a multi-site mutation represents an alteration of the composition of a DNA molecule over a region long enough to include several shorter mutated sites. Further studies of multi-site mutations in transforming DNA should prove helpful in elucidating this problem.

E. Intramolecular Recombination and Genetic Integration

Recombination is any process in which two parents differing by more than one genetic character give rise to progeny possessing a new combination of the parental characters (e.g., parent $ab \times$ parent $AB \rightarrow$ progeny $aB +$ progeny Ab). Obviously, recombination occurs in every

bacterial transformation in which only a few of the several genetic markers[5] of a DNA preparation are transferred from donor to recipient. Thus, an *Ab* transformant resulting from a "cross" between an *AB* donor and an *ab* recipient is the product of genetic recombination. When the markers involved, however, are borne on independently transferred DNA molecules, one may speak of intermolecular recombinations. When the markers involved, on the other hand, are borne by the same molecule of transforming DNA, one must conceive of intramolecular recombinations. Indeed, all transformations may involve intramolecular recombinations, since a given marker probably occupies only a relatively small length of the molecule that bears it, and the genetic integration of this marker requires only recombination away from the rest of the molecule. Such partial integration of DNA transforming molecules may very well be the rule, rather than the exception, since if it occurs when one is dealing with a multiply marked molecule (as in the case of a molecule bearing two or more mutated sites), it probably occurs even when there is but one known mutant marker on the molecule. It is obvious, nevertheless, that one can only measure intramolecular recombinations when a multiply marked DNA molecule is employed.

What is the nature of an intramolecular recombination? What is the relation of such an event to "crossing-over" in higher organisms? Of this one can say little at the present time, and current views on the mechanisms involved will be discussed in Section VI. Here it must suffice to present a factual account of that stage of the transformation process in which the integration of transferred genetic information takes place.

It has been known for some time that genetic integration of a transforming marker may occur within the first two generations following incorporation of DNA. Hotchkiss (1956) plated competent pneumococci exposed to DNA containing a streptomycin-resistance marker on agar devoid of antibiotic. This plating was carried out after allowing a given number of cell divisions to proceed after exposure to DNA. Colonies on the plain agar plate were tested as to whether or not they contained any streptomycin-resistant organisms by transferring samples by the velvet-replica technique (Lederberg and Lederberg, 1952). The proportion of resistant organisms in the colonies could then be determined by isolating such colonies on the plain agar plate as had been revealed to contain any resistant bacteria, suspending them in liquid medium, and plating aliquots in streptomycin agar and in plain agar. It was found that a very small proportion of the clones descending from infected bacteria were pure in respect to streptomycin resistance. Not only did the vast majority of these clones contain both sensitive and resistant bacteria,

[5] Markers are here taken as genetic factors that distinguish the donor from the recipient strain.

but most of the clones produced by bacteria resulting from the second division after infection were also mixed, although the proportion of sensitive bacteria per mixed clone was smaller. These findings were taken to mean that genetic integration may occur early, but in a significant fraction of infected bacteria integration may be delayed for three or more generations. That is to say, in the latter cases, the infecting DNA marker is effectively diluted out in the clone descending from the original recipient bacterium. Ravin (1958, 1960b) has obtained similar results in measuring the frequency of erythromycin- and streptomycin-resistant transformants produced in tubes inoculated with no more than one cell infected by the marker in question. His results were in accord with the assumption that many integrations occur early, before the second generation, but that other integrations occurred later, some as late as the seventh generation after infection. In Ravin's results there appeared to be no preferred generation of integration, but as generations elapse after infection, the fraction of infected cells that have not yet integrated their newly acquired marker diminishes rapidly. These results are important to bear in mind when considering other experiments performed with populations of infected cells. It has already been mentioned in Section IV,A that Fox and Hotchkiss (1960) have found that, fairly soon after penetration, replication of a newly introduced marker proceeds at a rate identical to that of the host genetic material. These results were obtained by isolating the DNA of a population of infected cells at various times after fixation and penetration of the transforming DNA. Thus, under the conditions prevailing in these experiments, if only a small fraction (25% or less) of the infected cells did not integrate the introduced marker by the second division after infection, the increase in the activity of the introduced marker would not be significantly different from that actually observed.

Ephrussi-Taylor (1958) determined the time at which there was a significant increase in the number of colony-forming pneumococcal units replicating the streptomycin-resistance markers with which they were infected. This was done by plating pneumococci exposed to a DNA containing a streptomycin-resistance agent, first in an agar medium devoid of streptomycin, then allowing sufficient time (2 hours) for development of resistance in the cells infected with the agent before overlayering with a similar agar medium containing streptomycin. The number of colony-forming units containing the introduced marker itself or a copy of the marker was found to increase at 90 minutes. Correcting for the average number of cells per colony-forming unit (three cocci per colony-forming unit), it was determined that after 55 minutes, i.e., by about the second generation following infection, replication of the marker had begun in most of the infected clones. The quantitative (al-

though not qualitative) disagreement with other results (Hotchkiss, 1956; Ravin, 1960b) may simply be due to the fact that, under different conditions of growth following infection, the time at which integration occurs in the average infected cell may vary. As will be seen, there is good evidence for such variability. Ephrussi-Taylor also learned that cells that had incorporated DNA in such a way as to shelter it from the action of DNase were nevertheless capable of being "cured" of the infected DNA. In other words, the transformation process could be blocked at the stage following penetration. The inhibition is brought about by treating the cells which had "fixed" their transforming DNA (making it resistant to DNase) with chloramphenicol. Cells so treated are thrown into metabolic imbalance, which may ensue in death. For a given exposure to chloramphenicol, however, a certain percentage of the surviving cells are "cured" of their transforming DNA, in that they no longer will give rise to a transformed progeny. It could be learned, by adding chloramphenicol at various times after DNA fixation, at what time transformation can no longer be reversed by this inhibitor. This analysis was aided by the fact that the cells were dividing synchronously. It turns out that different fractions of the infected population become insensitive to the "curing" action of chloramphenicol at different times, corresponding roughly to the times at which divisions occur in the population. Thus, a certain percentage of the infected cells is no longer curable after division 1, a certain percentage of the infected cells is no longer curable after division 2, etc. Ephrussi-Taylor then compared, in a series of experiments, the fraction of the population no longer curable after division 1 with the fraction of the population which replicates the marker at division 2. While there was considerable variability in these values from one experiment to the next, in any one experiment the two fractions were the same. This finding suggests quite strongly that the event which causes insensitivity to the "curing" action of chloramphenicol leads directly to replication of the infecting marker at the subsequent bacterial fission. This event has a certain probability of occurring by the first division; if it does not occur by the first division, it may by the second or some subsequent division. Since no detectable replication of the marker occurs at the first division, even though an appreciable proportion of infected cells have undergone the "irreversible" event,[6] one must suppose that the specificity of the introduced marker is not copied during the "irreversible" event. If it were, either the copy or the template would have to

[6] The "irreversible" event here mentioned refers simply to the event that shelters the transforming marker from the "curing" action of chloramphenicol. It is different from the "irreversible" event involved in DNA fixation to the recipient bacterium, which shelters the marker from the action of DNase.

be destroyed. It is conceivable, on the other hand, that the marker is directly integrated into a nascent genome, and that this recombinant genome replicates for the first time as such in the next division of the cell.

In this regard, the work of Ravin (1954, 1960b) and of Hotchkiss (1956) is especially relevant. Ravin found that, when a given molecule of transforming DNA bears two markers separable by recombination, the event which selects the length of the genome to be integrated is a single one. In other words, consistent with Ephrussi-Taylor's studies with chloramphenicol inhibition, no copying of the introduced marker appears to occur prior to genetic integration. If such copying did occur, all copies but the one eventually integrated into a bacterial genome must be destroyed. (This eventuality should not be overlooked, as there may be a brief interval during the cycle of growth and reproduction of a given bacterium when its endogenous DNase is free to attack whatever DNA is not protected within some organelle such as a chromosome.) Similarly, Hotchkiss found that when a pneumococcus is infected by a molecule bearing the linked *mann*+ and *str-r* factors, either factor, or both, is eventually integrated in the clone descending from a given recipient, but in no case is a clone mixed, containing some cells with one marker and other cells with the second marker. The only process of recombination likely to give such results is one in which some segment of the transforming DNA molecule, not always the same in different recipients, is substituted for a homologous segment in a corresponding DNA molecule. Replication of the substituted factor would not occur until the following cell division.

Fox (1960) and Voll and Goodgal (1961) have devised excellent systems for studying the relation of recombination to replication. Advantage is taken of the linkage of certain markers. In *Hemophilus influenzae,* for example, the markers for cathomycin resistance and streptomycin resistance are linked. By briefly exposing cells bearing one of the markers to DNA bearing the other marker, the recipient cells can then be lysed and assayed for DNA containing linked transforming activity. The results of Voll and Goodgal (1961) show that by 15 minutes (about half the generation time) after penetration of the DNA, half the maximum amount of recombination occurs between donor and recipient DNA.

The amount of time elapsing before half of the ultimate linked biological activity is attained, may very well be different, however for different pairs of linked markers. Fox (1960) has shown, for example, that, following very brief exposure of competent pneumococci carrying the *d* marker to transforming DNA bearing the *a* marker, 50% of the ultimate activity of the linked *a-d* (sulfanilamide-resistance) markers

is found in DNA reisolated from the recipients after only 6 minutes of incubation at 37°C. The rate of recovery, however, depends on the temperature at which the infected cells are incubated. Thus, when incubated at 30°C, half of the ultimate linked activity is not attained until 16 minutes. Fox (1960) showed, moreover, that the rate of recovery of linked activity was significantly slower than the rate of recovery of the activity of the single marker introduced into the recipient cells. In other words, two distinct processes follow penetration of transforming DNA. One results in a recovery of the transiently lost biological activity of the introduced marker; this process probably involves the release into the cell of the DNA bound at the time of fixation (Ephrussi-Taylor, 1960c). The other process results in the recombination of the introduced DNA with its endogenous homolog. According to Fox (1960), neither process requires extensive growth of the infected cells, since both processes are completed before there is a 10% increase in the turbidity of the culture. Moreover, concentrations of 5-fluorodeoxyuridine sufficient to inhibit growth fail to inhibit either process. Also the net new synthesis of DNA (as determined by P^{32} incorporation into DNA) in the infected bacteria is less than 5%. Voll and Goodgal (1961) have arrived at a similar conclusion regarding the lack of dependence of recombination on DNA replication in *Hemophilus*.

A summary of the results on integration would be useful now. It seems clear that the event of recombination occurring during transformation precedes replication of the introduced determinant, that it generally involves only a part of the transforming molecule, and that it is nonreciprocal in the sense that whatever portion of the molecule is not used for integration into a particular genome is discarded. In other words, the recovery of reciprocal products (an *aB* for every *Ab* in the cross $AB \times ab$), typical of conventional "crossing-over" in higher organisms, does not occur in intramolecular recombination.

What governs the frequency of recombination involving a particular marker? In part, the physiological state of the recipient cell appears to be important. Ravin (1954) provided evidence that varying environmental conditions alters the probability of integrating a particular marker from a given transforming molecule. Ephrussi-Taylor (1958) found that the fraction of recipient cells replicating the infecting marker at the second division varied with the culture employed. Similarly, Hotchkiss and Evans (1958) showed that the frequency of linked transfer of sulfanilamide-resistance determinants was affected by changes in the physiological state of the culture. Another factor that may play a role is the size of the marker that is integrated. Most workers find (Hotchkiss, 1956, 1957; Goodgal and Herriott, 1957b; Ravin and Iyer, 1961; Bryan, 1961) that the frequency of transformants acquiring two

linked markers is usually less than the frequency of transformants acquiring either one of the linked markers. Similarly, the frequency of transformants acquiring three linked markers is generally less than the frequency of transformants acquiring any pair of them (Hotchkiss, 1957, 1958; Ravin and Iyer, 1961). The probability of integrating a multi-site mutation appears to be less than that of the smaller mutations with which they are allelic (Lacks and Hotchkiss, 1960). These results suggest that the larger the segment integrated, the lower the probability of genetic integration. Consistent with this interpretation are the results of Ravin (1958, 1960b) and of Ephrussi-Taylor (1960a) which indicate that the probability of genetic integration by the second generation depends on the marker studied. Markers more sensitive to ultraviolet light, and hence apparently occupying a larger fraction of the DNA molecules bearing them, are apparently integrated with lower efficiency. Finally, there is good evidence that the regions of the transforming molecule adjacent to the marker in question also influence the probability of integration of the marker. Schaeffer (1956a, 1957b) studied the causes for the lower frequency of transfer of a given marker to recipients of a different *Hemophilus* species than to recipients of the same species as that of the donor. He showed it was not due to any lower probability of the marker penetrating cells of the different species, but rather was due to post-penetration difficulties encountered by the molecule bearing the marker in question. These properties of the molecule were generally removed whenever the marker was recombined into the recipient genome. Schaeffer proposed the plausible hypothesis that a necessary prelude to integration is synapsis involving a "fitting" of the endogenous DNA molecule with its exogenous homolog. Similarity of the structures of these two molecules determine the "goodness of fit." When a marker is recombined into a recipient genome, it is removed from the major part of the molecule bearing it. Thus, any marker of heterospecific origin that succeeds in being integrated will thereafter be relieved of whatever material contributed to the "poorness of fit." Green (1959) proposed a similar hypothesis to account for the depressed frequency of integration of a streptomycin-resistance marker in certain host strains of pneumococcus except that in this case the factor responsible for the lowered frequency of integration appeared to be tightly linked to the streptomycin-resistance factor and recombined away from it infrequently.

F. Phenotypic Expression of the Infecting Determinant

It is obvious that the only way in which the presence of a genetically active agent can be detected is through some influence on the phenotype of the cell containing it. Thus, one cannot directly detect hypothetical

potential transformants that have acquired by infection DNA determinants which have not yet brought about a transformed phenotype. Conversely, if a transformant is detected, it is because phenotypic expression of the transferred marker has occurred. Therefore, it is not easy to determine the relation between phenotypic expression of an infecting determinant, on the one hand, and genetic integration and/or replication of the determinant, on the other hand. If one exposes growing competent bacteria to transforming DNA for a brief period of time (10 minutes or less), destroys unincorporated DNA with DNAse, and then plates at various times afterwards in a medium selective for cells possessing the transformed phenotype, one observes: (a) an initial rapid rise in the number of cells which have at once expressed the new phenotype and become capable of giving rise to a clone of transformed bacteria; (b) a leveling-off of this number, followed by (c) a second rise in this number but at a slower rate equivalent to the rate of growth of the cells in the medium employed. The time at which the leveling-off occurs is taken to be the time at which all cells capable of integrating their newly acquired determinant have, in fact, undergone a phenotypic transformation. This time is around 90 minutes (ca. three generations) for streptomycin-resistance markers in pneumococcus (Hotchkiss, 1954; Green, 1959; Ephrussi-Taylor, 1958), and is about the same for some erythromycin-resistance markers, although the initial lag in expression is somewhat greater for these markers (Green, 1959). Ravin and Iyer (1961) found, however, an erythromycin-resistance marker requiring a considerably longer time for expression.

Fox (1959) has demonstrated that the streptomycin resistance acquired by transformation is expressed in an all-or-none fashion. The number of pneumococci possessing the newly acquired phenotype as a function of time is distributed normally, and this distribution is not affected by the concentration of transforming DNA. The general form of the kinetics of expression does not vary as a function of the challenging concentration of streptomycin used to select the expressed transformants, although the curves of expression are somewhat displaced in time. This time-displacement can be entirely explained by the different rates of killing at the different concentrations of streptomycin, permitting different numbers of survivors per unit time to complete the process of expression. It can be concluded that the change in phenotype induced by the acquisition of a transforming DNA factor occurs abruptly, in less than 3 minutes. The time at which this transition occurs is random, having a mean of 60 minutes. Abe and Mizuno (1959) independently obtained similar results, and arrived at a similar conclusion.

While current techniques do not permit detecting either cells that are replicating phenotypically unexpressed markers or cells possessing phenotypically active but nonreplicating markers, the available results accord with the view that phenotypic expression of a genetic determinant does not require prior integration. Consistent with this idea are the results of Hotchkiss (1956), who infected pneumococci with a streptomycin-resistance marker and then allowed them to undergo two divisions. The resulting progeny were treated with streptomycin briefly, and the survivors were assumed to be cells that had developed streptomycin resistance as a result of the phenotypic activity of their *str-r* determinants. Some of these survivors were found to give rise to a mixed clone of sensitive and resistant cells. It was concluded that phenotypically resistant cells may not have genetically integrated the infecting determinants. Indeed, one would have to conclude that, in pneumococcus, streptomycin resistance is dominant to streptomycin sensitivity. Ephrussi-Taylor (1960a) indicates, however, some complicating features of the streptomycin-resistance character that require some caution in the interpretation of results. Apparently, the first phenotypic effect of the presence of the streptomycin-resistance determinant in the cell is not the acquisition of true indifference to streptomycin but the ability to become indifferent. This latter state is manifested by the ability to survive although not reproduce in the presence of streptomycin, and it arises very early after DNA uptake, in some cells as early as 12 minutes after infection. The ability to reproduce in the presence of the antibiotic does not develop until about 3 hours after DNA uptake. The early state, termed "pseudoresistance" by Ephrussi-Taylor (1960a), probably does not require prior integration of the streptomycin-resistance marker. If this pseudoresistant phenotype is the result of a specific heterocatalytic function of the DNA molecule bearing the resistance marker, then it follows that this function of DNA can be exerted when the marker has not yet been integrated into a bacterial genome and before it is replicated.

VI. Mechanism of Recombination Occurring in Transformation

A. Copy-Choice vs. Breakage-Reunion

There are two classic schemes to account for "crossing-over" between homologous chromosomes in higher organisms. These are:

(1) Breakage-reunion, whereby homologous chromosomes break at homologous points along their respective lengths and reconstitution of the chromosomes occurs by the fusion of originally separate parts [see Fig. 6 (1)];

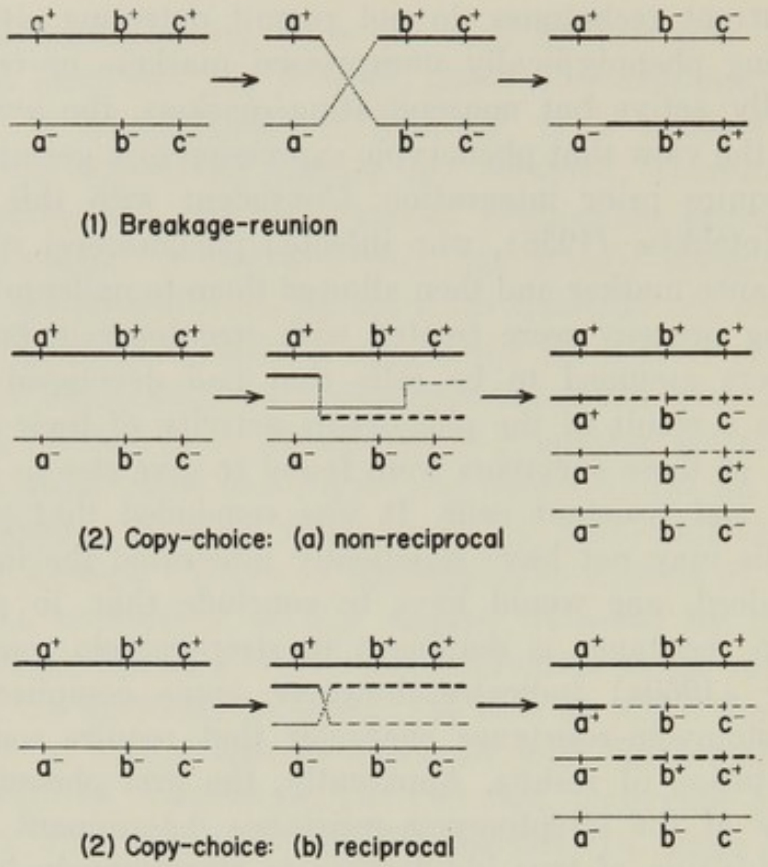


FIG. 6. Two models of genetic recombination. Heavy and light lines may be taken to represent either chromosomes or DNA molecules. If the latter, the lines (heavy and light) can be conceived of, on the Watson-Crick model, as single, noncomplementary strands of homologous molecules. (1) Breakage-reunion: dotted line represents physical exchange between homologs broken at homologous points. (2) Copy-choice: dotted line represents alternation in copying; in (a) there is nonsynchronous copying and alternation of copying; in (b) there is synchronous copying and alternation of copying.

(2) Copy-choice, whereby homologous chromosomes serve as templates, and the newly synthesized replicas are copied alternately from the two templates [see Fig. 6 (2)]. (a) If only one copy is synthesized per pair of templates, a nonreciprocal recombination occurs. Similarly, if one copy is made per template and copies are produced nonsynchronously such that alternation of copying does not occur at homologous points, nonreciprocal recombination may occur. (b) If, on the other hand, one copy is made per template, and copies are produced synchronously such that copying "switches" to alternate templates at homologous points, reciprocal recombinant products are produced.

It will be seen that the breakage-reunion model predicts reciprocal recombination, regardless of whether breakage and reunion occur before or after chromosomal replication (i.e., at the so-called 2-strand or 4-strand stage). Nonreciprocal recombination *could* occur by the copy-choice mechanism, but not obligatorily. If reciprocal recombinants did

occur as a result of the copy-choice mechanism, two parental for every two recombinant products would be observed. Thus, reciprocal recombination via a copy-choice mechanism would be semiconservative (Delbrück and Stent, 1957). "Crossing-over" in organisms more highly evolved than the bacteria is, in general, characterized by reciprocal recombination. It apparently occurs at the so-called four-strand stage when each chromosome consists of two strands, and a "cross-over" at a given point occurs between only a single pair of non-sister strands (Weinstein, 1928, 1936). These conclusions are based, in part, on the recovery of reciprocal recombinants in tetrad analysis, and on the fact that in a given tetrad the two parental types are generally found along with each pair of recombinant types (for review, see Pontecorvo, 1958). However, in microorganisms such as yeasts and molds, recombination analysis within short regions of the chromosome has indicated exceptional situations. While over long segments recombination is similar to "crossing-over" in higher organisms, within very short regions nonreciprocal recombinations are often produced (Mitchell, 1955; Roman, 1956; Case and Giles, 1958). Similarly, the probability of multiple cross-overs as a function of the distance between two sites of a chromosome is very different depending on the order of magnitude of the distance between those sites. The probability of multiple cross-overs decreases proportionally with decreasing distance between the sites (positive interference) until the distance is of the order of one Morgan unit (1% recombination) when the probability of multiple cross-overs suddenly increases (negative interference; Pritchard, 1955; Benzer, 1955; Chase and Doermann, 1958). It is possible, although by no means settled, that two distinct mechanisms of recombination occur: one resulting in reciprocal products and occurring over relatively long distances of the chromosome, and the other capable of giving rise to nonreciprocal products occurring at the microchromosomal, perhaps molecular level (see, however, Pritchard, 1960).

It is hazardous, however, to relate genetic recombination in higher organisms possessing a meiotic cycle with the recombinations observed in bacteria which lack such a cycle. In particular, one is not sure what relation exists, if any, between the recombinations that occur in bacteria as a result of genetic infections (via transformation, transduction, or conjugation) and the recombinations that occur in organisms possessing a conventional mode of sexual reproduction. It has been speculated that recombinations produced in bacteria by infectious agents are similar in mechanism to those occurring within short intervals of the chromosome of higher organisms (Hotchkiss, 1957; Roman and Jacob, 1958). In other words, one would imagine that both are intramolecular recom-

binations, different in mechanism from conventional "crossing-over" at the gross chromosomal level. While there is good reason to believe that the mode (or modes) of genetic recombination has become more complex during the evolution of "higher" forms of life and more complicated patterns of reproduction, it is nonetheless likely that the recombinations between DNA molecules known to occur in the bacteria possess their counterpart, at the molecular level, in the chromosomal interactions known to occur in the higher organisms. In this view, the process of intramolecular recombination has general genetic significance.

Returning to the two models that have been proposed to account for genetic recombination, it is obvious that they may operate either at the gross chromosomal level or at the molecular level. In other words, these two models may be considered to account for intramolecular recombination as well as "crossing-over" between homologous chromosomes. So long as we are ignorant of the manner in which DNA is organized within the chromosome of the "higher" organism, we must consider the two levels of recombination independently of each other and, indeed, accept the possibility that one model may be valid at the gross chromosomal level and invalid at the molecular level. With this caution in mind, let us now examine how the evidence on intramolecular recombination "fits" the proposed models.

The experimental conclusions outlined in Section V,E, bearing on intramolecular recombination as it occurs in transformation, may be summarized briefly:

1. The DNA molecule possesses many pieces of genetic information, but the amount of information actually integrated into the genome of a transformed cell may be but a small fraction of the total.

2. The DNA molecule as a whole is essential for effective contact with the recipient cell, i.e., is essential for the genetic material of transforming DNA to penetrate the recipient.

3. Immediately after fixation to the recipient cell, the biological activity of the transferred genetic material disappears, but before cell division, and apparently without requiring DNA synthesis, this activity completely reappears. Shortly after its reappearance, this activity is found linked to markers resident in the host genome. This linkage also appears not to require DNA synthesis.

4. Replication of the introduced marker does not precede the recombination event. When replication does occur, it proceeds at a rate identical to that of the resident genome. The fraction of infected clones in which replication of the marker commences by the second generation depends on environmental conditions and the physiological state of the cells. In some clones derived from infected cells, replication of the

marker appears to occur after several cell divisions have elapsed. It is possible to obtain conditions, however, in which the major fraction of infected clones have integrated the introduced markers into their genomes by the second generation.

5. Recombination is nonreciprocal, in that in the clone of the recipient cell infected by a doubly marked DNA molecule, one does not find reciprocal recombinant products. Some part of the infecting molecule is utilized, and the clone is homogeneous in respect to the part utilized.

The important conclusion relative to the alternative models of recombination is that intramolecular recombination does not require replication. This conclusion, supported as it is by several lines of evidence described above, would exclude the copy-choice mechanism of recombination. It may be worth postponing the demise of this hypothetical scheme of recombination, however, until the quantitative evidence on the independence of intramolecular recombination and DNA replication is compelling. For the time being, it seems worthwhile to investigate the explanatory possibilities of the breakage-reunion model. As has already been pointed out, this model would predict the recovery of reciprocal recombinant products, but this is not what is observed. It must be recalled, however, that the breakage-reunion model, as originally proposed, was dealing with homologous chromosomes, which were obviously conserved in meiosis regardless of whether recombination had occurred. The same situation may not prevail in the case of transformation processes. If one imagines, for example, that in a given recipient the exogenous DNA molecule is not conserved as such, and that only a part is utilized and the remainder discarded, a revised breakage-reunion model is obtained that is consistent with the experimental findings. In the revised model of breakage-reunion, the exogenous molecule is not conserved as a whole. A portion of this molecule is transferred directly into an existing molecule, by substituting for a homologous segment which is eliminated. One may even suppose that the utilized part of the exogenous DNA molecule is attached directly onto an incomplete copy of the endogenous DNA molecule, rather than being substituted into the latter itself. In this view (a sort of conciliation between the breakage-reunion and copy-choice models), a copy is made from the endogenous DNA molecule as a normal part of the metabolic processes of bacterial growth. This copy-making is not accomplished in one fell swoop, but is accomplished gradually, during which time an incomplete copy may exist transitorily. If an exogenous molecule enters the cell at a time when a partial copy exists, that part or parts which are still lacking in the copy may be "taken" directly from the exogenous mole-

cule. This acquisition of a part of the donor molecule could conceivably occur in the absence, or during the arrest of DNA synthesis. If this were so, it would explain how recombination could be independent of DNA synthesis going on at the time of recombination, although recombination would depend on prior synthesis of partial copies of DNA molecules. In any event, there are no findings in regard to transformation that are inconsistent with some form of the breakage-reunion hypothesis.

On the other hand, some recent evidence directly supports it. Fox (1960) and Voll and Goodgal (1961) find that linkage of two markers, one introduced from the transforming DNA, the other resident in the host genome, is achieved before appreciable DNA replication or cell division occur. In addition, Ephrussi-Taylor (1960a) has reported briefly on interesting findings in this regard. She has been studying in pneumococcus the integration of two unlinked markers, one (*str-r*) conferring resistance to streptomycin, the other (*opt-r*) conferring resistance to optochin. These markers behave differently in a number of respects. The *opt-r* marker is considerably more sensitive to ultraviolet light, and there is about an eightfold lower frequency of *opt-r* transformants than *str-r* transformants produced when a doubly marked DNA is used to infect a doubly sensitive recipient strain. Furthermore, while the percentage of *str-r* markers integrated by the first division after penetration is variable, and often is low, practically all of the *opt-r* markers that are eventually integrated are integrated by the first division. The *opt-r* marker, in other words, is either integrated by the time the first division occurs, or it is not integrated at all. Ephrussi-Taylor (1960a) reports that by blocking division and genetic integration immediately after DNA penetration, it is possible to measure the rate of disappearance of the *opt-r* and *str-r* markers from the population of infected cells. In this way, she found that the *opt-r* marker is at least as stable as the *str-r* marker within the infected cell. This leaves the possibility that the low frequency of integration of the *opt-r* marker and its failure to be integrated after the first division is due to the process of recombination itself. All of the preceding facts would be in harmony with the idea that the *opt-r* marker occupies a larger fraction of the DNA molecule bearing it than does the *str-r* marker, that the event of recombination is preceded by, or directly involves a disorganization of the DNA molecule, that the breaking of the molecule within the region occupied by a marker inactivates it, and that therefore the probability of successful recombination of the *opt-r* marker into a recipient genome is less than that of the *str-r* marker. This hypothesis was tested as follows. Competent pneumococci are treated for 3 minutes with a DNA containing both the *str-r* and *opt-r* markers, and at various

times after exposure to transforming DNA, the DNA of the infected cells is extracted and then assayed on another group of competent cells. It is found that up to three-fourths of the way toward completion of the first fission the activity of the *str-r* marker relative to that of the *opt-r* marker increases significantly, and remains high thereafter. This result is what would be expected if prior to, or concomitant with recombination, the *opt-r* markers were more often inactivated by breakage than the *str-r* markers.

In any event, at the present time, the breakage-reunion model of recombination is in accord with the experimental findings of intramolecular recombination, as it occurs in transformation, while the copy-choice mechanism is not. Whichever of these models, or modifications of them, proves to be valid, a more concrete formulation of intramolecular recombination will still be needed to predict the frequency of recombination between regions on homologous molecules in terms of a number of parameters, such as the sizes of the regions involved, the distances between them, etc. Ephrussi-Taylor (1960b) has furnished an interesting formulation of this kind which is amenable to test. Her formulation is based on the following assumptions: (1) homologous, effective pairing occurs all along the length of the molecules; (2) following recombination, only one of the molecules, that of the recipient, is conserved; (3) every detectable intramolecular recombination involves two (or an even number of) cross-overs between the recombining regions; (4) a cross-over is a "switch" from one molecule to its homolog (either as the result of copy-choice or of breakage-reunion); (5) the initial cross-over, the "point of attack," is a switch from the recipient to the donor molecule, and it occurs at random along the length of the molecule; (6) given a "point of attack," however, the second[7] cross-over, or "point of return" to the recipient molecule, has a fixed probability of occurring; (7) as a consequence of assumption (6), the lengths of donor sequences contained in the recombinant molecule, following attacks starting at a given point, are distributed normally. This formulation implies a nonlinear relationship between the distance separating two regions on homologous molecules and the frequency of recombination between them. It also accounts for the unequal frequencies of recombination observed in reciprocal crosses between maltase mutants of pneumococcus (Lacks and Hotchkiss, 1960). The formulation of Ephrussi-Taylor's should excite further studies on intramolecular recombination, as it occurs in transformation, which may in turn demonstrate a sufficient number of properties of this process as to reveal the underlying molecular mechanisms involved.

[7] Or the final even-numbered cross-over following the "point of attack."

For the physicochemical basis of intramolecular recombination is still far from being understood. An important step forward was made by the presentation of the Watson-Crick model for the structure of the DNA molecule. However, a great deal must yet be done to determine how, or if, such a structure can participate in the reactions which transforming DNA molecules are known to undergo. In the first place, something must be learned of the process of synapsis or pairing, which endogenous and exogenous DNA molecules must presumably undergo if recombination is to occur. It is hard to imagine how, with any efficiency, a segment of an introduced DNA molecule can be substituted for a homologous segment in a corresponding molecule in the cell's genome if synapsis between homologous sites of homologous molecules did not take place. What chemical forces between DNA molecules could account for such alignments? Indeed, what forces could be specific enough, since synapsis between nonhomologous molecules must be minimized? Thomas (1957) has proposed some possible chemical explanations for this phenomenon, and future chemical investigations may be able to test these hypotheses. In this connection, too, one wants to know whether the double-stranded helices of the DNA molecule become "unwound" prior to synapsis, so that synapsis occurs between single (presumably complementary) strands of interacting molecules. Or does synapsis occur between two double-stranded helices? The only current data that are relative to this question are those of Herriott (1961) who finds that he can link two (potentially linkable) markers of *Hemophilus* by heating a mixture of the DNAs containing the respective markers and then cooling slowly. Such a treated mixture can transfer a much greater proportion of the markers together than an untreated mixture of the DNAs, although not with the efficiency of a single DNA preparation containing both markers. If the linkage obtained by heating and slow cooling is due to the rewinding of originally separate complementary strands into a "hybrid" double helix, one strand of which carries the marker contributed by one DNA preparation and the other strand of which carries the marker contributed by the other DNA preparation, two conclusions follow: (1) the genetic information borne by the complementary strands of a double helix is redundant, in that either strand is sufficient for transfer of all the genetic information carried by the molecule as a whole, and (2) synapsis takes place between two double helices since in order to get two linked markers of a hybrid molecule integrated into a recipient's genome, they must be together at the time of recombination. If synapsis occurs between two double-stranded helices, as Herriott's results suggest, then recombination must involve the replacement of a segment of a double helix for a corre-

sponding segment of the homologous double helix. Otherwise, how could the linked markers of a "hybrid" molecule be integrated together? Finally, if this picture is the correct one, how do the two linked markers of a "hybrid" molecule, occurring as they do on complementary strands and involving *ex hypothesi* different, albeit complementary, nucleotide sequences, get to exist at last on the same strand—so that the DNA molecule becomes "homozygous" for a given pair of linked markers rather than "heterozygous"? One can imagine this happening if intramolecular recombination occurred subsequent to DNA replication in

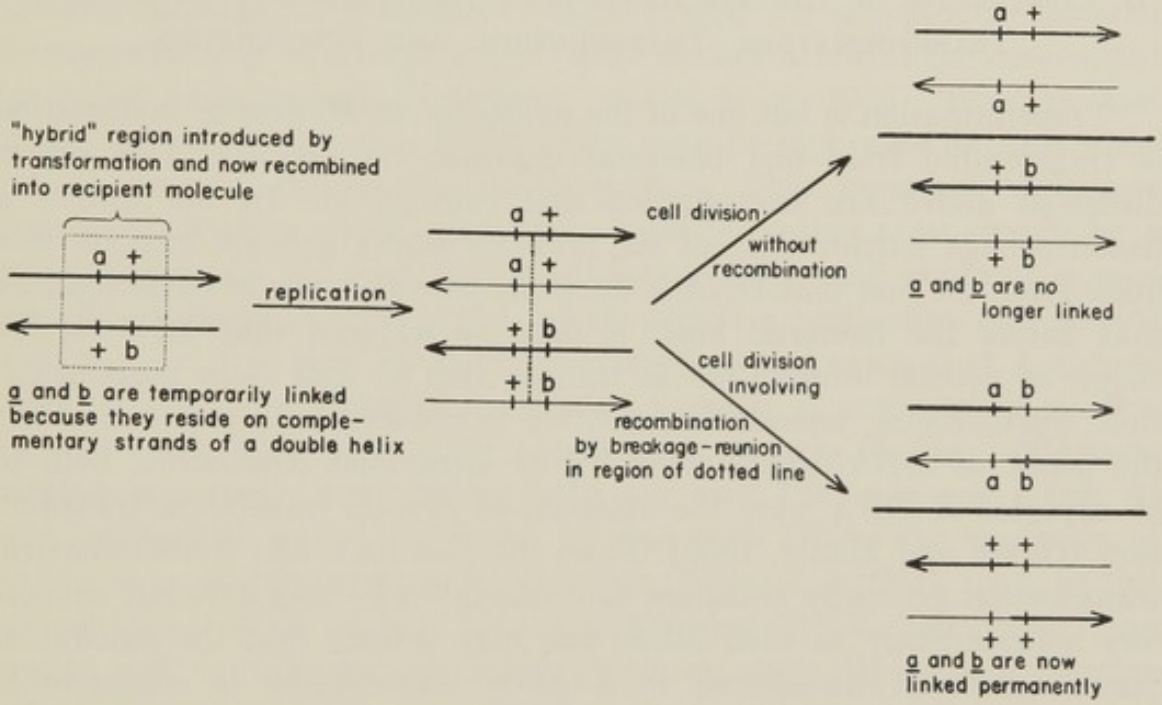


FIG. 7. A possible scheme to account for recombination of linked markers in a hybrid DNA molecule. Arrows pointing in opposite directions indicate the hypothetical complementary strands of a Watson-Crick double helix. Strands indicated by lighter type are strands produced by replication in the manner suggested by Watson-Crick.

the generation immediately following the introduction of the linked markers. This step would be essential if the double helix replicated by first "unwinding" into its constituent complementary strands, as Watson and Crick (1953b) suggested. It is obvious that any delay in the second intramolecular recombination would permanently remove the linkage of the markers (Fig. 7). These speculations, however, face certain difficulties. If intramolecular recombination occurred between double helices as a result of breakage-reunion, and this process were a general feature of cell division, one would expect a dispersion of material between molecules and no conservation of structure, even of a semiconservative kind, would prevail. This prediction does not appear to be borne out by the

elegant studies of DNA replication performed by Meselson and Stahl (1958).

Obviously, further physicochemical studies of transforming DNA are needed now in order to throw light on the structural basis of intramolecular recombination and on the adequacy of the Watson-Crick model. It is safe to say that, with regard to our understanding of the physicochemical nature of DNA replication and recombination, the situation is still fluid.

B. Comparison of the Mechanisms of Recombination Involved in Transformation, Transduction, and Conjugation

Transformation is but one of the modes by which genetic information is transferable from one bacterial organism to another. Two others discussed above are transduction and conjugation. In the former a bacteriophage carries part of its previous host's genome into its new host. From the fact that DNA is the principal substance of bacteriophage that enters the bacterial host, it may be deduced that DNA is the vector of genetic information in transduction as well as in transformation. Furthermore, transducing activity is reduced as a result of either the decay of DNA-incorporated P^{32} or ultraviolet irradiation, both to be expected if DNA were the material of genetic transfer in transduction (Garen and Zinder, 1955). From the fact that, like transformation, transduction generally transfers determinants affecting different characters independently of each other, one may deduce that the amount of genetic material transferred in a given transduction is considerably less than that of the host genome as a whole. In the case of phages (like PLT 22 in *Salmonella* hosts) which may carry any part of the host genome (generalized transduction), one as yet knows little about the mechanism of recombination between the infecting determinant and host genome. In the case of phages (like P1 in *Shigella-Escherichia* hosts or λ in the *Escherichia coli* K12 host) which carry only a specific part (or parts) of the host genome (restrictive transduction), it is now clear that such phages are defective in some one or more "phage" properties and lack a portion of the normal phage genome which has been replaced by a specific portion of the host bacterial genome. The portion of the host genome contained in the phage genome is that which is transduced, and it corresponds to the segment of the bacterial chromosome immediately adjacent to the site to which the phage was attached as a prophage in its previously lysogenized host (for brief review, see Luria, 1959). Unaided by the DNA of normal phages, the DNA of such transducing phages is incapable of vegetative multiplication inside the infected host, although it is still capable of donating the bacterial genes

it carries, presumably by pairing and recombining with the homologous region of the chromosome of the infected host. Although incapable of vegetative multiplication inside the host, the transducing DNA may nevertheless serve as an alternative template in copy-choice replication when synapsed with its endogenous homolog. However, nothing is yet known about the mechanism of recombination in transduction. It is known that transducing DNA may be replicated as such in synchrony with the intact genome of the infected bacterium, so that the descendants of the original recipient may each possess a copy of the transducing DNA in addition to an as-yet-unsubstituted bacterial genome. Recombination between the exogenotic fragment and the endogenous bacterial chromosome may be deferred, and occur in only a few of the descendant (so-called "heterogenotic") progeny (Morse *et al.*, 1956). Thus, at least in the case of transducing DNA, DNA may be replicated as such without necessarily undergoing recombination. This apparent difference from the situation which prevails in transformation may simply be a manifestation of the fact that transducing DNA still contains a considerable number of viral genes, which permit a replication coordinated with that of the host DNA. Furthermore, it is known that a normal viral genome in an infected recipient is capable of autonomous replication. Normal viral DNA, after penetration, triggers its own replication without apparently recombining with any host DNA; in any event, its own replication is not regulated by the host DNA, the synthesis of which, as a matter of fact, is inhibited. Thus, it is clear that, in this regard, viral DNA differs from transforming DNA. The latter may simply not contain enough information to order its own replication independent of its host.

In this regard, it is interesting to consider how recombination occurs between viral DNA molecules during the vegetative period (prior to maturation) of a virulent infection. When two or more related phage particles carrying different genetic markers are allowed to infect the same bacterium, recombinant phage progeny are found in the eventual "burst." The distribution of recombinant types in the "bursts" of bacteria infected by suitably marked parental phage indicates that a mechanism of nonreciprocal recombination is operating (Hershey, 1958). This fact would be in favor of a copy-choice mechanism of recombination, were it known that phage DNA, during the period of vegetative multiplication, remained in unfragmented form. Recently Meselson and Weigle (1961), as well as Kellenberger, Zichichi and Weigle (1961), were able to show, in crosses of bacteriophage suitably labeled with genetic markers and with isotopic nitrogen and carbon, that the DNA of recombinant bacteriophage contains discrete portions of parental phage DNA. Thus, as in transformation, recombination in bacteriophage involves the

breakage of DNA molecules and the subsequent reunion of fragments into complete molecules.

One other mode of genetic transfer, which has already been referred to, is conjugation. In this case, it is known that a very large part of the donor chromosome is transferred into the recipient bacterium. It is presumed that in conjugation, too, DNA bears the genetic information that is transferred, since eventual integration into the recipient genome is highly susceptible to the decay of P^{32} incorporated into donor DNA or to the ultraviolet irradiation of donor cells (Jacob and Wollman, 1958). In conjugation some kind of fragmentation process occurs either concomitant with transfer or subsequent to transfer (Wollman *et al.*, 1956), so that only a fraction of the donor genome is potentially capable of giving rise to recombinants. While many determinants affecting many different characters may enter a recipient cell, not all of them are eventually recombined into the recipient genome. Recombination in the case of conjugation, too, is nonreciprocal, as shown by the analysis of clones descending from recipient bacteria (Anderson and Mazé, 1957; Lederberg, 1957). While this fact would again suggest a copy-choice mechanism of recombination, nothing can be concluded until more is known about the process of fragmentation that occurs in conjugation and how it is related to the events of recombination.

VII. Mechanism of the Heterocatalytic Function of the Transforming Agent

One of the most important questions that has been posed by the development of the science of genetics is the manner in which the gene exerts its control over a specific character of the organism. In higher organisms this problem has been attacked in numerous ways, which have included the determination of the earliest steps affected by the mutant gene in the embryonic development of the organism. This kind of analysis has led to a realization of the pleiotropic effects of a genetically induced physiological disturbance occurring early in ontogeny. It has been hoped that, by studying the nature of the earliest physiological effect manifested by a gene mutation, the primary specific action of the gene would be elucidated. A necessary working assumption for this kind of approach is that there is but a single primary action of the gene, but that this action may entrain, through the interlocking of the complex processes of metabolism, a manifold system of effects. To date there has been no strong evidence presented to warrant a less simple assumption. However, as many authors have pointed out, it is an extremely difficult assumption to disprove, and the main difficulty has been, of course, in deciding the nature of the primary or immediate heterocatalytic activity of the gene.

An understandable approach has been to study the heterocatalytic function of the gene at the molecular level, and this kind of study has led to the general finding that gene mutation results in the alteration of some specific protein of the cell bearing that mutation. The evidence supporting this generalization is too lengthy to go into here (for review, see Beadle, 1945); suffice it to say that where it can be studied a gene mutation is generally accompanied by the absence or modification of a specific protein. It is too soon to conclude that the synthesis of a specific protein is the primary heterocatalytic action of the gene, however usefully it may serve as a simplified working hypothesis. One must be prepared for the possibility that future investigations will reveal intermediate processes occurring between the metabolic reaction in which the gene participates directly and specific protein synthesis.

What is most desirable to possess for the study of the primary action of the gene is a system in which the gene is essentially isolated, transferred into a cell lacking it, and the biochemical consequences of that transfer followed in detail. Most of the desiderata of such a system are fulfilled by the kinds of genetic infections that occur in bacteria. One may follow the biochemical changes ensuing from the transfer of a chromosomal fragment between two conjugating bacteria, or from the transfer of a virus from one bacterial host to another, or from the transfer of a molecule of transforming DNA. Only in the case of the latter process of infection does the transfer involve a less highly organized system than a chromosome, and when the day arrives that the various DNA molecules in the preparation obtained from a given cell-type are separated by chemical fractionation, it will be possible in principle to follow the biochemical consequences of the transfer of a single species of DNA molecule. It is true that the DNA molecule is large and complex, and may carry several genes (defined functionally). Nevertheless, this system represents a very close approach to the desired one for studying gene function.

There have already been some promising results obtained from the study of viruses and transforming DNA. In the case of viruses, the control of viral genes over the synthesis of specific viral proteins has been investigated. Several mutations are known in the bacteriophages T2 and T4 which affect the ability to synthesize proteins of either the so-called "head" or "tail" of the mature bacteriophage coat. These proteins constitute 60 to 70% of the proteins synthesized in the bacterium 12 minutes after phage infection. Brenner and Barnett (1959) are looking for changes in the amino acid sequence in the head protein as a consequence of mutations in a region of the bacteriophage chromosome which appears to control directly the structure of this protein. It is also

known that soon after infection by bacteriophage T2, and well before mature phage particles are formed, enzymes appear in the infected cell catalyzing the synthesis of specific nucleotide precursors of the phage DNA (i.e., nucleotides containing hydroxymethylcytosine instead of cytosine; Kornberg *et al.*, 1959). While it is highly likely that bacteriophage genes induce the appearance of these new enzymes, there is as yet no direct evidence, such as the existence of mappable mutations in the bacteriophage genome leading to altered synthesis of these enzymes.

With transforming DNA, the results of biochemical studies also support the view that the heterocatalytic function of the infecting gene is the ordering of the synthesis of a specific protein. One of the best-studied cases is the transfer of the ability to synthesize the enzyme mannitol phosphate dehydrogenase in pneumococcus (Marmur and Hotchkiss, 1955). Wild type bacteria are incapable of utilizing mannitol (*mann*—) and do not have the enzyme, or very little of it. Mutant bacteria that are genetically capable of utilizing mannitol (*mann*+) are capable of synthesizing mannitol phosphate dehydrogenase, but do so only in the presence of the substrate mannitol. The enzyme, in other words, is "adaptive," or inducible by its substrate, in *mann*+ bacteria. Bacteria transformed into *mann*+ by the appropriate transforming DNA also do not synthesize the enzyme until the substrate is added to the growth medium. The DNA of *mann*+ cells (mutant or transformed) growing in the absence of mannitol, and hence not synthesizing the enzyme, is just as effective in transforming *mann*— bacteria to the *mann*+ condition as the DNA from fully induced cells actively synthesizing the enzyme. This is a beautiful case of the difference between the hereditary capacity donated by a particular gene and the phenotype expressible in a specified environment. The *mann*+ gene, presumably a part of a DNA molecule, confers a genetic ability to synthesize mannitol phosphate dehydrogenase; it is replicated under conditions when the enzyme is not being synthesized; the enzyme is synthesized provided the genetic constitution and the intracellular environment of the cell permit it.

Several other cases of the control of a transforming DNA molecule over specific protein synthesis are known. The ability to synthesize a specific type of capsular polysaccharide (the character transformed in "classic" pneumococcal transformations) has been shown to be due to the ability to synthesize certain enzymes involved in the metabolism of polysaccharide precursors (Austrian and Bernheimer, 1959; Austrian *et al.*, 1959). Capsule-deficient mutants of the normal Type III strain are apparently unable to synthesize uridine pyrophosphoglucose dehydrogenase; synthesis of this enzyme is restored by infection with DNA

from the appropriate strain. Ephrussi-Taylor (1954) described a large colony-forming mutant, the DNA of which was capable of transforming "wild type" cells into the "large colony" type. The latter not only produced large colonies on blood agar, but had a diminished ability to respire in the presence of glucose, failed to oxidize lactic acid, and produced much less H_2O_2 in the course of glucose oxidation than "wild type" pneumococci (the latter property being presumably the cause of the larger colonies, since pneumococci poison themselves by H_2O_2 production). Udaka *et al.* (1959) showed that a lactic acid oxidase, a flavoprotein, is present in "wild type" cells but not in the large-colony-forming mutant; this enzyme catalyzes the oxidation of lactic acid to acetate and CO_2. The oxidation is reduced 50% upon removing H_2O_2 with catalase, under which conditions pyruvate is the end product. Using wild type DNA, Spizizen (1959) has been able to transform to the wild type condition mutant strains of *B. subtilis* incapable of effecting certain specific steps in the synthesis of tryptophan. These steps have been shown to be under the catalytic control of specific enzymes. Although enzyme studies have only just begun in this work, it may be presumed that the transforming DNA of *B. subtilis* imparts a specific protein-synthesizing ability to the cells it infects. Lacks and Hotchkiss (1960) have studied a group of eight linked mutations, each of which results in the absence of an active maltase enzyme in pneumococcus. This enzyme, found in wild type, permits the utilization of maltose by catalyzing the reaction: maltose + $(\text{glucose})_n \rightarrow$ glucose + $(\text{glucose})_{n+1}$.

Again in the case of transformation of *E. coli* requiring the help of defective phage (Kaiser and Hogness, 1960), the infecting DNA is responsible for endowing the recipients with the ability to synthesize at least two enzymes involved in the utilization of galactose: galactokinase and galactose-1-phosphate uridyl transferase. Since these enzymes have been isolated and purified and their action well studied (Kurahashi, 1957), and since the "galactose" segment of the infecting DNA has been well mapped (E. Lederberg, 1960), there is good reason to believe that this system of transformation will be useful in clarifying the relation of DNA structure to protein specificity.

Hotchkiss and Evans (1957, 1958) are investigating an extremely promising case for the elucidation of genetic control over protein specificity. The *a-d-b* group of linked markers for sulfanilamide resistance in pneumococcus appear to be mutations of a region in a DNA molecule which directs the synthesis of a *p*-aminobenzoic acid (PABA)-utilizing enzyme necessary for folic acid production. The reaction catalyzed by the enzyme seems to condense PABA with L-glutamine. Evidence has

been put forward indicating that the different levels of resistance to sulfanilamide characteristic of the markers are due to the characteristic alterations they impart to the substrate affinities of this enzyme. Thus, each marker, or combination of markers, imparts a certain quantitative PABA-binding capacity to the enzyme so that corresponding levels of sulfanilamide, a metabolic analog of PABA, are needed to displace the natural substrate. Furthermore, Hotchkiss and Evans have investigated a number of PABA analogs, varying systematically in structure, to determine their relative efficiency in inhibiting the PABA-utilizing enzyme. In this way, *d*-containing strains were found to be the most highly sensitive to carboxylated analogs of PABA, and the *b* marker was found to confer a high sensitivity to nuclear-substituted analogs. Since the relative order of the markers on the DNA molecule bearing them is known, it is hoped that changes in specific regions of the transforming molecule may be correlated with alterations in structurally specific regions of the enzyme.

From the studies on the transfer of ability to synthesize mannitol phosphate dehydrogenase in pneumococcus, it is clear that the transforming marker may replicate, i.e., carry out its autocatalytic function, without the enzyme under its control being synthesized. If direct participation in enzyme synthesis is the immediate heterocatalytic function of DNA, then it follows that DNA may be autocatalytically active while heterocatalytically inactive. Thus, these two functions of the gene may be uncoupled. Similarly, there is evidence that the transforming marker may be heterocatalytically functional while still unintegrated into its recipient genome. In Hotchkiss' (1956) analysis of progeny descending from pneumococci infected with the streptomycin-resistance marker, he learned that streptomycin-resistant recipients may give rise to a mixed clone of streptomycin-resistant and -sensitive progeny. These findings suggested: (1) that the streptomycin-resistance marker employed by Hotchkiss was dominant to its sensitivity-conferring allele, and (2) that the introduced marker may not be genetically integrated and yet be active in transforming the phenotype of its host. Ephrussi-Taylor (1960a) finds that the initial resistance to streptomycin conferred by the infecting marker is unlike the phenotypic effect eventually associated with it: the initial or "pseudo" resistance permits survival but not reproduction in the presence of the antibiotic. In any case, if this "pseudo" resistance is the direct result of the heterocatalytic activity of the transforming factor, the independence of its autocatalytic and heterocatalytic functions may be concluded.

The techniques now available for following the appearance in the cell of proteins possessing specific amino acid sequences as a conse-

quence of infection by DNA molecules possessing specific nucleotide sequences affords hope of relating nucleotide sequence to amino acid sequence, that is, of solving the so-called "coding problem." The role of ribonucleic acid (RNA) as an intermediate between DNA and protein cannot be overlooked (Brachet, 1960), and future investigations may be expected to look into this possibility.

VIII. Interspecific Transfer of Bacterial Genes and Bacterial Evolution

Are the processes of genetic infection—conjugation, transduction, and transformation—tricks played by the human investigator on the bacteria, or are they phenomena that occur in natural populations of bacteria under "normal" environmental conditions? There is not a great deal of evidence on this subject (for review, see Ravin, 1960a), but it is not unlikely that these processes serve, perhaps under certain environmental conditions, to increase the rate of genetic variety in bacterial populations by recombination of spontaneously occurring mutations. These processes, in this view, would be serving the same function in haploid, asexually (clonally) reproducing organisms as genetic recombination between chromosomes occurring during the fertilization-meiosis-gamete cycle of diploid, sexually reproducing organisms. If these processes, on the other hand, are "accidents" of the laboratory, one would have to explain the potentiality for genetic recombination existing in so many bacterial species. As discussed elsewhere (Ravin, 1960a), one would be forced to explain this potentiality as either the vestige of sexual processes occurring in primitive organisms ancestral to the bacteria or as a "preadaptation" resident in the structure and function of DNA and utilized only in the evolutionarily advanced forms of life.

It is obvious that, if they occurred in nature to a significant extent, conjugation, transduction, and transformation would speed the flow of genes through bacterial populations and would not only promote genetic variety but would also tend to produce a continuous spectrum of genetic differences through the bacterial universe. On the contrary, genetic discontinuity is a fact of the bacterial universe as well as of the universe of higher organisms. It is true, perhaps, that the decision is often difficult as to whether one clone of bacteria is sufficiently different from another clone of bacteria on morphological, physiological, and ecological grounds to warrant separate species designations for them—although the difficulty is probably no greater than that of distinctions made on similar grounds in the case of populations of higher organisms. Nevertheless, there are certain natural, genetically stable groups of bacteria, sufficiently distinct from each other to permit a fairly dependable system

of classification at the supra-species level. Such genetic discontinuity implies some barrier to gene flow, similar to the isolating mechanisms found in higher organisms (Dobzhansky, 1941).

Indeed, it has been observed generally that genetic transfer between bacteria—by any of the modes described—is not promiscuous. Some strains of conjugating bacteria will not mate with other strains. In other cases, however, the efficiency of conjugation (i.e., the physical act of mating) may be as high between bacteria of different strains, species, or genera as between bacteria of the same origin. In these cases, one often finds that the efficiency of recombination involving certain genes is extremely low between bacteria of different origin relative to the efficiency with which it occurs in bacteria of the same strain (Luria and Burrous, 1957). Thus, one must make a distinction between conjugation compatibility and another type of compatibility which may be referred to as pairing compatibility for reasons to be discussed below.

It is also a general finding that a given bacterial species is transformed with less efficiency by DNA of a different species than by autologous DNA (Schaeffer and Ritz, 1955; Leidy *et al.*, 1956; Bracco *et al.*, 1957; Pakula *et al.*, 1958a,b; Catlin, 1960a). Schaeffer (1956a, 1957a,b, 1958) has investigated the basis of the low frequency of heterospecific transformations relative to homospecific transformations. He found that the DNA of a streptomycin-resistant strain of *Hemophilus para-influenzae* (Y*str*) produced 300 times more transformants in streptomycin-sensitive recipients of this species (Y) than in streptomycin-sensitive recipients of *Hemophilus influenzae* (X). This difference could not be accounted for by differences in the competence of the recipient cultures or by differences in the rate of phenotypic expression of the streptomycin-resistance marker in the two species. Nor could it be attributed to specific inactivation of transforming DNA by X cells, since the DNA of a streptomycin-resistant strain of *H. influenzae* (X*str*) is even more efficient in transforming X bacteria than Y*str* DNA is in transforming Y bacteria. The lowered efficiency of the heterospecific "cross" must therefore reside in the structure of the heterospecific DNA. That it was due, not to the streptomycin-resistance marker itself, but rather to properties of the DNA molecule as a whole was shown by the following experiment. If DNA is extracted from X cells transformed by Y*str* DNA (such transformants to be referred to as X*str*Y) and then this DNA used to transform another group of X cells, it is found that the frequency of transformation is as high as that obtained with X*str* DNA (or with X*str*X DNA). X*str*Y DNA is different from Y*str* DNA in being relieved of whatever factors contributed to the low frequency of transformation of X by Y*str* DNA. That X*str*Y

DNA does retain some structures specific to Y, however, is suggested by the finding that higher frequencies of transformation are obtained in the cross Y × X*str*Y DNA than in the cross Y × X*str*X.

Schaeffer (1958) proposed the hypothesis that pairing between the endogenous (recipient) DNA and the exogenous (transforming) DNA is a necessary step before genetic integration of a particular marker borne on the exogenous DNA. The assumption is made that the exactness of pairing depends on the extent of structural homology between the endogenous and exogenous molecules, and that the less exact this pairing the less frequently is an exogenous marker integrated into a recipient genome. It is further assumed that structural homology is less between DNA molecules of different species than between DNA molecules of the same species. In this view, pairing incompatibility is held responsible for the relatively low efficiency of heterospecific transformations. When such a transformation occurs, however, the integrated marker may be freed from a considerable amount of the molecule on which it was borne. If the recipient is species X, the integrated marker will now be associated with elements having pairing properties specific to X and hence, thereafter be more readily transferable into the genomes of X cells although somewhat less readily transferable into Y cells.

Schaeffer (1957a,b) obtained experimental support for this finding by following the uptake of P^{32} from labeled DNA. He found that the same number of molecules is incorporated per competent cell when the latter is treated with either homospecific or heterospecific DNA. Indeed, DNA molecules of different species compete with each other to gain entrance into recipient cells of one of the species. Thus, the relatively low frequency of heterospecific transformations must be due to some event occurring after penetration.

Green (1959) found that similar difficulties in integrating certain markers into recipient genomes could arise within a single species of pneumococcus. A streptomycin-resistance marker was transferred four to ten times less readily than an unlinked erythromycin-resistance marker into a specific recipient strain, although there was little difference in the frequencies of transformation by the two markers in a very closely related second strain. In the strain in which the streptomycin-resistance transformations occurred less frequently, no difficulty in penetration by the marker could be observed. On the contrary, some process occurring after penetration, and presumably one leading to genetic integration, was found to be terminated earlier in the affected strain. The factor responsible for this low frequency of integration was found to be tightly linked to the streptomycin-resistance marker. Most, although not all, of the transformants produced in the affected strain

possessed a DNA which retained the factor responsible for reduced integration efficiency. Thus, in this case the hypothetical factors on the DNA molecule responsible for reduced pairing efficiency are more closely linked to the marker being integrated than in the case of heterospecific transformations in *Hemophilus*. This quantitative difference simply indicates that many degrees of pairing incompatibility may be anticipated. Since DNA factors may arise causing pairing incompatibility in transformations between closely related populations of the same species, it is tempting to speculate that these factors may be the raw materials from which stronger isolating mechanisms evolve.

Ravin (1960a) has suggested that, if the processes of genetic infection occur to a significant extent in natural populations of bacteria, the evolution of isolating mechanisms may bring about the genetic discontinuity between groups adapted for different ecological niches. Such isolating mechanisms may take the following forms:

1. Surface incompatibility: involving some difficulty in the superficial encounter between the recipient bacterium and the vector of genetic information.

a. Conjugation incompatibility (in conjugation): due, for example, to specific differences in fertility factors necessary for mating (Lederberg and Lederberg, 1956).

b. Adsorption and/or "injection" incompatibility (in transduction): due to incompatibility between specific structures on the surface of the recipient bacterium and specific adsorption or "injection" proteins on the bacteriophage tail (Adams, 1959).

c. Incompatibility for DNA fixation (in transformation): due to the elaboration of cell wall barriers[8] or to increased specificity of DNA receptor sites on the bacterial surface (see Section IV).

2. Pairing incompatibility: involving some difficulty in the synapsis between endogenous and exogenous genetic material, a step essential for genetic recombination. Such incompatibility would be presumably due to structural differences, of an as yet unknown nature, between the DNA molecules confronting each other.

Ravin (1960a) has also pointed out that, if the processes of genetic infection occur in nature with a frequency that is inconsequential evolutionarily, the differences in DNA structure resulting from evolutionary divergence could still be utilized by the taxonomist as an index of speciation.

[8] Such barriers would not only isolate a strain from the DNA of other strains, but from its own DNA as well, unless there were some specific selectivity in the barrier.

The possibilities for research into the mechanisms of bacterial evolution have only begun to be explored. The combination of genetic and chemical tools now possible for studying bacteria in their ecological niches may prove of great value in this direction.

References

Abe, M., and Mizuno, D., 1959. A contribution to the knowledge of pneumococcus transformation during the period between the incorporation of deoxyribonucleic acid and the appearance of streptomycin-resistance. *Biochim. et Biophys. Acta* **32,** 464–469.

Adams, M. H., 1959. "Bacteriophages." Interscience, New York.

Adler, J., Lehman, I. R., Bessman, M. J., Simms, E. S., and Kornberg, A., 1958. Enzymatic synthesis of deoxyribonucleic acid. IV. Linkage of single deoxynucleotides to the deoxynucleoside ends of deoxyribonucleic acid. *Proc. Natl. Acad. Sci. US.* **44,** 641–647.

Alexander, H. E., and Leidy, G., 1951. Determination of inherited traits of *H. influenzae* by desoxyribonucleic acid fractions isolated from type-specific cells. *J. Exptl. Med.* **93,** 345–359.

Alexander, H. E., and Leidy, G., 1953. Induction of streptomycin resistance in sensitive *Hemophilus influenzae* by extracts containing DNA from resistant *Hemophilus influenzae*. *J. Exptl. Med.* **97,** 17–31.

Alexander, H. E., and Redman, W., 1953. Transformation of type specificity of meningococci. *J. Exptl. Med.* **97,** 797–806.

Alexander, H. E., Leidy, G., and Hahn, E., 1954. Studies on the nature of *Hemophilus influenzae* cells susceptible to heritable changes by desoxyribonucleic acids. *J. Exptl. Med.* **99,** 505–553.

Alloway, J. L., 1933. Further observations on the use of pneumococcus extracts in effecting transformation of type *in vitro*. *J. Exptl. Med.* **57,** 265–278.

Anderson, T. F., and Mazé, R., 1957. Analyse de la descendance de zygotes formés par conjugaison chez *Escherichia coli* K12. *Ann. inst. Pasteur* **93,** 194–198.

Anderson, T. F., Wollman, E. L., and Jacob, F., 1957. Sur le processus de conjugaison et de recombinaison chez *Escherichia coli*. III. Aspects morphologiques en microscopie électronique. *Ann. inst. Pasteur* **93,** 450–455.

Auerbach, C., and Robson, J. M., 1944. Production of mutations by allyl *iso*-thiocyanate. *Nature* **154,** 81–82.

Austrian, R., 1952a. Observations on the transformation of pneumococcus *in vivo*. *Bull. Johns Hopkins Hosp.* **91,** 189–196.

Austrian, R., 1952b. Bacterial transformation reactions. *Bacteriol. Rev.* **16,** 31–50.

Austrian, R., 1953a. Morphologic variation in pneumococcus. I. An analysis of the bases for morphologic variation in pneumococcus and description of a hitherto undefined morphologic variant. *J. Exptl. Med.* **98,** 21–34.

Austrian, R., 1953b. Morphologic variation in pneumococcus. II. Control of pneumococcal morphology through transformation reactions. *J. Exptl. Med.* **98,** 35–40.

Austrian, R., and Bernheimer, H. P., 1959. Simultaneous production of two capsular polysaccharides by pneumococcus. I. Properties of a pneumococcus manifesting binary capsulation. *J. Exptl. Med.* **110,** 571–584.

Austrian, R., and Colowick, M. S., 1953. Modification of the fermentative activities

of pneumococcus through transformation reactions. *Bull. Johns Hopkins Hosp.* **92,** 375–384.

Austrian, R., and MacLeod, C. M., 1949a. Type-specific protein from pneumococcus. *J. Exptl. Med.* **89,** 439–450.

Austrian, R., and MacLeod, C. M., 1949b. Acquisition of M protein by pneumococci through transformation reactions. *J. Exptl. Med.* **89,** 451–460.

Austrian, R., Bernheimer, H. P., Smith, E. E. B., and Mills, G. T., 1958. Acquisition of new capsular type by pneumococcus, a multifactor transformation. *Cold Spring Harbor Symposia Quant. Biol.* **23,** 99–100.

Austrian, R., Bernheimer, H. P., Smith, E. E. B., and Mills, G. T., 1959. Simultaneous production of two capsular polysaccharides by pneumococcus. II. The genetic and biochemical bases of binary capsulation. *J. Exptl. Med.* **110,** 585–602.

Avery, O. T., MacLeod, C. M., and McCarty, M., 1944. Studies on the chemical nature of the substance inducing transformation of pneumococcal types. I. Induction of transformation by a desoxyribonucleic acid fraction isolated from pneumococcus type III. *J. Exptl. Med.* **79,** 137–158.

Balassa, R., 1954. Transformationsmechanismen der *Rhizobien*. I. - III. *Acta Microbiol. Acad. Sci. Hung.* **2,** 51–78.

Balassa, R., 1955. *In vivo* induzierte Transformationen bei *Rhizobien*. *Naturwissenschaften* **42,** 422–423.

Balassa, R., 1956. Durch Desoxyribonucleinsäuren induzierte Veranderungen an *Rhizobien*. *Naturwissenschaften* **43,** 133.

Balassa, R., 1957a. Durch desoxyribonukleinsauren induzierte veranderungen bei *Rhizobien*. *Acta Microbiol. Acad. Sci. Hung.* **4,** 77–84.

Balassa, R., 1957b. Einige bedingungen der ausbildung einer durch desoxyribonukleinsauren induzierten niedrigen streptomycinresistenz bei *Rhizobien*. *Acta Microbiol. Acad. Sci. Hung.* **4,** 85–95.

Balassa, R., 1960. Transformation of a strain of *Rhizobium lupini*. *Nature* **188,** 246–247.

Beadle, G. W., 1945. Biochemical genetics. *Chem. Revs.* **37,** 15–96.

Beiser, S. M., and Hotchkiss, R. D., 1954. Abnormal capsular transformation in pneumococci. *Federation Proc.* **13,** 1595.

Bendich, A., Pahl, H. B., and Beiser, S. M., 1956. Chromatographic fractionation of desoxyribonucleic acids with special emphasis on the transforming factor of pneumococcus. *Cold Spring Harbor Symposia Quant. Biol.* **21,** 31–48.

Benzer, S., 1955. The fine structure of a genetic region in bacteriophage. *Proc. Natl. Acad. Sci. US.* **41,** 344–354.

Bessman, M. J., Lehman, I. R., Adler, J., Zimmerman, S. B., Simms, E. S., and Kornberg, A., 1958. Enzymatic synthesis of deoxyribonucleic acid. III. The incorporation of pyrimidine and purine analogues into deoxyribonucleic acid. *Proc. Natl. Acad. Sci. US.* **44,** 633–640.

Boivin, A., 1947. Directed mutation in colon bacilli, by an inducing principle of desoxyribonucleic nature: its meaning for the general biochemistry of heredity. *Cold Spring Harbor Symposia Quant. Biol.* **12,** 7–17.

Boivin, A., Vendrely, R., and Lehoult, Y., 1945. L'acide thymonucléique hautement polymérisé, principe capable de conditionner la spécificité sérologique et l'équipement enzymatique des bactéries. Consequence pour la biochimie de l'hérédité. *Compt. rend. acad. sci.* **221,** 646–647.

Boivin, A., Delaunay, A., Vendrely, R., and Lehoult, Y., 1946. Sur certaines con-

ditions de la transformation du type antigénique et de l'équipement anzymatique d'un colibacille sous l'effet d'un principe inducteur de nature thymonucléique issu d'un autre colibacille (mutation "dirigée"). *Experientia* **2,** 139–140.

Bracco, R. M., Krauss, M. R., Roe, A. S., and MacLeod, C. M., 1957. Transformation reactions between pneumococcus and three strains of streptococcus. *J. Exptl. Med.* **106,** 247–259.

Brachet, J., 1960. Ribonucleic acids and the synthesis of cellular proteins. *Nature* **186,** 194–199.

Brenner, S., and Barnett, L., 1959. Genetic and chemical studies of the head protein of bacteriophages T2 and T4. *Brookhaven Symposia in Biol. No.* **12,** 86–94.

Brown, E. R., Cherry, W. B., Moody, M. D., and Gordon, M. A., 1955. The induction of motility in *Bacillus anthracis* by means of bacteriophage lysates. *J. Bacteriol.* **69,** 590–602.

Brown, G. B., and Roll, P. M., 1955. Biosynthesis of nucleic acids. *In* "Nucleic Acids" (E. Chargaff and J. N. Davidson, eds.), Vol. II, pp. 341–392. Academic Press, New York.

Brown, G. L., and Martin, A. V., 1955. Fractionation of the deoxyribonucleic acid of T2r bacteriophage. *Nature* **176,** 971–972.

Brown, G. L., and Watson, M., 1953. Heterogeneity of desoxyribonucleic acids. *Nature* **172,** 339–342.

Brown, G. L., McEwen, M. B., and Pratt, M. I., 1955. Macromolecular weight and size of deoxypentose nucleic acid. *Nature* **176,** 161–162.

Bryan, B. E., 1961. Genetic modifiers of streptomycin resistance in pneumococcus. *J. Bacteriol.* In press.

Butler, J. A. V., and James, D. W. F., 1951. Gel-like behavior of desoxyribonucleic acid solutions. *Nature* **167,** 844.

Butler, J. A. V., Phillips, D. M., and Shooter, K. V., 1957. The influence of protein on heterogeneity of DNA. *Arch. Biochem. Biophys.* **71,** 423–429.

Carlson, E. A., 1959a. Allelism, complementation and pseudoallelism at the dumpy locus in *Drosophila melanogaster*. *Genetics* **44,** 347–374.

Carlson, E. A., 1959b. Comparative genetics of complex loci. *Quart. Rev. Biol.* **34,** 33–67.

Case, M. E., and Giles, N. H., 1958. Recombination mechanisms at the *pan*-2 locus in *Neurospora crassa*. *Cold Spring Harbor Symposia Quant. Biol.* **23,** 119–135.

Catlin, B. W., 1960a. Interspecific transformation of *Neisseria* by culture slime containing deoxyribonucleate. *Science* **131,** 608–610.

Catlin, B. W., 1960b. Transformation of *Neisseria meningitidis* by deoxyribonucleate from cells and from culture slime. *J. Bacteriol.* **79,** 579–590.

Cavalieri, L. F., 1952. Studies on the structure of nucleic acids. V. On the mechanism of metal and enzyme interactions. *J. Am. Chem. Soc.* **74,** 1242–1247.

Cavalieri, L. F., 1957. Paucidisperse deoxyribonucleic acid and its use in the study of genetic determinants. *J. Am. Chem. Soc.* **79,** 5319.

Chargaff, E., 1955. Isolation and composition of the deoxypentose nucleic acids and and of the corresponding nucleoproteins. *In* "Nucleic Acids" (E. Chargaff and J. N. Davidson, eds.), Vol. I, pp. 307–372. Academic Press, New York.

Chargaff, E., Crampton, C. F., and Lipschitz, R., 1953. Separation of calf thymus desoxyribonucleic acid into fractions of different composition. *Nature* **172,** 289–292.

Chargaff, E., Schulman, H. M., and Shapiro, H. J., 1957. Protoplasts of *E. coli* as sources and acceptors of deoxypentose nucleic acid: rehabilitation of a deficient mutant. *Nature* **180,** 851–852.

Chase, M., and Doermann, A. H., 1958. High negative interference over short segments of the genetic structure of bacteriophage T4. *Genetics* **43,** 332–353.

Corey, R. R., and Starr, M. P., 1957a. Colony types of *Xanthomonas phaseoli*. *J. Bacteriol.* **74,** 137–140.

Corey, R. R., and Starr, M. P., 1957b. Genetic transformation of colony type in *Xanthomonas phaseoli*. *J. Bacteriol.* **74,** 141–145.

Corey, R. R., and Starr, M. P., 1957c. Genetic transformation of streptomycin resistance in *Xanthomonas phaseoli*. *J. Bacteriol.* **74,** 146–150.

Davis, B. D., 1948. Isolation of biochemically deficient mutants by penicillin. *J. Am. Chem. Soc.* **70,** 4267.

Davis, B. D., 1950. Nonfiltrability of the agents of genetic recombination in *Escherichia coli*. *J. Bacteriol.* **60,** 507–508.

Dawson, M. H., and Sia, R. H. P., 1931. A technique for inducing transformation of pneumococcal types *in vitro*. *J. Exptl. Med.* **54,** 681–699.

DeFilippes, F. M., and Guild, W. R., 1959. Irradiation of solutions of transforming DNA. *Radiation Research* **11,** 38–53.

Delbrück, M., and Stent, G. S., 1957. On the mechanism of DNA replication. *In* "Chemical Basis of Heredity" (W. McElroy and B. Glass, eds.), pp. 699–736. Johns Hopkins Univ. Press, Baltimore, Maryland.

Demerec, M., 1945. Production of *Staphylococcus* strains resistant to various concentrations of penicillin. *Proc. Natl. Acad. Sci. U.S.* **31,** 16–20.

Demerec, M., 1948. Origin of bacterial resistance to antibiotics. *J. Bacteriol.* **56,** 63–74.

Demerec, M., 1956. A comparative study of certain gene loci in *Salmonella*. *Cold Spring Harbor Symposia Quant. Biol.* **21,** 113–121.

Demerec, M., Hartman, P. E., Moser, H., Kanazir, D., Demerec, Z. E., Fitzgerald, P. L., Glover, S. W., Lahr, E. L., Westover, W. E., and Yura, T., 1955. Bacterial genetics. *Carnegie Inst. Wash. Yearbook.* **54,** 219–234.

Demerec, M., Lahr, E. L., Miyake, T., Goldman, I., Balbinder, E., Banic, S., Hashimoto, K., Glanville, E. V. and Gross J. D., 1958. Bacterial genetics. *Carnegie Inst. Wash. Yearbook.* **57,** 390–406.

Dobzhansky, Th., 1941. "Genetics and The Origin of Species," 2nd ed. Columbia Univ. Press, New York.

Doermann, A. H., Chase, M., and Stahl, F. W., 1955. Genetic recombination and replication in bacteriophage. *J. Cellular Comp. Physiol.* **45,** *Suppl. 2,* 51–74.

Doty, P., Marmur, J., and Suoeka, N., 1959. The heterogeneity in properties and functioning of deoxyribonucleic acids. *Brookhaven Symposia Biol. No.* **12,** 1–16.

Doty, P., Marmur, J., Eigner, J., and Schildkraut, C., 1960. Strand separation and specific recombination in deoxyribonucleic acids: physical and chemical studies. *Proc. Natl. Acad. Sci. U.S.* **46,** 461–476.

Drew, R. M., 1957. Induction of resistance to A-methopterin in *Diplococcus pneumoniae* by deoxyribonucleic acid. *Nature* **179,** 1251–1252.

Eagle, H., 1960. The sustained growth of human and animal cells in a protein-free environment. *Proc. Natl. Acad. Sci. U.S.* **46,** 427–431.

Englesberg, E., 1952. Mutagenic action of formaldehyde on bacteria. *J. Bacteriol.* **63,** 1–11.

Ephrati-Elizur, E., and Zamenhof, S., 1959. Incorporation of 5-bromouracil into

transforming principle of *Bacillus subtilis* and its biological effects. *Nature* **184,** 472–473.

Ephrati-Elizur, E., Srinavasan, P. R., and Zamenhof, S., 1961. Genetic analysis, by means of transformation, of histidine linkage groups in *Bacillus subtilis*. *Proc. Natl. Acad. Sci. U.S.* **47,** 56–63.

Ephrussi-Taylor, H. E., 1951a. Transformation allogenes du pneumocoque. *Exptl. Cell Research* **2,** 589–607.

Ephrussi-Taylor, H. E., 1951b. Genetic aspects of transformations of pneumococci. *Cold Spring Harbor Symposia Quant. Biol.* **16,** 445–456.

Ephrussi-Taylor, H. E., 1954. A new transforming agent determining pattern of metabolism and glucose and lactic acids in pneumococcus. *Exptl. Cell Research* **6,** 94–116.

Ephrussi-Taylor, H. E., 1955. Current status of bacterial transformations. *Advances in Virus Research* **3,** 275–307.

Ephrussi-Taylor, H. E., 1957. X-ray inactivation studies on solutions of transforming DNA from pneumococcus. *In* "Chemical Basis of Heredity" (W. McElroy and B. Glass, eds.), pp. 299–320. Johns Hopkins Univ. Press, Baltimore, Maryland.

Ephrussi-Taylor, H. E., 1958. The mechanism of desoxyribonucleic acid-induced transformations. *In* "Recent Progress in Microbiology" (G. Tunevall, ed.), pp. 51–68. C. C Thomas, Springfield, Illinois.

Ephrussi-Taylor, H. E., 1960a. On the biological functions of deoxyribonucleic acid. *Symposium Soc. Gen. Microbiol.* **10,** 132–154.

Ephrussi-Taylor, H. E., 1960b. Recombination analysis in microbial systems. "Growth: Molecule, Cell, Organism." Basic Books, New York.

Ephrussi-Taylor, H. E., 1960c. L'état du DNA transformant au cours des premières phases de la transformation bactérienne. *Compt. rend. soc. biol.* **CLIV,** 1951–1955.

Ephrussi-Taylor, H. E., and Latarjet, R., 1955. Inactivation, par les rayons X, d'un facteur transformant du pneumocoque. *Biochim. et Biophys. Acta* **16,** 183–197.

Fluke, D., Drew, R., and Pollard, E., 1952. Ionizing particle evidence for the molecular weight of the pneumococcus transforming principle. *Proc. Natl. Acad. Sci. U.S.* **38,** 180–187.

Fox, M. S., 1957. Deoxyribonucleic acid incorporation by transformed bacteria. *Biochim. et Biophys. Acta* **26,** 83–85.

Fox, M. S., 1959. Phenotypic expression of a genetic property introduced by deoxyribonucleate. *J. Gen. Physiol.* **42,** 737–748.

Fox, M. S., 1960. Fate of transforming deoxyribonucleate following fixation by transformable bacteria. II. *Nature* **187,** 1004–1006.

Fox, M. S., and Hotchkiss, R. D., 1957. Initiation of bacterial transformation. *Nature* **179,** 1322–1325.

Fox, M. S., and Hotchkiss, R. D., 1960. Fate of transforming deoxyribonucleate following fixation by transformable bacteria. I. *Nature* **187,** 1002–1003.

Fraser, D., Mahler, H. R., Shug, A. L., and Thomas, C. A., Jr., 1957. The infection of sub-cellular *Escherichia coli* strain B, with a DNA preparation from T2 bacteriophage. *Proc. Natl. Acad. Sci. U.S.* **43,** 939–947.

Garen, A., and Zinder, N. D., 1955. Radiological evidence for partial genetic homology between bacteriophage and host bacteria. *Virology* **1,** 347–376.

Gierer, A., and Mundry, K. W., 1958. Production of mutants of tobacco mosaic virus by chemical alteration of its ribonucleic acid *in vitro*. *Nature* **182,** 1457.

Goodgal, S. H., and Herriott, R. M., 1957a. Studies on transformation of *Hemophilus influenzae*. *In* "Chemical Basis of Heredity" (W. McElroy and B. Glass, eds.), pp. 336–340. Johns Hopkins Univ. Press, Baltimore, Maryland.

Goodgal, S. H., and Herriott, R. M., 1957b. A study of linked transformations in *Hemophilus influenzae*. *Records Genet. Soc. Am.* **26,** 372.

Goodgal, S. H., Rupert, C. S., and Herriott, R. M., 1957. Photoreactivation of *Hemophilus influenzae* transforming factor for streptomycin resistance by an extract of *Escherichia coli* B. *In* "Chemical Basis of Heredity" (W. McElroy and B. Glass, eds.), pp. 341–343. Johns Hopkins Univ. Press, Baltimore, Maryland.

Green, D. McD., 1959. A host-specific variation affecting relative frequency of transformation of two markers in pneumococcus. *Exptl. Cell Research* **18,** 466–480.

Griffith, F., 1928. Significance of pneumococcal types. *J. Hyg.* **27,** 113–159.

Guild, W. R., and DeFilippes, F. M., 1957. Ionizing radiation and ultrasonic evidence for a minimum unit of transforming principle DNA. *Biochim. et Biophys. Acta.* **26,** 241–251.

Hall, C. E., 1956. Visualization of individual macromolecules with the electron microscope. *Proc. Natl. Acad. Sci. U.S.* **42,** 801–806.

Hamilton, L. D., Barclay, R. K., Wilkins, M. H. F., Brown, G. L., Wilson, H. R., Marvin, D. A., Ephrussi-Taylor, H. E., and Simmons, N. S., 1959. Similarity of the structure of DNA from a variety of sources. *J. Biophys. Biochem. Cytol.* **5,** 379–404.

Hayes, W., 1953. The mechanism of genetic recombination in *E. coli*. *Cold Spring Harbor Symposia Quant. Biol.* **18,** 75–93.

Hayes, W., 1960. The bacterial chromosome. *Symposium Soc. Gen. Microbiol.* **10,** 12–38.

Herriott, R. M., 1961. Formation of heterozygotes by annealing a mixture of transforming DNAs. *Proc. Natl. Acad. Sci. US.* **47,** 146–153.

Hershey, A. D., 1958. The production of recombinants in phage crosses. *Cold Spring Harbor Symposia Quant. Biol.* **23,** 19–46.

Hershey, A. D., and Chase, M., 1952. Independent functions of viral protein and nucleic acid in growth of bacteriophage. *J. Gen. Physiol.* **36,** 39–56.

Hotchkiss, R. D., 1948. Études chimiques sur le facteur transformant du pneumocoque. "Les unités biologiques douées de continuité génétique." pp. 57–65. C.N.R.S., Paris.

Hotchkiss, R. D., 1951. Transfer of penicillin resistance in pneumococci by the desoxyribonucleate derived from resistant cultures. *Cold Spring Harbor Symposia Quant. Biol.* **16,** 457–461.

Hotchkiss, R. D., 1952. The role of desoxyribonucleates in bacterial transformations. "Phosphorus Metabolism" (W. McElroy and B. Glass, eds.), Vol. II, pp. 426–436. Johns Hopkins Univ. Press, Baltimore, Maryland.

Hotchkiss, R. D., 1954. Cyclical behavior in pneumococcal growth and transformability occasioned by environmental changes. *Proc. Natl. Acad. Sci. U.S.* **40,** 49–55.

Hotchkiss, R. D., 1955. The biological role of the deoxypentose nucleic acids. *In* "Nucleic Acids," Vol. II (E. Chargaff and J. N. Davidson, eds.), pp. 435–473. Academic Press, New York.

Hotchkiss, R. D., 1956. The genetic organization of the deoxyribonucleate units functioning in bacterial transformations. "Enzymes: Units of Biological Structure and Function" (O. H. Gaebler, ed.), pp. 119–130.

Hotchkiss, R. D., 1957. Criteria for quantitative genetic transformations of bacteria. *In* "Chemical Basis of Heredity" (W. McElroy and B. Glass, eds.), pp. 321–325. Johns Hopkins Univ. Press, Baltimore, Maryland.

Hotchkiss, R. D., 1958. Size limitations governing the incorporation of genetic material in bacterial transformations and other nonreciprocal recombinations. *Symposia Soc. Exptl. Biol.* **12,** 49–59.

Hotchkiss, R. D., and Ephrussi-Taylor, H. E., 1951. Use of serum albumin as source of serum factor in pneumococcal transformation. *Federation Proc.* **10,** 200.

Hotchkiss, R. D., and Evans, A. H., 1957. Genetic and metabolic mechanisms underlying multiple levels of sulphonamide resistance in pneumococci. "Drug Resistance in Microorganisms," Ciba Foundation Symposium, pp. 183–196. Little, Brown, Boston, Massachusetts.

Hotchkiss, R. D., and Evans, A. H., 1958. Analysis of the complex sulfonamide resistance locus of pneumococcus. *Cold Spring Harbor Symposia Quant. Biol.* **23,** 85–97.

Hotchkiss, R. D., and Marmur, J., 1954. Double marker transformations as evidence of linked factors in deoxyribonucleate transforming agents. *Proc. Natl. Acad. Sci. US.* **40,** 55–60.

Imshenetskii, A. A., Perova, K. Z., Zaitseva, T. A., and Belozerskii, A. N., 1959. Transmission of streptomycin resistance in staphylococci by means of DNA. *Mikrobiologya* **28,** 187–190.

Jackson, S., MacLeod, C. M., and Krauss, M. R., 1959. Determination of type in capsulated transformants of pneumococcus by the genome of non-capsulated donor and recipient strains. *J. Exptl. Med.* **109,** 429–438.

Jacob, F., and Wollman, E. L., 1958. Genetic and physical determinations of chromosomal segments in *Escherichia coli*. *Symposia Soc. Exptl. Biol.* **12,** 75–92.

Kaiser, A. D., and Hogness, D. S., 1960. The transformation of *Escherichia coli* with deoxyribonucleic acid isolated from bacteriophage λ dg. *J. Mol. Biol.* **2,** 392–415.

Kellenberger, G., Zichichi, M. L., and Weigle, J. J., 1961. Exchange of DNA in the recombination of bacteriophage. *Proc. Natl. Acad. Sci. US.* **47,** 869–878.

Kelner, A., 1949. Effect of visible light on the recovery of *Streptomyces griseus* conidia from UV-irradiation injury. *Proc. Natl. Acad. Sci. US.* **35,** 73–79.

Klein, D. T., and Klein, R. M., 1953. Transmittance of tumor-inducing ability to avirulent crown-gall and related bacteria. *J. Bacteriol.* **66,** 220–228.

Klein, D. T., and Klein, R. M., 1956. Quantitative aspects of transformation of virulence in *Agrobacterium tumefaciens*. *J. Bacteriol.* **72,** 308–313.

Kohiyama, M., and Saito, H., 1960. Stimulation of genetic transformation in *Bacillus subtilis* by biologically inactive DNA and polyphosphate. *Biochim. et Biophys. Acta* **41,** 180.

Kornberg, A., 1957. Pathways of enzymatic synthesis of nucleotides and polynucleotides. *In* "Chemical Basis of Heredity" (W. McElroy and B. Glass, eds.), pp. 579–608. Johns Hopkins Univ. Press, Baltimore, Maryland.

Kornberg, A., Zimmerman, S. B., Kornberg, S. R., and Josse, J., 1959. Enzymatic synthesis of DNA. VI. Influence of bacteriophage T2 on the synthetic pathway of host cells. *Proc. Natl. Acad. Sci., US.* **45,** 772–785.

Kurahashi, K., 1957. Enzyme formation in galactose-negative mutants of *Escherichia coli*. *Science* **125,** 114–116.

Lacks, S., and Hotchkiss, R. D., 1960. A study of the genetic material determining an enzyme activity in pneumococcus. *Biochim. et Biophys. Acta.* **39,** 508–518.

Latarjet, R., 1956. Effects of radiation and peroxides on viral and bacterial functions linked to DNA specificity. "Ionizing Radiation and Cell Metabolism," Ciba Foundation Symposium, pp. 275–297. Little, Brown, Boston, Massachusetts.

Lederberg, E. M., 1960. Genetic and functional aspects of galactose metabolism in *Escherichia coli* K12. *Symposium Soc. Gen. Microbiol.* **10,** 115–131.

Lederberg, J., 1955. Recombination mechanism in bacteria. *J. Cellular Comp. Physiol.* **45,** *Suppl. 2,* 75–107.

Lederberg, J., 1956. Conjugal pairing in *Escherichia coli. J. Bacteriol.* **71,** 497–498.

Lederberg, J., 1957. Sibling recombinants in zygote pedigrees of *Escherichia coli. Proc. Natl. Acad. Sci. U.S.* **43,** 1060–1065.

Lederberg, J., and Lederberg, E. M., 1952. Replica plating and indirect selection of bacterial mutants. *J. Bacteriol.* **63,** 399–406.

Lederberg, J., and Lederberg, E. M., 1956. Infection and heredity. *In* "Cellular Mechanisms in Differentiation and Growth" (D. Rudnick, ed.), pp. 101–124. Princeton Univ. Press, Princeton, New Jersey.

Lederberg, J., and St. Clair, J., 1958. Protoplasts and L-type growth of *Escherichia coli. J. Bacteriol.* **75,** 143–160.

Lederberg, J., and Tatum, E. L., 1946. Novel genotypes in mixed cultures of biochemical mutants of bacteria. *Cold Spring Harbor Symposia Quant. Biol.* **11,** 113–114.

Lederberg, J., and Zinder, N. D., 1948. Concentration of biochemical mutants of bacteria with penicillin. *J. Am. Chem. Soc.* **70,** 4267–4268.

Lederberg, J., Lederberg, E. M., Zinder, N. D., and Lively, E. R., 1951. Recombination analysis of bacterial heredity. *Cold Spring Harbor Symposia Quant. Biol.* **16,** 413–443.

Leidy, G., Hahn, E., and Alexander, H. E., 1953. *In vitro* production of new types of *Hemophilus influenzae. J. Exptl. Med.* **97,** 467–482.

Leidy, G., Hahn, E., and Alexander, H. E., 1956. On the specificity of the desoxyribonucleic acid which induces streptomycin resistance in *Hemophilus. J. Exptl. Med.* **104,** 305–320.

Lerman, L. S., 1955. Chromatographic fractionation of the transforming principle of the pneumococcus. *Biochim. et Biophys. Acta.* **18,** 132–134.

Lerman, L. S., 1956. In discussion following paper by Bendich *et al. Cold Spring Harbor Symposia Quant. Biol.* **21,** 46–47.

Lerman, L. S., and Tolmach, L. J., 1957. Genetic transformation. I. Cellular incorporation of DNA accompanying transformation in pneumococcus. *Biochim. et Biophys. Acta.* **26,** 68–82.

Lerman, L. S., and Tolmach, L. J., 1959. Genetic transformation. II. The significance of damage to the DNA molecule. *Biochim. et Biophys. Acta.* **33,** 371–387.

Leslie, I., 1955. The nucleic acid content of tissues and cells. *In* "Nucleic Acids," Vol. II (E. Chargaff and G. N. Davidson, eds.), pp. 1–45. Academic Press, New York.

Levine, L., Murakami, W. T., Van Vunakis, H., and Grossman, L., 1960. Specific antibodies to thermally denatured deoxyribonucleic acid of page T4. *Proc. Natl. Acad. Sci. U.S.* **46,** 1038–1043.

Levinthal, C., and Crane, H. R., 1956. On the unwinding of DNA. *Proc. Natl. Acad. Sci. U.S.* **42,** 436–438.

Litman, R .M., and Ephrussi-Taylor, H., 1959. Inactivation et mutation des facteurs génétiques de l'acide désoxyribonucléique du pneumocoque par l'ultraviolet et par l'acide nitreux. *Compt. rend. acad. sci.* **249,** 838–840.

Litman, R. M., and Pardee, A. B., 1956. Production of bacteriophage mutants by a disturbance of deoxyribonucleic acid metabolism. *Nature* **178,** 529–532.

Litt, M., Marmur, J., Ephrussi-Taylor, H. E., and Doty, P., 1958. The dependence of pneumococcal transformation on the molecular weight of deoxyribose nucleic acid. *Proc. Natl. Acad. Sci. U.S.* **44,** 144–152.

Lucy, J. A., and Butler, J. A. V., 1954. Fractionation of deoxyribonucleoprotein by successive extraction with constant salt concentration. *Nature* **174,** 32–33.

Luria, S. E., 1959. Genetic transfers by viruses. *Brookhaven Symposia in Biol.* No. 12, 95–102.

Luria, S. E., and Burrous, J. W., 1957. Hybridization between *Escherichia coli* and *Shigella*. *J. Bacteriol.* **74,** 461–476.

McCarty, M., 1945. Reversible inactivation of the substance inducing transformation of pneumococcal types. *J. Exptl. Med.* **81,** 501–514.

McCarty, M., 1946a. Purification and properties of desoxyribonuclease isolated from beef pancreas. *J. Gen. Physiol.* **29,** 123–139.

McCarty, M., 1946b. Chemical nature and biological specificity of the substance inducing transformation of pneumococcal types. *Bacteriol. Rev.* **10,** 63–71.

McCarty, M., and Avery, O. T., 1946a. Studies on the chemical nature of the substance inducing transformation of pneumococcal types. II. Effect of desoxyribonuclease on the biological activity of the transforming substance. *J. Exptl. Med.* **83,** 89–96.

McCarty, M., and Avery, O. T., 1946b. Studies on the chemical nature of the substance inducing transformation of pneumococcal types. III. An improved method for the isolation of the transforming substance and its application to pneumococcus types II, III and VI. *J. Exptl. Med.* **83,** 97–104.

McCarty, M., Taylor, H. E., and Avery, O. T., 1946. Biochemical studies of environmental factors essential in transformation of pneumococcal types. *Cold Spring Harbor Symposia Quant. Biol.* **11,** 177–183.

MacLeod, C. M., and Krauss, M. R., 1947. Stepwise intra-type transformation of pneumococcus from R to S by way of a variant intermediate in capsular polysaccharide production. *J. Exptl. Med.* **86,** 439–453.

MacLeod, C. M., and Krauss, M. R., 1956. Transformation reactions with two non-allelic R mutants of the same strain of pneumococcus type VIII. *J. Exptl. Med.* **103,** 623–638.

Mahler, H. R., and Fraser, D., 1959. Studies in partially resolved bacteriophage-host systems. IV. Some properties of the protoplast-infecting agent derived from T2 bacteriophage. *Virology* **8,** 401–424.

Marmur, J., and Doty, P., 1959. Heterogeneity in deoxyribonucleic acids. II. Dependence on composition of the configurational stability of deoxyribonucleic acids. *Nature* **183,** 1427–1429.

Marmur, J., and Fluke, D. J., 1955. Uniformity of ionizing radiation on several transforming factors of pneumococcus. *Arch. Biochem. Biophys.* **57,** 506–514.

Marmur, J., and Hotchkiss, R. D., 1955. Mannitol metabolism, a transferable property of pneumococcus. *J. Biol. Chem.* **214,** 383–396.

Marmur, J., and Lane D., 1960. Strand separation and specific recombination in deoxyribonucleic acids: biological studies. *Proc. Natl. Acad. Sci. U.S.* **46,** 453–461.

Meselson, M., and Stahl, F. W., 1958. The replication of DNA. *Cold Spring Harbor Symposia Quant. Biol.* **23,** 9–12.

Meselson, M., and Weigle, J. J., 1961. Chromosome breakage accompanying genetic recombination in bacteriophage. *Proc. Natl. Acad. Sci. U.S.* **47,** 857–868.

Mirsky, A., 1947. Discussion following paper by Boivin. *Cold Spring Harbor Symposia Quant. Biol.* **12,** 16.

Mitchell, M. B., 1955. Aberrant recombination of pyridoxine mutants of *Neurospora. Proc. Natl. Acad. Sci. U.S.* **41,** 215–220.

Morse, M. L., Lederberg, E. M., and Lederberg, J., 1956. Transductional heterogenotes in *Escherichia coli. Genetics* **41,** 758–779.

Nester, E. W., and Lederberg, J., 1961. Linkage of genetic units of *Bacillus subtilis* in DNA transformation. *Proc. Natl. Acad. Sci. U.S.* **47,** 52–55.

Odaka, T., and Watanabe, T., 1959. Transformation reaction of pneumococci in the absence of serum factor. *Nature* **184,** 471–472.

Pakula, R., Fluder, Z., Hulanicka, E., and Walczak, W., 1958a. Studies on transformation of streptococci. *Bull. acad. polon. sci., Classe (II)* **6,** 319–323.

Pakula, R., Hulanicka, E., and Walczak, W., 1958b. Transformation between streptococci, pneumococci and staphylococci. *Bull. acad. polon. sci., Classe (II)* **6,** 325–328.

Pakula, R., Hulanicka-Bankowska, E., and Walczak, W., 1960. Photoreactivation of UV-damaged streptomycin resistance marker in transforming DNA of streptococci and pneumococci. *Bull. acad. polon sci., Classe (II)* **8,** 269–274.

Phillips, J. H., Braun, W., and Plescia, O., 1957. Studies on the antigenicity of bacterial DNA. *Records Genet. Soc. Am.* **26,** 389.

Pontecorvo, G., 1958. "Trends in Genetic Analysis." Columbia Univ. Press, New York.

Prestidge, L. S., and Pardee, A. B., 1957. Induction of bacterial lysis by penicillin. *J. Bacteriol.* **74,** 48–59.

Pritchard, R. H., 1955. The linear arrangement of a series of alleles in *Aspergillus nidulans. Heredity* **9,** 343–371.

Pritchard, R. H., 1960. The bearing of recombination analysis at high resolution on genetic fine structure in *Aspergillus nidulans* and the mechanism of recombination in higher organisms. *Symp. Soc. Gen. Microbiol.* **10,** 155–180.

Rapaport, J. A., 1946. *Compt. rend. acad. sci. U.R.S.S.* **54,** 65.

Rappaport, H. P., and Guild, W. R., 1959. Defined medium for two transformable strains of *Diplococcus pneumoniae. J. Bacteriol.* **78,** 203–205.

Ravin, A. W., 1954. A quantitative study of autogenic and allogenic transformations in pneumococcus. *Exptl. Cell Research* **7,** 58–82.

Ravin, A. W., 1956. The properties of bacterial transforming systems. *Brookhaven Symposia in Biol.* No. 8, 33–49.

Ravin, A. W., 1957. The significance of bacterial transformations to studies of the neoplastic process. *Ann. N.Y. Acad. Sci.* **68,** 335–348.

Ravin, A. W., 1958. Specificity in the efficiency of response by pneumococcus to different transforming agents. *Proc. Intern. Congr. Genet. 10th Congr., Montreal* **2,** 228–229.

Ravin, A. W., 1959a. Reciprocal capsular transformations of pneumococci. *J. Bacteriol.* **77,** 296–309.

Ravin, A. W., 1959b. Reversions at loci affecting capsule synthesis and erythromycin-resistance in pneumococcus. *Bacteriol. Proc. (Soc. Am. Bacteriologists)* **59,** 36.

Ravin, A. W., 1960a. The origin of bacterial species. *Bacteriol. Rev.* **24,** 201–220.

Ravin, A. W., 1960b. Linked mutations borne by deoxyribonucleic acid controlling the synthesis of capsular polysaccharide in pneumococcus. *Genetics* **45,** 1387–1404.

Ravin, A. W., and Iyer, V. N., 1961. The genetic relationship and phenotypic expression of mutations endowing pneumococcus with resistance to erythromycin. *J. Gen. Microbiol.* **66,** 1–25.

Reichmann, M. E., Rice, S. A., Thomas, C. A., and Doty, P., 1954. The molecular weight and shape of desoxypentose nucleic acid. *J. Am. Chem. Soc.* **74,** 3203–3204.

Rolfe, R., and Meselson, M., 1959. The relative homogeneity of microbial DNA. *Proc. Natl. Acad. Sci. US.* **45,** 1039–1043.

Roman, H., 1956. Studies of gene mutation in *Saccharomyces. Cold Spring Harbor Symposia Quant. Biol.* **21,** 175–183.

Roman, H., and Jacob, F., 1958. A comparison of spontaneous and ultraviolet-induced allelic recombination with reference to the recombination of outside markers. *Cold Spring Harbor Symposia Quant. Biol.* **23,** 155–160.

Rosenberg, B. H., Sirotnak, F. M., and Cavalieri, L. F., 1959. On the size of genetic determinants in pneumococcus and the nature of the variables involved in transformation. *Proc. Natl. Acad. Sci. US.* **45,** 144–156.

Rupert, C. S., 1960. Photoreactivation of transforming DNA by an enzyme from Baker's yeast. *J. Gen. Physiol.* **43,** 573–595.

Rupert, C. S., and Goodgal, S. H., 1960. Shape of ultraviolet inactivation curves of transforming desoxyribonucleic acid. *Nature* **185,** 556–557.

Rupert, C. S., Goodgal, S. H., and Herriott, R. M., 1958. Photoreactivation *in vitro* of ultraviolet-inactivated *Hemophilus influenzae* transforming factor. *J. Gen. Physiol.* **41,** 451–471.

Schaeffer, P., 1956a. Transformation interspécifique chez des bactéries du genre *Hemophilus. Ann. inst. Pasteur* **91,** 192–211.

Schaeffer, P., 1956b. Analyse génétique de la résistance à la streptomycine chez le pneumocoque. *Ann. inst. Pasteur* **91,** 323–337.

Schaeffer, P., 1957a. Existence d'une compétition entre molécules d'acides désoxyribonucléiques pour la pénétration dans les bactéries transformables. *Compt. rend. acad. sci.* **245,** 230–231.

Schaeffer, P., 1957b. La pénétration de l'acide nucléique dans les bactéries réceptrices au cours des transformations interspécifiques. *Compt. rend. acad. sci.* **245,** 375–377.

Schaeffer, P., 1957c. L'inhibition de la transformation comme moyen de mesure de la "compétence" bactérienne. *Compt. rend. acad. sci.* **245,** 451–453.

Schaeffer, P., 1958. Interspecific reactions in bacterial transformations. *Symposia Soc. Exptl. Biol.* **12,** 60–74.

Schaeffer, P., and Ionesco, H., 1959. Sur la transformation de *Bacillus subtilis. Compt. rend. acad. sci.* **249,** 481–482.

Schaeffer, P., and Ritz, E., 1955. Transfert interspécifique d'un caractère héréditaire chez des bactéries du genre *Hemophilus. Compt. rend. acad. sci.* **240,** 1491–1493.

Schaeffer, P., Ionesco, H., and Jacob, F., 1959. Sur le déterminisme génétique de la sporulation bactérienne. *Compt. rend. acad. sci.* **249,** 577–578.

Schmidt, G., 1955. Nucleases and enzymes attacking nucleic acid components. *In* "Nucleic Acids" (E. Chargaff and J. N. Davidson, eds.), Vol. I, pp. 555–626. Academic Press, New York.

Setlow, R., and Doyle, B., 1954. The action of radiation on dry desoxyribonucleic acid. *Biochim. et Biophys. Acta.* **15,** 117–125.

Sirotnak, F. M., Lunt, R., and Hutchison, D. J., 1960a. Genetic studies on amethopterin resistance in *Diplococcus pneumoniae. J. Bacteriol.* **80,** 648–653.

Sirotnak, F. M., Lunt, R., and Hutchison, D. J., 1960b. Transformation of resistance to 8-azaguanine in *Diplococcus pneumoniae*. *Nature* **187,** 800–801.

Spizizen, J., 1957. Infection of protoplasts by disrupted T2 virus. *Proc. Natl. Acad. Sci., U.S.* **43,** 694–701.

Spizizen, J., 1958. Transformation of a biochemically deficient strain of *Bacillus subtilis* by deoxyribonucleate. *Proc. Natl. Acad. Sci. U.S.* **44,** 1072–1078.

Spizizen, J., 1959. Genetic activity of deoxyribonucleic acid in the reconstitution of biosynthetic pathways. *Federation Proc.* **18,** 957–965.

Sterne, M., and Proom, H., 1957. Induction of motility and capsulation in *Bacillus anthracis*. *J. Bacteriol.* **74,** 541–542.

Stocker, B. A. D., Krauss, M. R., and MacLeod, C. M., 1953. Quantitative experiments on pneumococcal transformation. *J. Pathol. Bacteriol.* **66,** 330–331.

Suoeka, N., 1960. Mitotic replication of deoxyribonucleic acid in *Chlamydomonas reinhardi*. *Proc. Natl. Acad. Sci. U.S.* **46,** 83–91.

Suoeka, N., Marmur, J., and Doty, P., 1959. Dependence of the density of desoxyribonucleic acids on guanine-cytosine content. *Nature* **183,** 1429–1431.

Swift, H. H., 1953. Quantitative aspects of nuclear nucleoproteins. *Intern. Rev. Cytol.* **2,** 1–76.

Szybalski, W., Opara-Kubinska, Z., Lorkiewicz, Z., Ephrati-Elizur, E., and Zamenhof, S., 1960. Transforming activity of deoxyribonucleic acid labelled with 5-bromouracil. *Nature* **188,** 743–745.

Talmadge, M. B., and Herriott, R. M., 1960. A chemically defined medium for growth, transformation and isolation of nutritional mutants of *Hemophilus influenzae*. *Biochem. Biophys. Research Commun.* **2,** 203–206.

Taylor, H. E., 1949a. Transformations réciproques des formes R et ER chez le pneumocoque. *Compt. rend. acad. sci.* **228,** 1258–1259.

Taylor, H. E., 1949b. Additive effects of certain transforming agents from some variants of pneumococcus. *J. Exptl. Med.* **89,** 399–424.

Taylor, J. H., Woods, P. S., and Hughes, W. L., 1957. The organization and duplication of chromosomes as revealed by autoradiographic studies using tritium-labeled thymidine. *Proc. Natl. Acad. Sci. U.S.* **43,** 122–128.

Thomas, R., 1954. Recherches sur la dénaturation des acides désoxyribonucléiques. *Biochim. et Biophys. Acta* **14,** 231–240.

Thomas, R., 1955. Recherches sur la cinétique des transformations bactériennes. *Biochim. et Biophys. Acta* **18,** 467–481.

Thomas, R., 1957. Transferts génétiques à l'échelle macromoléculaire. "Mecanismes d'Autoréproduction," pp. 253–291. Masson, Paris.

Thomas, R., 1960. Observations relatives au role des protéines du milieu dans les phénomènes de transformations bactériennes. *Biochim. et Biophys. Acta* **40,** 50–61.

Udaka, S., Koukol, J., and Vennesland, R., 1959. Lactic oxidase of pneumococcus. *J. Bacteriol.* **78,** 714–725.

Voll, M. J., and Goodgal, S. H., 1961. Recombination during transformation in *Hemophilus influenzae*. *Proc. Natl. Acad. Sci. U.S.* **47,** 505–512.

von Borstel, R. C., and Wolff, S., 1955. Photoreactivation experiments on the nucleus and cytoplasm of the *Habrobracon* egg. *Proc. Natl. Acad. Sci. U.S.* **41,** 1004–1009.

Wacker, A., 1959. Bakterien-Transformation. *In* "Chemie der Genetik," 9. Colloquium der Gesellschaft für physiologische chemie, pp. 85–93. Springer, Berlin.

Wacker, A., and Laschet, L., 1960. Übertragung der Streptomycin-Empfindlichkeit bei *E. coli*. *Arzneimittel-Forsch.* **10,** 488–489.

Watson, J. D., and Crick, F. H. C., 1953a. A structure for desoxyribosenucleic acids. *Nature* **171,** 737–738.

Watson, J. D., and Crick, F. H. C., 1953b. Genetical implications of the structure of desoxyribosenucleic acids. *Nature* **171,** 964–967.

Watson, J. D., and Crick, F. H. C., 1953c. The structure of DNA. *Cold Spring Harbor Symposia Quant. Biol.* **28,** 123–131.

Weil, A. J., and Binder, M., 1947. Experimental type transformation of *Shigella paradysenteriae* (Flexner). *Proc. Soc. Exptl. Biol. Med.* **66,** 349–352.

Weinstein, A., 1928. Four strand crossing over. *Anat. Record.* **41,** 109–110.

Weinstein, A., 1936. The theory of multiple strand crossing over. *Genetics* **21,** 155–199.

Weygand, F., Wacker, A., and Dellweg, H., 1952. Stoffwechseluntersuchungen bei Mikroorganismen mit Hilfe radioktiver Isotope. II. Kompetitive und nicht-kompetitive Enthemmung von 5- ^{82}Br-Uracil. *Z. Naturforsch.* **7b,** 19–28.

Wilkins, M. H. F., Stokes, A. R., and Wilson, H. R., 1953. Molecular structure of desoxypentose nucleic acids. *Nature* **171,** 738–740.

Wollman, E. L., Jacob, F., and Hayes, W., 1956. Conjugation and genetic recombination in *Escherichia coli* K12. *Cold Spring Harbor Symposia Quant. Biol.* **21,** 141–162.

Zamenhof, S., 1956. Biology and biophysical properties of transforming principles. *Progr. in Biophys. and Biophys. Chem.* **6,** 85–120.

Zamenhof, S., 1957. Properties of the transforming principles. *In* "Chemical Basis of Heredity" (W. McElroy and B. Glass, eds.), pp. 351–377. Johns Hopkins Univ. Press, Baltimore, Maryland.

Zamenhof, S., and Griboff, G., 1954. Incorporation of halogenated pyrimidines into the deoxyribonucleic acids of *Bacterium coli*. *Nature* **174,** 306.

Zamenhof, S., Leidy, G., Alexander, H. E., Fitzgerald, P. L., and Chargaff, E., 1952. Purification of the desoxypentose nucleic acid of *Hemophilus influenzae* having transforming activity. *Arch. Biochem. Biophys.* **40,** 50–55.

Zamenhof, S., Alexander, H. E., and Leidy, G., 1953. Studies on the chemistry of the transforming activity. I. Resistance to physical and chemical agents. *J. Exptl. Med.* **98,** 373–397.

Zamenhof, S., Leidy, G., Hahn, E., and Alexander, H. E., 1956. Inactivation and unstabilization of the transforming principle by mutagenic agents. *J. Bacteriol.* **72,** 1–11.

Zamenhof, S., Leidy, G., Greer, S., and Hahn, E., 1957. Differential stabilities of individual hereditary determinants in transforming principle. *J. Bacteriol.* **74,** 194–199.

Zinder, N. D., and Lederberg, J., 1952. Genetic exchange in *Salmonella*. *J. Bacteriol.* **64,** 679–699.

SOME CONTRIBUTIONS TO POPULATION GENETICS RESULTING FROM THE STUDY OF THE LEPIDOPTERA

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Page

I. Introduction 165
II. Fluctuations in Selective Value 167
III. Industrial Melanism 170
IV. Protective Coloration 177
A. Cryptic Coloration 177
B. Eye Spots 180
C. Warning Coloration 181
D. Müllerian Mimicry 181
E. Batesian Mimicry 182
V. The Evolution of Dominance 188
VI. Sex-Controlled Inheritance 192
VII. The Evolution of Super-Genes 196
VIII. Disruptive Selection 197
IX. Polygenically Controlled Characters in *Euphydryas aurinia* 200
A. Fluctuations in Population Size of the Colony of *Euphydryas aurinia* 201
B. Variability of Wing Pattern between 1894–1935 201
C. Fluctuations in Population Size and Evolution 202
X. Selection and Polygenically Controlled Characters in *Maniola jurtina* . 203
A. The Spot-Frequency Distribution in Southern England 203
B. The Spot-Frequency Distributions in Northern Scotland, Ireland, the Isle of Man, and Normandy 204
C. The Spot-Frequency Distributions in West Devon and the Cornish Mainland 204
D. The Spot-Frequency Distribution in the Isles of Scilly 206
E. Natural Selection and Spot-Number 207
XI. Polygenically Controlled Characters in *Panaxia dominula* 209
XII. Summary 211
Acknowledgments 212
References 212

I. Introduction

Opinions based on mathematical models concerning the mechanisms of evolution can be confirmed or refuted only by observation and experiment in laboratory and wild populations. For this purpose the genus *Drosophila* is admirably suited for detailed genetic analysis of particular

situations. However, any general views on evolutionary stability or change, based on the study of one genus, must be highly biased, particularly so in the case of *Drosophila,* where the ecology of most species is a complete mystery.

Unfortunately, so many people have worked with *Drosophila* and so few with other organisms that we are not able, at the present time, to make a full comparative study of the mechanisms of evolution. It has only been possible for those not primarily concerned with the genetics of this genus to investigate a number of loosely related evolutionary problems for which *Drosophila* provides exceptionally poor material. Consequently any account of population genetics in the Lepidoptera is of necessity somewhat disjointed and must be divided up under a number of separate headings. Moreover, in the main the results obtained concern the genetics and evolutionary significance of color patterns, for the study of which butterflies and moths provide particularly good material. The physiological effects of genes have been little examined except insofar as these are revealed in the color pattern differences between forms. Problems of cytological interest are also hardly investigated since the chromosomes of the Lepidoptera are small and numerous. However, should the new techniques developed for human chromosomes prove modifiable for use with butterflies and moths, large advances can be expected. The facts that (1) the taxonomy of the Lepidoptera is well understood, (2) the distribution and ecology of many species are accurately known and, (3) hybrids between species as well as subspecies can often be produced, should make the Lepidoptera potentially useful for cytological studies of evolution.

In this review I shall refrain from discussing in detail the results obtained from interspecific hybridization in the Lepidoptera except where these throw light on the evolution of species differences. Most hybridization experiments have been concerned with problems of sex determination (see Cockayne, 1938), a subject requiring a separate review. It may be noted in passing, however, that if there is a deficiency of one sex among the offspring of such a cross it is usually the females, the heterogametic sex, which are rare or absent (see Clarke and Sheppard, 1955a,b, 1956, 1957; Aé, 1959; and Remington, 1959). The production of intersexes in such crosses, a phenomenon so cleverly exploited by Goldschmidt (1934) in the analysis of sex determination, seems to be rather uncommon.

I shall also not cover much of what is known about the genetics of polymorphism in the Lepidoptera. Ford (1953) has given an excellent general account of this subject and Remington (1954) has discussed the genus *Colias.* It would be a complete waste of space for me to restate

what they have said. Consequently, in this review, I shall not concern myself with information already supplied by them, except insofar as such knowledge is essential for understanding new advances.

II. Fluctuations in Selective Value

In the early days of population genetics Wright (1931, 1932), as a result of his mathematical models, concluded that genetic drift was essential for sustained evolutionary change to occur. Fisher (1930), although not ignoring the effect of random sampling errors on gene-frequency, believed that they were unimportant in evolution. Wright's argument was based on the assumption that selective values are small, usually 1% or less, and comparatively constant in direction.

In order to investigate these two assumptions Fisher and Ford (1947) decided in 1939 to study the frequency of a particular gene in a population of the scarlet tiger moth, *Panaxia dominula,* and to assess the maximum contribution of genetic drift to gene-frequency change by estimating the size of the population from year to year. The gene chosen seemed particularly apt for their purpose, since (1) the heterozygote var. *medionigra* is recognizably distinct from the two homozygotes var. *bimacula* and the normal wild form, *dominula;* (2) the gene was only known from the one colony near Oxford (except as an extreme rarity); (3) the species lives in isolated colonies with little or no migration from one to another; and (4) it was believed to be distasteful to birds and warningly colored, so that any selection detected was unlikely to reflect differential predation on the three phenotypes, but to result from the physiological effects of the allelomorphs concerned.

Fisher and Ford showed that the gene-frequency changed from generation to generation and that the change could not be accounted for by genetic drift alone. Further studies (Sheppard, 1951, 1952, 1953a, 1956) have demonstrated not only that the changes in gene-frequency could not be due to drift [a view challenged by Wright (1948)] but also that the selective value of the genotypes fluctuated in direction and intensity from year to year. All the characters controlled by the gene and subject to selection have not yet been revealed, and consequently the reasons for the changes in frequency have not been determined. However, it is known that the inherited factor affects color pattern, mating preference under laboratory conditions, male fertility, and survival in the larval stages. The gene does not apparently affect the mean date of emergence of the insects.

The study of this colony has continued until the present time, thus providing one of the most extensive records of population size and gene-frequency from generation to generation for any wild animal (see Table

1). It will be seen that initially there was a rapid fall in gene-frequency, but that since 1947 there has been no constant trend but fluctuation about a mean frequency of approximately 3%.

Quite early in the investigation it became apparent that the polymorphism was not being maintained by the heterozygote being at an advantage to both homozygotes. The fluctuations in the gene-frequency

TABLE 1

Quantitative Data from the Colony of *Panaxia dominula* at Cothill *

Year	Phenotypes found			Frequency of *medionigra* gene (%)	Estimated population size
	dominula	*medionigra*	*bimacula*		
Up to 1928	164	4	0	1.2	—
1939	184	37	2	9.2	—
1940	92	24	1	11.1	—
1941	400	59	2	6.8	2,000– 2,500
1942	183	22	0	5.4	1,200– 2,000
1943	239	30	0	5.6	1,000
1944	452	43	1	4.5	5,000– 6,000
1945	326	44	2	6.5	4,000
1946	905	78	3	4.3	6,000– 8,000
1947	1,244	94	3	3.7	5,000– 7,000
1948	898	67	1	3.6	2,600– 3,800
1949	479	29	0	2.9	1,400– 2,000
1950	1,106	88	0	3.7	3,500– 4,700
1951	552	29	0	2.5	1,500– 3,000
1952	1,414	106	1	3.6	5,000– 7,000
1953	1,034	54	1	2.6	5,000–11,000
1954	1,097	67	0	2.9	10,000–12,000
1955	308	7	0	1.1	1,500– 2,500
1956	1,231	76	1	3.0	7,000–15,000
1957	1,469	138	5	4.6	14,000–18,000
1958	1,289	94	4	3.7	10,000–20,000
1959	460	19	1	2.2	5,500– 8,500
1960	182	7	0	1.9	1,000– 4,000

* The data are from Fisher and Ford (1947), Sheppard (1951, 1953a, 1956), and Ford and Sheppard (unpublished).

are too large to be accounted for by changes in the relative selective values of the two homozygotes, when one remembers that on this hypothesis both have to be at a disadvantage to the heterozygote. This aspect of the work has been considered in detail by Williamson (1961).

It soon became obvious that the selective values of the three genotypes could not be estimated with any accuracy, since the heterozygote *medionigra* and particularly the rare homozygote *bimacula* had become too uncommon for accurate measurements of their frequency to be

made. Consequently it was decided to establish a new colony in a suitable place, where the insect was not to be found, and to ensure that the gene-frequency was high enough for that purpose. The artificial colony was started in a damp valley near Oxford where the food plants of *P. dominula* are abundant, and where the ecological conditions seemed very suitable for the species (Sheppard, 1956). About 4000 eggs derived from the backcross between *medionigra* and *dominula* were put down in the valley in the summer of 1951. A sample of thirty-one larvae was collected the next April, when the larvae were full grown, and the frequency of *medionigra* determined from the adults produced. There was a significant deficiency ($P < 0.05$) of *medionigra* from the 1:1 ratio of *medionigra* to *dominula* expected. It was known that in the laboratory a good 1:1 ratio is always obtained so that the results indicate that, in this year under the conditions prevailing, the *medionigra* eggs or larvae were at a great disadvantage. The colony was small at this time and grew slowly in size. It was therefore decided to sample the gene-frequency from year to year by collecting, marking, and releasing the adults, rather than from collections of larvae. However, although a number of larvae were seen each spring no adults were observed between 1952 and 1956, when observation stopped. However, by 1959 when the colony was revisited the larvae had become quite common and a sample of them was taken (Table 2). The frequency of *medionigra*

TABLE 2

Phenotypes of Moths Obtained from Larvae in an Experimental Colony * of *Panaxia dominula* near Oxford †

Date of taking larvae	*dominula*	*medionigra*	*bimacula*	Gene-frequency (%)
April 1952	21	9	0	15.0
April 1959	20	3	0	6.5
April 1960	269	32	3	6.2

* Colony started in July 1951 with a gene-frequency of 25.0%.

† The data from Sheppard (1953a, and unpublished).

proved to be of sufficient interest for me to take a large sample in April 1960, the larvae being very abundant and the colony obviously large. It will be noted from the table that between 1951, when the gene-frequency was 25.0%, and 1960, there had been a considerable drop in the frequency of the "mutant" gene, indicating strong selection against it. However, the greatest change occurred during the first generation, although there has been a significant reduction in gene-frequency ($P < 0.01$) between 1952 and 1960. Consequently we must conclude

either (1) that *medionigra* is at a great disadvantage in the egg or larva, but at an advantage in the pupa and/or imago, or (2) that the selective value of the genotypes fluctuated wildly between 1951 and 1960. It is necessary to continue the experiment to distinguish between these two possibilities, and to establish new artificial colonies. One such colony is now being started in Cheshire, where the insect is not found. Because of the method of founding the colony it is hoped that the results from it will prove more satisfactory than those from the other experimental population. The method may prove useful elsewhere, and therefore a short account of it seems appropriate.

Two of the disadvantages of the original experiment are (1) the small initial size of the population, and (2) the change in the ecology of the habitat as a result of the larvae modifying the growth of their main food plant by eating out the young shoots in spring. Such a change might well alter the selective value of the genotypes, making it different in the second and subsequent generations of the experiment. To avoid these difficulties the second colony was started by putting out eggs from the cross *dominula* × *bimacula*. All the larvae were in consequence heterozygotes and no differential selection could take place in the first year when the plants were unmodified. The second advantage of this plan is that all the matings between the first season's adults must be between heterozygotes. The population size could be further augmented, therefore, by mating *medionigra* with *medionigra* and releasing the resulting eggs into the artificial colony. Thus, not only will the colony be larger in the second generation when selection can act, but the expected ratio of the phenotypes is exactly one *dominula* to two *medionigra* to one *bimacula*, which allows a very sensitive test for any effects of selection.

III. Industrial Melanism

The allelomorph responsible for the forms *medionigra* and *bimacula* in the scarlet tiger moth increases the amount of melanin on the wings. But, as already explained, this does not have much effect on the liability of the moth to attack by predators. There is some evidence, however, that the black *bimacula* is slightly more liable to attack by birds because of its increased conspicuousness when at rest and on the wing (Sheppard, 1953a).

There are a large number of species of moth, however, which have a black and a nonblack form, where the two are often subject to very different rates of attack, and therefore have very different selective values. The spread of such black forms is particularly noticeable in the manufacturing towns of industrial countries, and the phenomenon

is known therefore as industrial melanism. Since the sequence of events has been much the same in the British Isles (where about 70 species have such melanics), in Europe, and in North America, it is convenient to describe what has occurred in England, where it has been studied in most detail, particularly in one species, the peppered moth, *Biston betularia.*

Until the middle of the last century this species, which rests exposed on tree trunks and branches during the day, was typically a highly cryptic form with a black and white speckled pattern. Just prior to 1850 a black form *carbonaria* (Fig. 1) was caught in the industrial city

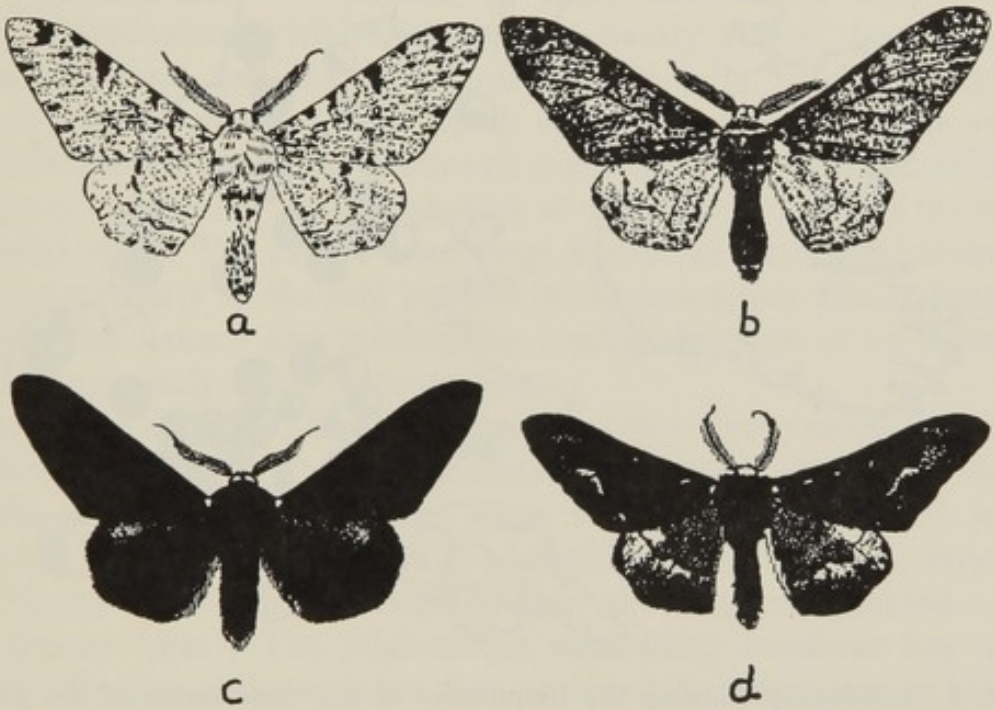


FIG. 1. There are three main forms of the black and white peppered moth, *Biston betularia:* the typical form (a) and the two dominant melanics (b) *insularia,* and (c) and (d) *carbonaria.* The two specimens of *carbonaria* are not identical; (c) is a heterozygote (black being dominant) and (d) is a moth caught in the middle of the nineteenth century and probably a heterozygote. The melanic *insularia* (b), although looking intermediate between typical and *carbonaria,* is in fact controlled at a different locus.

of Manchester. It was soon found in other large cities, and in all of them it increased rapidly in frequency. By 1906 the form had become common in a large number of places (see Kettlewell, 1958) and, in fact, far outnumbered the original speckled one in several industrial towns. Kettlewell (1958) has investigated the present distribution and frequency of *carbonaria,* which has been shown to be an autosomal dominant (see Bowater, 1914). Kettlewell's map (Fig. 2) and tables reveal that in many manufacturing districts the frequency of the black form



FIG. 2. Sketch-map showing the frequencies of the three forms of the peppered moth, *Biston betularia,* in Great Britain. The circles show the localities in which collections were made and their size indicates the number of moths in the sample. The largest circles represent collections of over 100 moths and the smallest samples of between 25 and 50 moths. The size of the black area of each circle gives the percentage of *carbonaria,* the hatched area the percentage of *insularia,* and the white area the percentage of the typical form. A single dot in the center of the circle means that only one moth of that variety was taken in the collection. Note that the proportion of *carbonaria* is high in industrial regions and to the east of them but decreases rapidly to the west, south, or north. The proportion of *insularia* compared with typical behaves in much the same way, showing that it also is an industrial melanic.

is well over 90%, and attains only slightly lower values in rural areas far to the east of such centers (Lowestoft, Suffolk, 75%; see Table 3). At the present time *carbonaria* is rare or absent only in the southwest of England, in the north of Scotland, in Ireland and, perhaps, in parts of Wales.

The speed at which the black form has spread indicates that it must be at a very considerable advantage in manufacturing districts, and Kettlewell (1955, 1956) has investigated the factors involved. The method he employed was to release males of both forms near Birmingham, a very large industrial city, and in Dorset, a rural area, and record the proportions of the forms that were recaptured subsequently. He also kept watch on moths which he had released on to tree trunks. He showed that in Dorset, when the trees are covered with lichens, the black form is seen and devoured by many species of birds more frequently than the speckled variety, which at a distance is almost indistinguishable from the lichens. In sharp contrast to this, he found that in Birmingham visible lichens were nearly absent, because of the smoke pollution, and that the trees were heavily blackened by soot. Here it was the speckled form which was the more conspicuous and the more often eaten by birds. Kettlewell pointed out that this selection was capable of explaining the distribution of *carbonaria,* even to the east of industrial cities. The smoke from large towns can drift for hundreds of miles, particularly to the east because of the prevailing winds, destroying many of the lichens and putting the black specimens at an advantage even in apparently rural environments.

Industrial melanism in the peppered moth is made more complex by the fact that there is a second autosomal dominant, *insularia,* which is also an industrial melanic. The locus is apparently unlinked to that producing *carbonaria. Insularia* is a speckled form (Fig. 1), very variable in appearance but far darker than the original speckled variety (the typical form). The allelomorph controlling *insularia* has no detectable effect in *carbonaria,* so that an insect, heterozygous or homozygous for *carbonaria,* is black, whether it be carrying the gene for *insularia* or not.

Kettlewell's (1958) data show that the gene controlling *insularia* never reaches the high frequency of that producing *carbonaria*: seldom as much as 50% of the non-*carbonaria* insects are *insularia.* Moreover, its frequency is very variable even in highly industrialized areas. Thus, on Caldy Common, near Hoylake, Cheshire, where the proportion of *carbonaria* is high, *insularia* is uncommon compared with the typical form, whereas in London, and in Bradford, Yorkshire, it is common (see Table 3).

It seems possible that the lower frequencies of the gene controlling *insularia,* compared with those of the allelomorph determining *carbonaria,* are the result of its being at an advantage in the heterozygote compared with the homozygote, for some physiological reason. However, this does not explain the absence of a good correlation between the

frequency of *insularia* and *carbonaria* in Kettlewell's data. Nor can genetic drift account for the variability since the populations concerned must be very large and migration between adjacent ones common. It seems more likely that the gene has physiological effects which give different selective values in different areas, but that in very rural en-

TABLE 3

The Per Cent Frequencies of the Non-Melanic Form and the Two Melanics, *insularia* and *carbonaria*, of *Biston betularia* from Some Selected Localities

Environment and locality	Date	T*	C*	I*	Total
Industrial					
Caldy, Cheshire	1959	6.01	93.29	0.71	283
	1960	4.89	94.22	0.89	225
Manchester	1952–56	0.00	98.00	2.00	350
Bradford, Yorkshire	1952–56	1.97	95.67	2.36	508
Glasgow	1952–56	10.33	89.66	0.00	29
London area	1952–56	5.81	90.21	3.98	327
Semi-industrial					
Oxford	1952	45.65	28.26	26.09	46
	1954	42.50	34.20	23.50	219
	1956	34.82	39.70	25.40	224
Penrith, Cumberland	1952–56	60.71	26.79	12.50	56
"Rural," to the east of industrial areas					
Fritton, Norfolk	1952–56	14.60	77.37	8.03	137
Lowestoft, Suffolk	1952–56	17.86	75.00	7.14	56
"Rural," to the north or west of industrial areas					
Kinloch Rannoch, Scotland	1952–56	100.00	0.00	0.00	285
Torquay, Devon	1952–56	100.00	0.00	0.00	100
Dolgelley, North Wales	1952–56	95.37	2.78	1.85	108

*T, C, and I signify the non-melanic, *carbonaria*, and *insularia* forms, respectively. Data from Kettlewell (1958) and Clarke and Sheppard (unpublished).

vironments visual selection against the conspicuous *insularia* outweighs any physiological advantage it may have. What does seem clear is that *insularia* is at a visual advantage to the typical form in areas suffering mild pollution, where the more extreme *carbonaria* is not yet at the huge advantage (probably of the order of 15% or more), which it is in highly industrial regions.

That the allelomorphs producing industrial melanics in some other species possess physiological effects subject to natural selection has long been recognized. Ford (1940) showed that the black form of *Cleora*

repandata, the mottled beauty, is more hardy than the pale one during the immature stages, particularly if the larvae are starved. Bowater (1914) came to the same conclusion in respect of *Gonodontis bidentata,* the scalloped hazel. However, his data did not show a significant excess of melanics (Ford, 1953). More recent work by Bowater (1955, unpublished) which he has kindly allowed me to use, shows a much larger nonsignificant excess of melanics in his backcross broods when brood 51.9 is excluded (Table 4). If it is included, there is a significant excess

TABLE 4

Modern Backcross Broods of *Gonodontis bidentata* and *Biston betularia* Segregating for Melanics and Non-melanics *

Brood	Melanic	Non-melanic	Total
G. bidentata			
Melanic × non-melanic			
50.7	5	5	10
51.8	50	31	81
51.9	30	3	33
51.10	20	12	32
52.3	38	32	70
52.10	45	50	95
52.11	42	44	86
52.12	27	20	47
53.6	8	7	15
53.13	25	30	55
54.19	9	6	15
55.25	1	2	3
55.27	11	12	23
55.28	4	6	10
	315	260	575
B. betularia			
Melanic × non-melanic			
4409 (Males)	31	22	53
4409 (Females)	28	52	80
	59	74	133

* Data for *G. bidentata* from Bowater (unpublished) and for *B. betularia* from Clarke and Sheppard (unpublished). Brood 51.9 appears to be heterogeneous with the other *G. bidentata* broods. The melanic in brood 4409 was *carbonaria.*

of melanics but also heterogeneity between broods. Bowater's 1914 F_2 data give a very close agreement to the expected 3:1 ratio, but the 1955 results show a nonsignificant excess of melanics (Table 5).

Kettlewell (1958), using the peppered moth, has compared the

backcross broods between melanic *carbonaria* and the typical form, obtained between 1900 and 1905, with his broods produced fifty years later. He finds that they are significantly different, and that the early broods show a nonsignificant deficiency of melanics, whereas his own show a significant excess of them. Thus, in both these comparisons modern broods show an excess of melanics (though not a significant one in the case of *G. bidentata*), and there is some evidence that in an earlier stage in the evolution of industrial melanism this excess did not exist or

TABLE 5

Modern Broods of *Gonodontis bidentata* Expected to Segregate for Melanics and Non-melanics in a 3:1 Ratio *

Brood	Melanic	Non-melanic	Total
50.1	14	3	17
50.3	6	1	7
50.4	32	5	37
50.5	36	4	40
52.2	50	23	73
52.9	5	1	6
53.14	31	3	34
53.18	12	2	14
53.20	35	5	40
53.25	7	4	11
53.26	15	1	16
53.28	48	19	67
53.31	5	4	9
54.1	5	3	8
54.6	10	6	16
55.12	11	4	15
55.16	3	4	7
	325	92	417

* Data from Bowater (unpublished).

was much smaller. There is therefore some reason to believe that the melanics, which are now at some physiological advantage to the original form, were not at an advantage in the beginning, and that this advantage has evolved by a reconstruction of the gene-complex. That such a reorganization can happen rapidly in some insects is demonstrated by Dobzhansky's work on *Drosophila* inversions.

It is clear, however, that much more work is urgently needed on this subject, since (1) Clarke, in a brood of *Biston betularia* reared from an industrial stock and twice as large as any of Kettlewell's, obtained a good 1:1 segregation with, if anything, a deficiency of melanics (Table 4). If this brood is included with the other modern broods there is heterogeneity between them ($P < 0.01$). Clarke's brood did, however,

show a significant difference in the frequency of *carbonaria* in the two sexes ($P < 0.02$), a point which needs further study; (2) Bowater's 1955 excess of melanics is not great enough to be significant unless a backcross brood which is obviously heterogeneous with the others is included.

The study of industrial melanism not only demonstrates the very large selective values which can result from a difference in camouflage between insects, but also shows that single genes affecting pattern can also have very important effects on the physiology of the animal. Moreover, there is some evidence that the spread of a major gene can lead to the reorganization of part of the gene-complex of an organism (see also below).

Nearly all industrial melanics are dominant or semidominant, although recessive nonindustrial melanics are known. There may be several reasons for this, the two most important probably being that dominance may be evolved if the form is at a high frequency in the population for a long time (see below); and secondly, if two genes have the same mutation rate, but one is recessive in effect whereas the other is dominant (or semidominant), the latter will have a better chance of establishing itself, since it will be exposed to selection from the moment the gene first mutates. However, despite this advantage of dominants, there is one recessive melanic which has spread to a small extent in the Oak Eggar, *Lasiocampa quercus*. The point of particular interest in this example of recessive industrial melanism is that there are a number of allelomorphs involved, or more probably closely linked loci, some of which affect melanism in the larva. Thus, in Yorkshire, black moths are usually produced from black larvae (black again being recessive), although a small proportion come from brown larvae. Black larvae are almost certainly less conspicuous in this particular Yorkshire locality than are brown ones. In the north of Scotland, where similar black moths are also to be found, black larvae are not present (Kettlewell, 1959a). It may well be that there is a super-gene involving more than one locus since, in the Lepidoptera, mutants affecting one stage in the life history very rarely affect others. This would be expected because the ecology of any two stages is quite different, and mutants advantageous in, say, the larva, would be likely to be deleterious in some other stage, say, the adult, if they had any effect.

IV. Protective Coloration

A. Cryptic Coloration

Industrial melanism illustrates the importance of cryptic coloration in a most spectacular way, because of the great ecological changes

produced by the Industrial Revolution. However, many other methods of camouflage are used in the Lepidoptera, and most of these must have been in existence, not for a mere 150 years, but for many millions of years. Consequently, very pronounced selection must have been acting continuously for a vast period of time on such characters. General accounts of protective devices will be found in the works of many authors, including the recent reviews by Cott (1940) and, with special reference to the Lepidoptera, by Ford (1955a), Kettlewell (1959b), and Sheppard (1959a). The degree of specialization evolved in the Lepidoptera to guard against attack by predators will be familiar to many naturalists. However, a few examples may be helpful to illustrate the complex adaptations which have been evolved as a result of selection by predators. It will be realized that during the development from egg to adult, a butterfly or moth will live in a series of very different environments, in each of which it must be protected. Consequently the method of protection may change from stage to stage. Thus, the larva of the pine hawk moth, *Hyloicus pinastri,* when small, sits on the pine needles on which it feeds. If the larva were plain green it would be a conspicuous object, since it would be far thicker than a needle. However, the caterpillar at this stage has yellow lines running longitudinally from head to tail, and these divide the green into a series of stripes of about the same width as the pine needles. This disruptive pattern makes the larva exceedingly difficult to see. It is also of interest to note that some other pine feeders use exactly the same device to render them cryptic. The full-grown larva is too large to be concealed in this way, and it changes its appearance to one of two alternative patterns. It either becomes a mottled brown all over, or it develops a mottled brown band along the dorsal surface and is mottled green on the sides. The mottled brown form is almost exactly like the branch on which it sits, and the head is a good imitation of a bud. The brown and green form also imitates a branch, but a much thinner one, the green sides of the larva blending with the green needles. The brown form is better concealed sitting exposed on the branches, whereas the brown and green one is more difficult to see when it is resting along a twig with needles on it. There is some evidence that the two types of larvae tend to take up a position appropriate to the particular form. This polymorphism, which may be environmentally controlled, would enable the relative advantages of the two patterns in the two types of resting position to be investigated experimentally. Special behavior patterns associated with cryptic coloration are well known in the Lepidoptera. L. P. Brower and J. V. Brower (1956) showed that a brightly colored moth, *Rhododipsa masoni,* which rests on the flower heads of *Gaillardia aristata,* orientates itself in such

a way that its pattern coincides with that of the flower. If it did not do this it would be far more conspicuous. The pupa of the pine hawk moth is found in the earth and is a blackish brown, as are most unexposed pupae. The moth is grey and extremely inconspicuous on the pine trunks on which it rests during the day.

An even more complex series of changes can be seen in many species, as for example, in the African swallowtail butterfly, *Papilio dardanus*. Its larva sits exposed on the upper surface of the broad leaves of its food plant. When young it is black with white marks at both the posterior and anterior end of the body. Later, as it grows larger, an extra white patch develops on the dorsal surface, halfway along the body. Both these patterns are good imitations of a bird dropping. When the caterpillar grows too large for this protective device to work, it becomes green like the leaf on which it rests, but some of the white on the middle of its back remains (but not that at the anterior and posterior ends) and gives the appearance of highlights reflected from a shiny leaf. The larva also becomes countershaded, being darker on its dorsal surface and paler low down on its sides. The pupa, when formed, is suspended horizontally under a leaf, with the ventral side of the animal uppermost. It is green and flattened, making it look much like a leaf. It also is countershaded, but it is the ventral surface, not the dorsal one, which is darker, since this is the upper surface of the pupa. The adult butterfly gains protection in many areas by being an extremely good mimic of distasteful species (see below). Thus, here again, a series of protective patterns has evolved, each appropriate to a different stage of the life cycle.

De Ruiter (1956) has demonstrated that countershading affords a larva some protection from birds. He has also demonstrated (1952) that a very small reduction in the resemblance to a twig can put larvae which imitate sticks at a considerable disadvantage. However, the advantage gained by resembling a bird dropping has never been investigated experimentally to my knowledge. Nevertheless, there is some indirect evidence that the advantage is considerable. It is a general rule, not only in the genus *Papilio*, but throughout the Lepidoptera, that when larvae resemble bird droppings they only do so for the first few instars, while they are small, and subsequently forfeit this guise for some other method of protection. Pupae and adult moths which resemble bird droppings are also small, as for example, the pupa of the black hairstreak, *Strymonidia pruni*, and the imago of the Chinese character, *Cilix glaucata*, and the lime-speck pug, *Eupithecia centaureata*. (See Ford, 1945, p. 106; 1955a, pp. 98–99, and Plate 2). Kettlewell (personal communication) tells me that he has observed for many years that by the

middle of the day, usually earlier, most of the Lepidoptera on the outside of his moth trap have been eaten by birds. By midsummer the trap is, of course, always covered with bird droppings, and among these the only surviving moths, usually in numbers, are the Chinese character and the lime-speck pug (see also Howarth, 1960).

Remington (1959) has noted that the larvae of many species of swallowtail which feed on the Rutaceae, particularly *Citrus,* look very like one another in color pattern, and that the larvae of the *machaon* group of swallowtails, which feed mainly on Umbelliferae, have a quite different pattern. One of these *Citrus* feeders, *Papilio demodocus,* also feeds extensively on Umbelliferae in South Africa, as well as on *Citrus.* In Kenya the larvae of *P. demodocus* are of the typical "*Citrus* pattern" and closely resemble those of its Asiatic relative, *P. demoleus.* However, in South Africa there is a second form of the larva which has a far more complex pattern than the rather simple one of the *Citrus* type. The difference between these two is controlled by a single gene, which in crosses with Kenya material showed no dominance. Furthermore, there is evidence (Clarke and Sheppard, unpublished) that in South Africa there are modifiers enhancing the effect of the gene producing the more complex pattern. It seems likely that the *Citrus* pattern is highly cryptic on the broad, shiny leaves of the Rutaceae but that the more complex pattern is better adapted to umbelliferous plants. The presence of the two forms of larvae, both of which will feed on *Citrus* and umbelliferous plants, should make it easy to measure the relative cryptic advantages of the two larval forms in the wild.

B. Eye Spots

There are a number of other methods which have evolved in the Lepidoptera for avoiding attack by predators, and the protection afforded by some of these has been investigated in the laboratory (for a short review see Sheppard, 1959a, p. 146–154). Thus, Blest (1957a,b) has shown that the large eyelike markings on many butterflies and moths can give some protection. These marks are usually concealed when the insect is at rest, but are suddenly exposed if it is disturbed. The sudden appearance of these "eyes," which closely resemble those of a large vertebrate, can put small birds to flight in the wild as well as in the laboratory (Kettlewell, 1959b). Blest has also shown that even a very poor resemblance to an eye can give some protection. However, the better the resemblance, the more effective is this mode of defense. Consequently such eyelike patterns can evolve gradually.

Blest has also shown that small eyelike spots can protect by a quite

different method. They deflect attack away from a vulnerable part of the body.

C. Warning Coloration

Insects which are distasteful to a large number of vertebrate predators often employ bright patterns which are simple and easily learned (warning coloration) to advertise the fact that they are not suitable as food. In this way they avoid being attacked by those predators who have learned that the pattern signifies that the insect is distasteful, or is protected by a sting, irritant hairs, or some other device. Behavior patterns which accentuate the warning pattern also seem to have been evolved on many occasions (Blest, 1960). That warningly colored insects are often highly distasteful to potential predators has been demonstrated on a number of occasions (see Carpenter and Ford, 1933; J. V. Brower, 1958a,b,c; Rothschild and Lane, 1960). Moreover, Brower has demonstrated that birds learn to avoid such patterns.

D. Müllerian Mimicry

An extension of the system of warning coloration is found in Müllerian mimicry, where several distasteful species have much the same pattern. This common pattern gives considerable protection to the species that possess it. When a predator has learned to avoid a particular warning pattern, it avoids other species with the same pattern, thus reducing the number of animals of each species destroyed in the learning process. For example, it has been shown that birds that have experienced wasps and learned to avoid them, also tend not to attack the black and orange striped larvae of the cinnabar moth, *Hypocrita jacobaeae* (Windecker, 1939).

Glass (1959) has pointed out that under certain circumstances there might be mimicry between a number of species, all of which were quite edible. He suggested that such a situation might arise if the predators did not take their various species of prey in proportion to their abundance, but tended to take a proportionately smaller number of the very common forms. Now Tinbergen (1960) has produced ecological evidence to show that some birds do take proportionately fewer of the very rare and the very common species. That is to say, they seem to concentrate on taking species of medium abundance. If this is a general phenomenon, and if birds recognize their prey species by sight, then one might expect one of two situations to evolve in a moderately common species. It might either become highly variable (Sheppard, 1959a, pp. 90–91; Fisher, 1958) or, if a rather similar common species was present, it

might come to mimic this common species. Either course would reduce predation if Tinbergen's thesis is generally applicable. However, it must be remembered that for either the polymorphism or the mimicry to evolve, the species must be a very important source of food to the predators. Moreover, it must not have a very cryptic appearance initially (or have an appearance highly adapted in some other way) since any departure towards a polymorphic or a mimetic state would tend to destroy the special adaptation and therefore release counterselection more powerful than that towards the polymorphism or mimicry.

Van Someren and Jackson (1959) have come to much the same conclusion as Glass, and give examples of edible species of butterflies and moths which are all much alike. Many of the examples they give are of species which are closely related and, thus, just those that one would expect to evolve this type of mimicry, since related species are more likely initially to resemble one another to some degree. If such mimicry does evolve it should lead to uniformity, not polymorphism, since the more individuals of one color pattern present, the more efficient the mimicry in protecting the species involved. However, it seems unlikely that this type of mimicry can evolve in butterflies, since they are probably never a sufficiently important source of food to elicit the necessary strong "avoiding reaction."

E. Batesian Mimicry

In Batesian mimicry a relatively edible species resembles a distasteful one in appearance, and, being mistaken for it by a potential predator, gains protection from attack. The evolution of Batesian mimicry, partially because of the spectacular resemblance between different species that is found, has aroused more interest than the evolution of almost any other protective device. The theory of Batesian mimicry is reviewed by Ford (1953) and Sheppard (1959a,b). However, because the phenomenon is the subject of active research at the present time, and because a great deal of information of general interest has come to light since Ford's (1953) excellent review, it is necessary to consider some aspects of the subject in detail.

1. *Laboratory Experiments on the Effectiveness of Batesian Mimicry*

J. V. Brower (1958a,b,c), working with several species of North American mimetic butterflies and their models (the distasteful species the mimic resembles), showed that the Florida scrub jay found the models distasteful and was unable readily to distinguish by sight the mimics from the models. Consequently, many of the edible mimics were not attacked by the birds, even when they were offered to them for as

long as 2 minutes. In another series of experiments using starlings as predators and mealworms as prey, she showed (1960) that a sufficiently distasteful model (mealworms dipped in a 66% aqueous solution of quinine dihydrochloride) could give a high degree of protection to a mimic (mealworms dipped in distilled water) even when the mimic was very much commoner than the model. It seems likely that the number of mimics that can be protected by a certain number of models will be determined in part by the inedibility of the models (see Sheppard, 1959b, 1961).

J. V. Brower (1958c) also obtained evidence that a rather imperfect resemblance to a model could confer some advantage, but presumably less than that given by a very good likeness to the model. This suggestion has been confirmed by Sexton (1960), using the distasteful firefly, *Photinus pyralis,* as the model, and the mealworm, *Tenebrio molitor,* marked by gluing various parts of *Photinus* to its dorsal surface, as the mimic, and the lizard, *Anolis carolinensis,* as the predator. He found that when tested together with *Photinus,* only those mimics marked with both the prothorax and elytra of *Photinus* escaped some predation. However, when tested together with unmarked *Tenebrio,* the mimics marked only with elytra, as well as those marked with both elytra and prothorax, escaped some predation. Since, in nature, the predator is unlikely always to be able to compare both model and mimic at the same time, these experiments indicate that imperfect mimicry can be effective in giving protection to an edible species.

Schmidt (1958, 1960) has also investigated the protection afforded by imperfect mimicry. In his studies the predators were chicks, and these were trained to avoid certain colored pictures of a hypothetical butterfly. Schmidt found that (1) some mimics resembling the model only slightly were avoided; (2) there was a tendency for the predator to learn not to avoid a mimic if it was presented frequently; (3) in general, the more elements of pattern there were common to both model and mimic, the greater the protection received by the mimic; (4) there was evidence for a threshold effect. In the experiments the presence of a small area of black had no detectable effect on avoidance of the mimic, but above a certain amount of black avoidance was elicited. Above this threshold extra black had no further protective effects. (5) Size and wing shape (the presence or absence of tails on the hind wings), did not seem to have any effect on discrimination between models and mimics by the chicks.

Schmidt's results again show that imperfect mimicry can bestow some advantage on the mimic, and that more perfect mimicry is even more advantageous. It is not very surprising that even a remote resem-

blance to a model was advantageous in his experiments, since he was working with the chick. Had he used a wild insectivorous species, a much more perfect resemblance might not have deceived the predator. His finding that the presence or absence of a tail did not alter the predation rate also may not apply in the wild for the same reason. In fact, there is genetic evidence that the presence or absence of a tail can be very important (see below).

2. *The Effectiveness of Mimicry in the Wild*

There is a mass of data in the literature which shows that mimics are seldom found outside the range of their models. However, very few random samples have been taken which show the relationship between the frequency of the forms of a polymorphic mimetic species and the frequency of their models in the same place. One such study by Carpenter has been reviewed by Sheppard (1959b), who analyzed Carpenter's data and showed that in the very polymorphic mimetic species *Pseudacraea eurytus,* there was a very significant correlation between the frequency of the mimics and that of the models. Moreover, the data suggested that the polymorphism was wholly maintained by the mimicry, there being no evidence of any physiological advantage of the heterozygotes disturbing the correlation. However, in some examples of mimicry there are almost certainly selective forces operating other than those caused directly by the mimicry, and these maintain the polymorphism. Such physiological selection almost certainly accounts for the presence of polymorphism in *Papilio dardanus* in Kenya where most of the models are rare.

If the resemblance between mimics and their models is maintained by natural selection and there is no advantage in mimicry when the models are very rare, as suggested on theoretical grounds by Fisher (1930), and by the experimental work of J. V. Brower (1960) and Schmidt (1958), one would expect more variability in the mimetic patterns where the models are rare than where they are common. Carpenter and Ford (1933) pointed out that in random samples of *Pseudacraea eurytus* and *Papilio dardanus* there were more imperfect mimics in populations where models were rare than where they were common. Sheppard (1959b) has taken the data given by Carpenter and by Ford and shown that there is a highly significant correlation between the frequency of imperfect mimics among mimics and the frequency of the mimetic species in random samples of models and mimics. J. V. Brower (1960) also records that in North America the mimetic resemblance between *Papilio troilus* and the model, *Battus philenor,* becomes less perfect when the model is rare. Thus, there is good evidence that in

nature mimicry protects the individual against predation to some extent, and that as the model becomes rare the mimicry progressively loses its protective value and the resemblance between model and mimic tends to break down.

3. *The Inheritance of Mimetic Patterns*

The mode of inheritance of mimetic patterns in polymorphic butterflies has been reviewed by Ford (1953). In all the species he discusses, the forms differ from one another by one or more major genes. If a species is common one would expect the evolution of a polymorphism controlled genetically by major genes acting as a switch mechanism. Ford points out that among the monomorphic species no information is available, either with regard to the mode of inheritance of the patterns, or as to why the species are not polymorphic. He says, "Among moths, monomorphic species of the genus *Sesia* have achieved a striking general resemblance to hornets and wasps, involving extreme transformation of the superficial characters of the Lepidoptera. Perhaps it has not been possible to maintain so great a change within the sphere of action of one or two major genes which can act as a switch in determining alternative forms." Although no monomorphic species showing as extreme mimicry as that in the genus *Sesia* has been investigated genetically, it has been shown (Clarke and Sheppard, 1955a) that the mimicry in *Papilio polyxenes* is controlled, in the main, by a single dominant gene together with modifiers (See also the work of Remington (1958) on *Limenitis,* where in two instances, one involving two subspecies each monomorphic and mimetic, the other two species each monomorphic but in which only one is mimetic, he found that the members of each pair differ from one another in the main by two major genes.)

Papilio polyxenes, which has been studied by J. V. Brower, is mimetic on the underside of the wings in both sexes, and on the upper surface in the females only. The upper surface of the males shows a rather poor mimetic resemblance owing to the presence of a broad band of yellow marks on the forewings. The black ground color of the wings gives rise to the major part of the resemblance to the model, *Battus philenor,* and in this respect the mimic differs from its yellow relatives of the *machaon* group by the presence of a single autosomal gene, dominant in effect. The blue scaling on the upper surface and the red on the under surface of the hind wing, important components of the mimicry, are apparently controlled polygenically. It is interesting to note that outside the range of the model there are two black related species, *P. brevicauda* and *P. nitra,* in which the mimicry has broken down in the female, since the yellow band of spots characteristic of the male of

P. polyxenes is present. Moreover, in *P. nitra,* whose taxonomic position is not fully understood (Remington, 1956), the mimicry on the underside of the wings is also poor, the red spots characteristic of the model being yellow or, at most, only slightly tinged with red.

Battus philenor supports two other mimetic species, *P. troïlus,* which is monomorphic, and *P. glaucus.* In *P. troïlus* the mimicry becomes less perfect outside the range of the model as was mentioned above. In *P. glaucus* the male is non-mimetic and has a black and yellow pattern. There are two forms of the female, one like the male, and a second black form which is the mimic. This black form is absent, or almost absent, from Canada, where the model is also absent, but reaches a high frequency further south where the model is found. In the Florida peninsula the black form again becomes rare, as does the model. The inheritance of black is not fully understood, but the factor responsible is certainly either Y-linked, cytoplasmic, or sex-controlled and partially sex-linked. It cannot be an autosomal sex-limited dominant as suggested by Remington (1954). Most of the evidence presented by Clarke and Sheppard (1959a) indicates that it is Y-linked, as does more recent unpublished data. Total Y-linkage (or alternatively, cytoplasmic inheritance) is not unknown in the Lepidoptera. One of the black female forms of *Phigalia pilosaria,* the pale brindled beauty, is inherited in this way, and may be an industrial melanic (Ford, 1955a). The difference between the white pattern of the green-veined white *Pieris napi* and the pattern of *P. bryoniae,* which is yellowish, heavily suffused with black, also seems to be controlled entirely by the genotype of the female. Ford (1937) has suggested that the results obtained may be due to abnormalities of segregation in the interspecific hybrid. However, against this it should be noted that in the extensive hybridization experiments using the genera *Papilio* and *Limenitis* (Clarke and Sheppard, 1955a; Remington, 1959; Aé, 1960) no such abnormal segregation has been noted.

If the inheritance of black in *Papilio glaucus* is controlled by the Y-chromosome (or is cytoplasmic), as I believe it is, the maintenance of the polymorphism for the two types of female in some areas is of considerable interest, since an advantage of the heterozygote cannot be invoked to account for it. The fact that there is a very steep morph-ratio-cline both near the northern limit of the range of the black form, and near its southern limit in Florida (Remington, 1954), suggests that between the two boundaries the black form is at an advantage because of the presence of the model, and that north and south of this central area the black form is at a disadvantage. Some migration of females across the border could then account for the presence of the inappro-

priate forms at low frequency. L. P. Brower has already shown that the closely related western allopatric species *P. rutulus, P. multicaudatus,* and *P. eurymedon* can move 10 miles or more in the course of a few days. Such movement could account for considerable gene flow. If the movement of *P. glaucus* could be similarly estimated together with the phenotype frequencies in a north-south transect of the species range, the relative selective values of the two forms in different areas might be estimated; the elimination of the inappropriate females would have to be exactly balanced by immigration if a stable morph-ratio-cline is found.

Although this approach might be profitable, the method suggested must be applied with caution, since (1) it may be that the polymorphism is maintained by the relative abundance of models and mimics, a possibility which could be assessed by taking random samples of models and mimics and comparing their relative frequencies, and (2) Acton (1957a) has found that *Chironomus tentans* populations can be cytologically polymorphic for at least three types of male which differ in their Y-chromosomes. In this species chromosomes of two different pairs may act as a Y (not in the same individual) and these may or may not be marked by particular inversions. Consequently here, as in *Papilio glaucus,* there is polymorphism in one sex which can not be maintained by an advantage of the heterozygote, since it is a Y-chromosome polymorphism.

Since Ford's (1953) review the genetics of several species with mimetic forms has been investigated. The most extensive of these studies concerns the forms of the mimetic butterfly, *Papilio dardanus* (Clarke and Sheppard, 1959b, 1960a,b). In this species there are a number of geographical races. The males are everywhere black and yellow, have tails on the hind wings, and are non-mimetic. The females in Madagascar and Grand Comoro are like the males, as are the majority in Abyssinia; elsewhere the females are tailless, unlike the males, and highly polymorphic. Clarke and Sheppard have investigated the genetic control of this polymorphism in some detail, and show that there is an autosomal multiple allelomorphic series consisting of at least twelve allelomorphs which control the major variants, both mimetic and non-mimetic (some female forms are neither malelike or mimetic). There is a second locus, unlinked with the first, which has two allelomorphs controlling the presence and absence of tails on the hind wing. The effects of both these loci are sex-controlled, exerting their effect only in the female (see below). Thus, as in other examples of butterfly mimicry the forms differ by single major genes, but in contrast to other

species, where more than one locus is usually concerned in controlling the several forms (see Ford, 1953), only one chromosome is involved as far as color pattern is concerned in *dardanus*.

4. *The Evolution of Mimicry*

The control of mimetic patterns by single genes has prompted several people to maintain that the mimicry must arise fully perfected at one step, because the major genes must have arisen by mutation at one step. Goldschmidt (1945) argued that this was the only reasonable interpretation of the evolution of mimicry. However, Fisher and Ford (see Ford, 1953) argued that although the allelomorph responsible for the mimicry arose by mutation its effect could be modified subsequently by the selection of modifiers which improved the mimicry. When Ford (1953) discussed Goldschmidt's hypothesis and produced powerful arguments against it, no critical genetic data were available which would decisively distinguish between the views put forward by Goldschmidt on the one hand and Fisher and Ford on the other. Such evidence is now available and, at least in respect of the origin of mimicry in *Papilio dardanus*, Goldschmidt's hypothesis can be decisively rejected (Clarke and Sheppard, 1960d). The evidence is twofold: (1) It has been shown that the forms *hippocoonides* and *hippocoon*, which respectively mimic the subspecies *dominicanus* and *niavius* of *Amauris niavius*, differ from one another by several genetic factors (polygenically). This finding is fatal to Goldschmidt's hypothesis because, if these two mimics were produced at one step by mutation, they should only differ by one (or at most two) loci. (2) It has been found that when a major gene producing a mimetic pattern is put into an "unfamiliar" gene-complex, by hybridization with a race of *P. dardanus* not possessing the mimetic form, the pattern produced by the gene is changed and the mimicry becomes less perfect. Recent evidence (Clarke and Sheppard, unpublished) shows that this breakdown of mimicry occurs in an extreme form in some F_2 and backcross hybrids, involving the mimetic South African race and the nonmimetic Madagascan race, in which all the females are malelike. On Goldschmidt's hypothesis one would not expect this breakdown in mimicry unless one also postulated that imperfect mimics could never be at an advantage, and that *P. dardanus* just happened to have the correct gene-complex for the correct mimetic pattern to be produced in, and only in, those areas where the models are also found!

V. The Evolution of Dominance

Work on the genetics of mimicry in *Papilio dardanus* has also given information on the evolution of dominance (Clarke and Sheppard,

1960c). It was shown that there is usually complete dominance between morphs which are present in the same subspecies of *P. dardanus* (forms that are sympatric). But when races are crossed in such a way that major genes not found together in nature (allopatric allelomorphs) are combined in a heterozygote, dominance tends to break down and intermediate phenotypes are formed. Moreover, it was also shown that the dominance of the malelike female form over *hippocoonides* and *cenea* in the Abyssinian race, where all three occur, breaks down slightly in the F_1 hybrid with the South African race in which the malelike female form is not found. It could be restored, however, by one generation of backcrossing into Abyssinian stock. The breakdown was much more pronounced on crossing South African material with the Madagascan race in which *hippocoonides* and *cenea* do not exist. The absence of dominance between allopatric but not between sympatric forms suggests that it has been evolved by natural selection.

This evolution of dominance would be expected in polymorphic mimetic species because an intermediate heterozygote would be at a disadvantage, since it would be likely to be a poor mimic. If, however, it were a good mimic, then one of the homozygotes would not have the correct mimetic pattern, and there would be selection for this homozygote to look like the better adapted heterozygote. In other words, there will be disruptive selection in mimicry which will tend to eliminate patterns intermediate between fully mimetic ones (see also below). The absence of dominance between allopatric forms controlled by a single gene has frequently been noted in other Lepidoptera. Thus, Remington (1958) has shown that *Limenitis archippus floridensis* and *L. a. archippus* differ by at least two genes, which show no dominance with respect to one another. Ford (1955a) also records its absence between the normal black form of the males of the muslin moth, *Cycnia mendica*, and the allopatric cream-colored males found in the Irish race. The females are everywhere white with black dots, and the difference between the males is controlled by a single sex-limited gene which produces an intermediate F_1 heterozygote in crosses between the British and Irish races. The heterozygotes in the F_2 are even more variable than the F_1, indicating a considerable segregation of modifiers of dominance.

Clarke and Sheppard (1955c) showed that the difference between the larval pattern of *Papilio machaon* and *P. hospiton* was in part due to a single gene showing no dominance. Moreover, they also showed (1956) that the difference between the larva of *P. machaon* from Europe and the allopatric race *saharae* from North Africa was due to a single gene, again showing no dominance. They also suggested that the gene responsible for the larval pattern of *P. hospiton* and of *P. machaon* race *saharae* might be the same.

It appears, therefore, that the absence of dominance between allopatric forms controlled by allelomorphs at a single locus is characteristic of many species and is not just confined to *P. dardanus* where mimicry is involved. In contrast with this situation, most polymorphic forms show complete dominance with respect to one another. There are, however, certain exceptions to the proposition that dominance is absent between allopatric forms, but not between sympatric ones. The most notable of these exceptions is that found in crosses involving the gene controlling the black pigmentation of *Papilio polyxenes* and *P. brevicauda*. In crosses between these species and their yellow relatives, black is found to be fully dominant, or very nearly so (Clarke and Sheppard, 1955a; Remington, 1959).

Even more convincing evidence for the evolution of dominance in polymorphic species of the Lepidoptera has been obtained by Ford (1953, 1955b). He investigated polymorphism in the lesser yellow underwing, *Triphaena comes*. There is a pale variety of this species which is the only one present over most of England. However, in Scotland there is also a melanic form, which does not replace but is found with the pale one. This melanic form is semidominant, the heterozygote being on the average paler than the dark homozygotes, but not sufficiently so for the two to form distinct classes. The darkest melanics are homozygotes and the palest melanics heterozygotes, but the intermediate forms may be either homozygous or heterozygous. The pale homozygote is quite distinct from the melanic forms, and intermediates do not normally occur. Thus there is a variable melanic form, and a constant and distinct pale form which is a homozygote. Ford crossed dark forms from two quite distinct isolated populations, one from the Orkneys and the other from the Outer Hebrides. He found that in hybridization experiments between the insects from these two populations, the clear distinction between the melanic form, *curtisii*, and the pale homozygous form vanished. He concluded therefore, that the semidominance of *curtisii* had been evolved independently in the two areas, and that different modifiers of dominance had been utilized in each.

That dominance may be evolved rapidly is indicated by the work on the peppered moth, *Biston betularia*. Many of the earliest industrial melanics (Fig. 1) had much more white on them than present-day heterozygotes in industrial areas (Kettlewell, 1958). Since these early melanics were almost certainly heterozygotes, the melanic form being rare at the time, it seems certain that the heterozygotes at least have become darker in the last hundred generations. If the early homozygotes were darker than the heterozygotes, then dominance has been evolved.

Most industrial melanics are dominant, and many may have been so from the beginning, since one dose of black pigment may produce as black an insect as do two. However, some industrial melanics are not sufficiently black for this explanation of dominance to be acceptable. Kettlewell (1959c) has pointed out that not all industrial melanics have arisen as a result of selection for a previously rare mutant. In several species the melanics which have spread in industrial areas are not absent in all rural environments, but form stable polymorphisms with non-melanic types. In such a situation dominance may have been evolved, owing to the presence of the stable polymorphism, before the Industrial Revolution. Consequently, on their first appearance as industrial melanics they were completely dominant.

The evolution of dominance in polymorphic populations has important theoretical implications. Caspari (1950) has investigated the effects of the allelomorphs controlling red and brown testis color in *Ephestia kühniella*. The two allelomorphs have several effects and each is dominant for its advantageous ones, and recessive for those that are disadvantageous. Caspari accounted for the polymorphism in his populations by pointing out that because of the dominance relationships, the heterozygotes were always at an advantage to both homozygotes. The latter always produced some disadvantageous effects, but the heterozygotes did not. Sheppard (1953a) pointed out that most major genes have more than one effect. Consequently, if those effects which are advantageous become dominant, whereas those which are disadvantageous become recessive in a polymorphic situation, the heterozygote will invariably become superior to both homozygotes. One would therefore expect that when a polymorphism is maintained by ecological factors, as in the case of mimicry, a stable polymorphism will be evolved, even if not initially present, with the heterozygote being at an advantage to both homozygotes. Several mimetic polymorphisms do, in fact, appear to be maintained by the heterozygote being at an advantage to the homozygotes (Ford, 1953). Such a stability probably explains the presence of a polymorphism in *P. dardanus* in Kenya despite the absence of appropriate models.

Dominance may even be evolved when an advantageous form is replacing an ancestral type, as in *Biston betularia*. Consequently, we might expect the transient polymorphism of *B. betularia* to evolve into a stable one if dominance is evolved sufficiently rapidly. Kettlewell (1958) has estimated the selective advantage of the melanic *carbonaria* for some populations, both in the early years of its existence and in more recent times. His estimates indicate that the advantage of *carbonaria*

is waning. This is exactly the result one would expect if the polymorphism is developing into a stable one, with the heterozygote at an advantage to both homozygotes.

It has often been stated that even in constant conditions a stable polymorphism maintained by heterozygous advantage will not persist because, eventually, an allelomorph will arise which, as a homozygote, is at an advantage to the heterozygote maintaining the polymorphism. However, if dominance is evolved in the way I have suggested, the original heterozygote is likely to be becoming more advantageous as time goes on, making it more and more unlikely that a new mutant can replace the original two allelomorphs. However, even if such a mutant arises, it itself is likely to produce a new polymorphism, and not replace both the original genes. Only a change in the environment is likely to cause the loss of one of the allelomorphs. Thus, it is not surprising that many polymorphisms have existed longer than the species in which they are now found, having been present in their common ancestor.

VI. Sex-Controlled Inheritance

Many mimetic polymorphisms in butterflies are confined to the female sex and almost none to the male sex (see Remington, 1954). It has often been maintained that this is a phenomenon characteristic of mimicry. However, nothing could be further from the truth. In fact, it is a characteristic of butterfly polymorphism, whether associated with mimicry or not, that it is often confined to one sex, and if it is, then it is the female sex which is polymorphic.

Sex-controlled inheritance is usually considered to result from the ability or inability of a particular allelomorph to produce its effect in the genetic environment of one of the two sexes. That is to say, there is an interaction between the sex-controlled gene and the sex chromosomes. Before considering the significance of sex-limited polymorphism, it is necessary to discuss specific hypotheses of the nature of sex-controlled inheritance.

Cockayne (1938), discussing the autosomal dominant producing the green variety *valezina* of *Argynnis paphia*, the pale *alba* form of *Colias philodice*, as well as some sex-controlled varieties of *Papilio polytes* and *Papilio memnon*, pointed out that Goldschmidt's explanation of the inheritance of these forms, namely, "that they are unable to appear in the male in the same way as ordinary female secondary sexual characters, such as winglessness in some of the Bistoninae," would not fit the facts. Cockayne pointed out that *valezina*-like males of *A. paphia* and pale *alba*-like males of *C. philodice* are known. He thought that the

sex-limited forms are determined by an autosomal dominant gene which only produces a visible effect in conjunction with a gene on the Y-chromosome, and that this activating gene is present on every Y-chromosome. He pointed out that the occasional *valezina* males could be due to nondisjunction, giving an XXY male. However, such an explanation does not account for the breeding results with rare white males, or the *alba*-colored patches in some *gynandromorphs* in *Colias* (see Remington, 1954).

Ford (1937), on the other hand, favors Goldschmidt's interpretation and remarks that Cockayne's explanation would be satisfactory "if we could see any reason why the auxiliary factor in Y should more often be activating than inhibiting." He also pointed out that "the control of the ordinary accessory sexual characters, alike in male and female, shows us that the operation of genes must often be inhibited (or activated) by the internal environment provided by one of the sexes." He explains the abnormal males as being analogous to the occasional heterozygous manifestation of genes normally recessive in effect.

Recently, Stehr (1959) has interpreted the genetic control of sex-controlled polymorphisms in another way. He worked with the color of the hemolymph of two species of moth, the spruce budworm, *Choristoneura fumiferana,* which is polymorphic for green and yellow hemolymph, but in which the frequency of yellow is often different in the two sexes, and the jack pine budworm, *Choristoneura pinus,* in which the females have green and the males yellow hemolymph. From his extensive breeding results with *C. fumiferana* he concluded that the polymorphism is controlled both by a sex-linked and by an autosomal locus having epistatic effects, which depend on the potency of the various allelomorphs in the individual. Moreover, he shows that the results from the hybrids between this species and *C. pinus* are consistent with the sexual dimorphism in *C. pinus* being controlled by the same two loci, but with the allelomorphs having different potencies from those of *C. fumiferana.* The *valezina* and *alba* males in *Argynnis* and *Colias* he explains by the presence of rare allelomorphs having much greater potency. Stehr points out that the system he proposes is similar to the method of control of sex itself. He says, "Where the determination of a character is divided between an autosomal protagonistic, and an X-chromosomal antagonistic gene, the conditions for a sexual dimorphism are given on the basis of the X-chromosomal/autosomal potency balance, in perfect parallel to the determination of sex as such. However, if there exist, as in *C. fumiferana,* alternative alleles of different relative potencies on one or the other of these loci, many forms of sex-controlled polymorphism and even some that seem not to

be sex-controlled, may be created. Such polymorphism thus becomes a phenomenon akin to intersexuality." However, a polymorphism seems to differ from intersexuality in one important respect, namely that there are clear-cut segregating classes. This can only mean on Stehr's hypothesis that the gene-complex has been adjusted by selection, so that the genes produce an all-or-none effect, as with sex itself.

Stehr's results are of the very greatest importance, since he is able to generalize his hypothesis and show that the genetic mechanism he postulates can account for most types of polymorphism found in the Lepidoptera. Moreover, it can explain the departure from the expected 3:1 ratio found in some crosses in *Argynnis* and *Colias*, which previously had been accounted for by postulating the presence of a lethal in coupling with the sex-controlled dominant *valezina* or *alba*, respectively.

The most important conclusion reached by Stehr from the point of view of population genetics, is that the system of genetic control he suggests would ensure that sex-controlled polymorphisms would be more common in the heterogametic sex (the females) than in the homogametic sex, because of the system of potencies. Consequently, if we accept Stehr's view, there is apparently no difficulty in accounting for the fact that sex-controlled polymorphisms in butterflies are almost invariably confined to the females. This still does not tell us why so many sex-controlled polymorphisms have evolved. In order to reach some decision on this point we must take certain facts into account.

(1) In butterflies and moths sex-controlled polymorphisms are far more commonly controlled by an autosomal pair of allelomorphs than by a sex-linked pair.

(2) In *Papilio dardanus* the autosomal locus apparently has at least twelve allelomorphs.

(3) Sex-controlled polymorphisms are far more frequent in butterflies than in moths.

(4) Sex-controlled polymorphisms are almost always confined to the female sex in butterflies. However, in moths a number of polymorphisms are known which are confined to the males.

Stehr's hypothesis does not explain why sex-controlled polymorphisms are so seldom determined by a pair of sex-linked genes. From his view one would expect them to be about as common as autosomally controlled ones, since in every case a sex-linked locus, as well as an autosomal one, must be involved. Moreover, it is extremely difficult to explain the polymorphisms in *P. dardanus* by postulating genes of different potencies, since the autosomal locus involved appears to have certainly twelve, and probably more than twelve, allelomorphs.

Points three and four above, although perfectly consistent with

Stehr's hypothesis, are not explained by it. To find a hypothesis which will account for them, we must consider the ecology of butterflies and moths rather than the actual genetic control of the polymorphism itself. Color pattern plays an important part in courtship in butterflies, but not in moths, where olfactory stimuli are of major importance. Ford (1953) has pointed out that under these circumstances a novel color pattern, although advantageous in some respects, may cause the female to reject the male during courtship, and therefore put such a pattern at a severe disadvantage in males. Female choice must be important in avoiding interspecific matings, since many male butterflies will readily court females of a different species (see Lorković, 1958). L. P. Brower (1959), Stride (1958), and Magnus (1958) have all shown that female color is important in butterfly courtship. Brower exposed both black and yellow females of *Papilio glaucus* to males of three yellow species allopatric to it, but sympatric to each other. The males courted the yellow *glaucus* but ignored the black female. It would be of the greatest importance to the understanding of sex-controlled mimicry to repeat the same type of experiment using *glaucus* males from areas with different frequencies of black and yellow females. Magnus, working with *Argynnis paphia* males, concluded that the *valezina* females did not attract the males at all. However, despite this finding, it is the experience of entomologists that female *valezina* collected in the wild have nearly always mated before capture. Thus, a novel pattern in the females, even if it does not attract the males as well as the old pattern, may, nevertheless, not thereby be at a great disadvantage. In butterflies, females can be fully fertilized as the result of a single copulation, and males can fertilize many females. Consequently, a female pattern which was ignored by five out of six males would not be very disadvantageous, since the female would almost certainly be mated by at least one male. However, a male which failed to copulate with five out of six of the females with which one of the other color would have succeeded, would be at a huge disadvantage. Thus the nature of butterfly courtship suggests that Ford's hypothesis to account for the commonness of polymorphism sex-controlled to the female in butterflies is the correct one. A new mutant will have a far better chance of establishing itself in many species of butterflies, if its effect is sex-limited to the female. This is not to say that polymorphisms in both sexes cannot be established, since the females of some species will be less discriminating than those of others, and no doubt new behavior patterns can be evolved by the females. It would be of the greatest interest to know how females react to different types of males in species which are polymorphic in both sexes, and in those which are polymorphic only in the female.

VII. The Evolution of Super-Genes

Fisher (1930) pointed out that if there are two pairs of allelomorphs in a population which control contrasting characters, *C*-*c* and *D*-*d*, respectively, then if *C* is at an advantage to *c* in the presence of *D*, but at a disadvantage in combination with *d*, and *D* is advantageous with *C*, but not with *c*, linkage will increase between the two loci as a result of selection, provided that polymorphism is maintained. Sheppard (1953b) has reviewed a number of examples of polymorphism in which there are a large number of allelomorphs or closely linked genes involved, and proposed that some of these have resulted from selection for increased linkage as suggested by Fisher. Mather (1955) has extended the hypothesis, and has suggested that disruptive selection, which can arise when there is more than one optimum phenotype, can lead to the establishment of a polymorphism, and that in the course of time more and more genes will be incorporated into the switch-gene controlling the polymorphism, thus resulting in the evolution of a super-gene.

It is now well known (see Pontecorvo, 1959) that many "genes" contain several sections of chromosome which can mutate independently, and that there can be crossing-over between "allelomorphs" within a cistron. However, there are at least three reasons for believing that many of the super-genes found in polymorphisms are not just single loci with occasional crossing-over within the cistron.

(1) There is no apparent reason why many allelomorphs at one locus should be maintained at high frequency in a population but no other loci contribute to the polymorphism. One would have expected that many loci would each produce one or two mutants which could be maintained. Thus, no hypothesis of gene action accounts for all twelve of the inherited factors which control the color patterns in the mimetic butterfly, *Papilo dardanus,* being at one locus. It would be more reasonable to expect that several distinct loci would, in the past, have produced advantageous patterns which could be maintained.

(2) In polymorphisms the members of the super-gene often control quite different types of character, but characters which are affected by the same selective forces. Thus, on the one hand, the characters, being different, are unlikely to be controlled by a series of allelomorphs at one locus, but are of a type where only certain combinations are advantageous, thus allowing for selection for linkage.

(3) In many polymorphisms such as those in the snail, *Cepaea,* and in the grouse locusts, the cross-over values observed are far larger than those typical of allelomorphs at a single locus. Consequently, there is

reason to believe that at least some of the switch mechanisms controlling polymorphisms have been evolved gradually by the incorporation of more and more cistrons.

Besides the evidence from the snail, *Cepaea,* and the grouse locust, *Paratettix,* that certain combinations of linked genes can be at an advantage to others in the wild, various other organisms have also been found to show the same phenomenon. Thus, Acton (1957b) has shown that in *Chironomus dorsalis* there appears to be a nonrandom association, apparently maintained by natural selection, between an inversion and the male-determining gene, which is outside the limits of the inversion. Levitan (1958) has found nonrandom associations between inversions which must also be maintained by natural selection. Dobzhansky (1959) has repeatedly pointed out that inversions are probably maintained in wild populations because they hold together certain advantageous combinations of genes; that is to say, because they produce a super-gene.

Clarke and Sheppard (1960e) have argued that in *P. dardanus* the super-gene controlling the mimicry has gradually evolved. They point out that if one examines the mimetic patterns one finds that all are composed of a limited number of elements. However, only certain combinations of elements produce mimetic patterns; the others do not. Consequently, during the evolution of mimicry each new element which was first produced as the result of mutation could only be retained in a polymorphic state if it were initially on the chromosome containing the switch mechanism. Thereafter, linkage between the two loci could be increased. Clarke and Sheppard also point out that such a hypothesis will also account for control of the presence or absence of a tail on the hind wing being controlled by a super-gene also affecting wing pattern in *P. memnon.* No super-gene has apparently been evolved in *Papilio polytes* where two independent loci control the non-mimetic and two mimetic female forms (see Ford, 1953). In this example the epistatic interactions between the loci ensure the absence of disadvantageous patterns.

VIII. Disruptive Selection

When talking about disruptive selection, we have so far considered major genes and how these can be built up into super-genes controlling a switch mechanism. However, as Mather has repeatedly pointed out, polygenes can also be built up into balanced linkage groups, and under disruptive selection one might expect a population to become multimodal for a particular character, with two or more modes at the two or more optimum values for the character. Thoday (1959, 1960) and

Thoday and Boam (1959) have selected for high and low sternopleural chaetae number in *Drosophila melanogaster* using various mating systems. They have shown that under disruptive selection (1) the variance of the population increases; (2) a bimodal distribution for chaetae number can develop; (3) a normally disadvantageous gene can become fixed because it increases the difference between the effect of two chromosomes on chaetae number; and (4) both polygenes and major genes can be maintained linked in coupling because of their effects on the character under selection. The experiment was not designed to give selection for increased linkage.

Although disruptive selection has been shown to be effective in the laboratory, where very heavy selection could be made to operate, this does not necessarily mean that it will be effective in nature. Clarke and Sheppard (1960d) have pointed out that *Papilio dardanus* provides an opportunity for studying the effects of disruptive selection on a metric character in nature. Over the whole of its distribution the species has males which have a black and yellow non-mimetic color pattern. They also have long tails on the hindwings, a feature characteristic of the genus *Papilio*. Over most of Africa the females have a pattern quite unlike the males, which is usually mimetic, and they also lack the tails characteristic of the males. However, in Madagascar all the females are like the males, both with respect to color pattern and the presence of tails, and in Abyssinia a majority of females are tailed and like the males, but a minority (about 20%) are tailed but have a mimetic pattern.

Clarke and Sheppard (1960c,e) have argued that the presence of tails is advantageous in those *P. dardanus* with a yellow and black non-mimetic pattern, since (1) the males have retained their tails everywhere; (2) in Madagascar the females have tails as long as those of the males; and (3) the variance of tail length is low in males and in the Madagascan females. They have also argued that the absence of tails must be advantageous in the mimics, since the models are tailless and the mimics have lost their tails over most of Africa. They account for the presence of tails in Abyssinian females by pointing out that the semidominant gene eliminating the tails in the females, but not in the males, is not linked to that controlling the mimetic pattern. Consequently, this gene, sex-controlled in effect, will be at an advantage in the minority of females (the mimics), but at a disadvantage in the majority (the malelike forms), and therefore, on the average, at a disadvantage. Elsewhere in Africa it is the mimics which are in the majority, and therefore the gene removing the tails is at an advantage. Had the locus been on the same chromosome as that controlling color

pattern, or had a mutant having the same effect occurred on that chromosome, one would have had the opportunity for the evolution of a super-gene, allowing the mimics in Abyssinia to be tailless, and the non-mimetic forms tailed. The evolution of such a super-gene would also, of course, have necessitated the evolution of the dominance of the tailed condition, since the mimetic pattern is recessive to the black and yellow form.

These arguments suggest that one might find in Abyssinia, at the present time, disruptive selection which has failed to produce a super-gene but, nevertheless, is still acting to reduce tail length in the mimics, but not in the malelike forms. If such selection is effective in the wild, one would expect from Thoday's results to find (1) an increase in the variance of tail length in Abyssinia compared with Madagascar, and (2) a difference in the mean tail length of the mimics and the malelike females, the latter having the longer tail. Clarke and Sheppard (1960c) have shown that this is exactly what is found in Abyssinian females. Moreover, they have made various crosses between Abyssinian butterflies and the main African and Madagascan stocks. The crosses were so designed as to allow any modifiers present in the Abyssinian stock to segregate. As expected, the difference between the tail length of the tailed mimics and malelike forms vanished (or was so reduced as to be undetectable with the material available) when segregation was allowed. Thus, they have shown that the Abyssinian population differs from the other African populations in possessing a polygenic system of modifiers which are sex-controlled in effect, and most of which reduce tail length only in the recessive mimics.

In Abyssinia, disruptive selection has resulted in the evolution of a complex of modifiers with rather specialized epistatic interactions with a major gene pair. In Thoday's experiments the bimodality was achieved by the evolution of sets of linked polygenes without marked epistatic interactions except possibly for one major gene *vg*. I believe that the difference in the genetic mechanisms controlling the bimodality in the two situations is of major importance and demonstrates the desirability of field investigations for comparison with laboratory findings.

In Thoday's experiment there was no difference in the external environment in the lines selected for high and for low bristle number. Consequently, there was no opportunity for the selection of genes which decreased bristle number in the environment in which low bristle number was being selected, and increased it in the alternative environment. However, once a genetic mechanism such as that produced by Thoday has evolved, then specific modifiers can be selected which increase the bimodality. In Thoday's (1960) experiment a single major gene *vg*. or

modifiers linked to it, acted in this way. In nature, because of the large number of chromosomes found in most organisms, the initial genetic differentiation is likely to be controlled by one or two major genes as in mimicry, since it would be difficult to produce the same effect with polygenes, unless only one or two chromosomes are utilized.

Most plants do not move from place to place during the greater part of their existence, and many animals are also nonmotile during part of their life cycle. If the environment is heterogeneous one would expect disruptive selection. When the offspring of an organism are not likely to develop in the same environment as their parents, genetic differentiation into ecotypes, as the result of disruptive selection, is unlikely to occur. However, selection for a gene-complex which interacts with the environment in such a way that an organism reacts appropriately during development can occur and will be highly adaptive. It may be noted that those pupae of butterflies and moths which are exposed to attack by birds are often highly cryptic. In some species there is a polymorphism for green and brown forms. It has been possible to produce green and brown pupae almost at will in *Papilio machaon* by altering the environment in which the larva pupates. Nevertheless, Clarke and Sheppard (unpublished) also found that there are genetic differences between subspecies with respect to the ease with which this can be done. In one hybrid cross it was found that an environment which normally produced 90% green pupae, in this particular hybrid produced green pupae which were all female, and brown pupae which were all male. There therefore seems little doubt that the environmental switch mechanism controlling this polymorphism has a genetic basis, and has been evolved as a result of disruptive selection (see Sheppard, 1959a).

The consideration of laboratory experiments and genetic investigations in wild organisms, particularly the Lepidoptera, leads to the conclusion that disruptive selection can be effective in the wild, and that it is likely to lead to a phenotypic flexibility (Thoday, 1953) in sessile organisms (see also Sheppard, 1959a, pp. 111–114) but may lead to genetic polymorphism in more motile ones.

IX. Polygenically Controlled Characters in *Euphydryas aurinia*

E. B. Ford (H. D. Ford and E. B. Ford, 1930; Ford, 1945), besides investigating polymorphism, has also studied quantitative characters in the marsh fritillary butterfly, *Euphydryas aurinia,* and has obtained evidence of strong selective forces acting in nature. The species in the British Isles is a colony-forming insect with an annual life cycle, and shows considerable variation in wing pattern from population to population. The Irish forms have even been referred to a separate subspecies

from the English ones. The particular population studied by Ford was confined to a few swampy fields in Cumberland, and data on the variability of wing pattern and abundance of the butterflies in this colony is available over the period 1881–1935. The data for the early years (up to about 1916) comes from the notes of local collectors and the preserved specimens they amassed. Subsequently, the colony was studied by Ford himself.

A. Fluctuations in Population Size of the Colony of *Euphydryas aurinia*

The species was common in 1881 and from year to year the numbers increased steadily until 1896, when the colony reached the height of its abundance and the insects were to be found in vast numbers. Thereafter the number decreased steadily, and associated with this reduction there was a steady rise in the proportion of larvae which were parasitized. By 1912 the species had practically disappeared from the locality where formerly it had been so common. Between 1912 and 1920 the insect had become so rare that only two or three adults were seen (never more than one in a year), even though the locality was extensively examined.

In 1920 about sixteen larvae were found and from then onward the numbers to be seen increased with extreme rapidity up to 1924, by which time the species was again very abundant, and the numbers tended still to increase slightly up to about 1930, and then remained approximately constant until 1935.

B. Variability of Wing Pattern between 1894–1935

During the period 1894–1912, for which adequate data are available, the color pattern of the insects was of a distinct type and constant, there being little variation to be found. No information is available on the pattern of the insects for 1912 to 1920, but the larvae collected in 1920 gave rise to small dark insects. Over the next three or four years, while the population was rapidly expanding, there was a spectacular burst of variation, hardly any two insects looking alike. Such variability has never been observed before or since this time. Some of the insects were so deformed that they could hardly fly, and this applied to nearly all the specimens which differed from the normal color pattern to an extreme degree. Such imagines would certainly be at a great disadvantage under normal circumstances. After 1924 the variability decreased rapidly and a new and constant pattern was evolved. Moreover, this new pattern was quite unlike that found in the period between 1894 and 1912.

Two features are of particular interest: (1) the evolution of a new color pattern after 1920, a pattern which once evolved remained constant up until 1935, when the study was discontinued; (2) the outburst of variability between 1920 and 1924 at a period when the rate of expansion of the population size was many times greater than at any other time.

C. Fluctuations in Population Size and Evolution

At first sight it might be thought that the observed changes in the pattern and its variability might be accounted for by the action of genetic drift in a small population. However, this argument cannot be sustained since the variability was low while the population was small and declining. Had genetic drift been the causative agent for the observed changes, one would have expected the increase in variability and the change of pattern to appear first when the population was small and declining, owing to the consequent increased inbreeding. In fact, the variability appeared while the population was increasing, not decreasing. This further demonstrates that considerable genetic variability was retained by the population despite its extremely low numbers between 1912 and 1920. Moreover, the new pattern was evolved and stabilized while the insect was common, not rare. Only powerful selection could account for the rapid decrease in variability after 1924 and the evolution of a new pattern.

H. D. Ford and E. B. Ford (1930) pointed out that while the population size was increasing slowly (1881–1896, 1925–1930), decreasing (1897–1912), or remaining constant and very small (1913–1920), selection was likely to be intense, thus reducing variability to a minimum. However, much reduced selection between 1920 and 1924 would have allowed the population to increase very rapidly and at the same time show a great burst of variability. They further point out that this variability would allow new genetic combinations to be formed and tested, thus facilitating the evolution of a new and better adapted form. It is of course possible to argue that during the period 1912–1920 the gene-pool of the population changed as the result of genetic drift, and that the new pattern produced after 1924 resulted from the readjustment of this gene-pool as the result of powerful selection in the larger population, on the lines discussed by Mayr (1954) and Dobzhansky and Pavlovsky (1957). Against this interpretation it should be noted that selection was probably intense during the period 1912–1920, thus much reducing any effect drift might have had.

X. Selection and Polygenically Controlled Characters in *Maniola jurtina*

E. B. Ford and his co-workers for many years have been making an extensive study of the spots on the underside of the hind wings of the meadow brown butterfly, *Maniola jurtina,* in the British Isles where it always has an annual life cycle. The spots, which are mainly black, are situated on the pale submarginal band on the underside of the hind wing. They vary in number from 0 to 5 (very rarely 6), and also in size. Their position on the wing, if they are present, is rather constant, although there is some variation in their distance from the margin of the wing. The main study has concerned the frequency distribution of the number of spots rather than their size. In fact, work on the size of the spot would be difficult to undertake and would probably be less rewarding at the present time.

If one numbers the spots from 1 to 5 starting with that nearest the costal margin, one can also record the frequency distribution of the various possible patterns of spots. A five-spotted butterfly would be recorded as 12345, one with spot 3 absent as 12045, one with only spot 2 present as 02000, and one with no spots as 00000. This pattern variation has been studied in recent years by E. B. Ford and his colleagues but the results are as yet unpublished.

A. The Spot-Frequency Distribution in Southern England

Creed *et al.* (1959), Dowdeswell and Ford (1952, 1953), and McWhirter (1957) have reported on the spot-frequency distribution in males and females of *M. jurtina* in southern England. In the males the distribution of spot-numbers is always unimodal and has a marked mode at 2 spots. There is considerable heterogeneity between the spot frequencies from collecting stations in southern England, but this is not sufficiently marked to disturb the constancy of the mode at 2 spots (McWhirter, 1957).

The female spot-distributions are quite unlike those of the males. In all the southern English populations there is a marked mode at 0 spots with a rapidly decreasing frequency of the higher spot-numbers. Moreover, from 1950 to 1952, samples taken at widely separated localities, between mid-Devon in the west and Ipswich in the east, were homogeneous both between years and between populations, as distinct from the males, despite the very different ecological and climatic conditions found at the various stations. On resampling in 1956 and 1957 (Creed *et al.,* 1959) it was found that the situation had somewhat altered. In the 1950–2 samples the proportion of females with 0 spots

was greater than 60% of those with 2 spots or less, and there were never more individuals with 2 spots than there were with 1. This Creed *et al.* called the O.E. (Old English) type of distribution. In 1956 most southern English populations were of the N.E. (New English) type in which less, not more, than 60% of the females have 0 spots but again fewer insects have 2 spots than have 1. By 1957 most of the populations (except in the west) were back to the O.E. type of distribution.

In 1955, 1956, and 1957 it was noticed that butterflies emerging early in the season tended to have more spots than those caught later in some but not all localities. There also seemed to be some evidence that this seasonal change tended to be present, or at least more pronounced, where a population had just changed its spot-distribution from the O.E. to the N.E. type. Thus, although the southern England female spot-frequencies have not remained absolutely constant, the changes have seldom been so great as to disturb the characteristic mode at 0 spots.

B. The Spot-Frequency Distributions in Northern Scotland, Ireland, the Isle of Man, and Normandy

Forman *et al.* (1959) have investigated samples of *Maniola jurtina* from North Sutherland in Scotland. They found that they are similar to the southern English samples in that the males are unimodal at 2 spots and the females at 0 spots. The latter conform to the O.E. type of distribution. In Ireland (Dowdeswell and Ford, 1953; Creed *et al.*, 1959) the females, as far as is known, are also unimodal at 0 spots but with an even more pronounced mode than the southern English one. The males also tend to be less spotted and in some samples were bimodal at 0 and 2 spots. Samples from the Isle of Man are intermediate for each sex between the Irish and the southern English populations. The one sample from Normandy is similar to southern English samples.

C. The Spot-Frequency Distributions in West Devon and the Cornish Mainland

There is a very sharp contrast between the very constant picture for female spot-distribution in southern English localities and that found in Cornwall. The "Cornish" localities can for convenience be divided into three groups: (1) those in the east near the Devon and Cornwall border; (2) those in the west near Falmouth, Hayle, and Lands End; (3) those in the Isles of Scilly, which lie some 30 miles out in the Atlantic off Lands End.

1. *The East Cornish Populations*

Work by Creed *et al.* (1959) has revealed a most remarkable situation in the region of the Devon-Cornwall border. By 1951 it had been found that although male spot-distributions were uniformly unimodal at 2 spots throughout the south of England, the female distribution was unimodal at 0 spots only up to Devon, and further to the west in Cornwall populations bimodal at 0 and 2 spots with the greater mode at 0 were to be found. Further investigation in 1952 revealed that the change from a unimodal to a bimodal distribution was not gradual, as might have been expected, but was abrupt in the region of the county boundary near Plymouth. Moreover, on each side of this abrupt change the populations were homogeneous over a wide area (bimodal to the west, unimodal to the east). In 1956 work showed that inland between Lydford and Lewannick the same sharp discontinuity occurred between the spot-frequency distributions to the east and to the west. Moreover, detailed investigations showed that the change-over from one to the other type of population occurred in a distance of a few yards at most (at a hedge which was no barrier to the movements of the butterflies). There was also some evidence that the difference between the southern English and East Cornish spot-frequency distributions was greatest at the border between them.

This remarkable discontinuity between contiguous populations was reinvestigated in 1957 with even more startling results. It was found that since the previous generation the boundary separating the two types of population had moved about 3 miles to the east and that the change-over now took about 150 yards, with an intermediate population in the intervening territory. Moreover, there was still evidence for the difference between the two types of distribution being greatest close to the border between them.

2. *The West Cornish Populations*

A number of populations have been investigated in West Cornwall but to a lesser extent than in the east of the county. On the Lands End peninsula the female spot-distribution is unimodal at 2 spots like that of the males, but with a smaller mode. Further east, in the region of Falmouth the population is probably bimodal at 0 and 2 spots but significantly different from the East Cornish distribution. To the north, on the Hayle sandhills of the coast, there is a very large isolated population which showed a significant change in spot-frequency distribution between 1951 and 1952, the two years in which samples were taken.

This is a unique sandhill locality, where differences in rainfall produce extreme effects upon the vegetation.

D. The Spot-Frequency Distribution in the Isles of Scilly

The Isles of Scilly, about 30 miles off Lands End, consist of about 140 islands, some of which are mere rocks exposed only at low tide. The islands inhabited *by M. jurtina* can be divided into three groups (Dowdeswell *et al.*, 1957): (1) the three large inhabited islands of St. Mary's, St. Martin's, and Tresco; (2) three islands of intermediate size, Bryher, St. Agnes (which has not been properly studied), and Samson; (3) a number of small islands, of which the most intensively studied is Tean. The other small islands investigated are Great Ganilly, Arthur, White Island, and St. Helen's. On all the islands the males' spot-distribution tends to have a single mode at 2 spots. However, there is heterogeneity between islands as there is between populations on the mainland. This heterogeneity is more pronounced on the islands and in some populations the mode at 2 spots is barely maintained.

It is in the female spot-frequency distributions that the greatest interest of the work on the Isles of Scilly lies.

1. *Small and Intermediate-Sized Islands*

The small and intermediate-sized islands in the Isles of Scilly are formed from rocky hills. When an island has more than one such hill, as most do, there is a low sandy bar between them. These bars are covered with short vegetation closely grazed by rabbits, uninhabited by permanent populations of *M. jurtina*, and a partial barrier to the butterflies' movements. Consequently many of the islands have two or more semi-isolated populations living on them.

On examining the female spot-distribution of the populations on the small and intermediate-sized islands we find (Dowdeswell and Ford, 1955; McWhirter, 1957; Dowdeswell *et al.*, 1949, 1960) that there are four types of distribution: (1) unimodal with a mode at 2 spots: St. Helen's, St. Agnes, Samson (South), and Gweal Hill on Bryher; (2) bimodal at 0 and 2 spots with the larger mode at 2: Tean area 1 and 3, Arthur, Samson (North), and Old Man (North); (3) bimodal at 0 and 2 spots with the larger mode at 0: Old Man (South); (4) unimodal with a large mode at 0 spots: Bryher (South) and White Island.

Although there are these four main types of distribution to be found, within any one type there is considerable heterogeneity in the actual frequencies of the spot classes from population to population.

2. *The Large Islands; the Main Populations*

In contrast to the remarkable heterogeneity between the populations on the small and medium-sized islands and even between the semi-isolated populations on any one island, the populations in the main collecting areas of the three large islands remained homogeneous among themselves and from year to year between 1950 and 1955. Moreover, unlike all the other populations they showed a "flat top" distribution with about equal number of butterflies with 0, 1, and 2 spots. Between 1955 and 1957 the population on St. Mary's, the largest and most diverse island, retained its distribution, but those on Tresco and St. Martin's changed, giving a distribution with a slight mode at 0 and about equal numbers of insects with 1 and 2 spots in 1956, and a more marked mode at 0 in 1957.

3. *The Large Islands; the Peripheral Populations*

Besides the main populations on the large islands there are a number of peripheral wholly isolated populations which differ both from one another and from the main populations. Thus, these populations behave like the small island populations. One, "the Farm Area" on Tresco, is quite distinct from the main area since it is unimodal at 2 spots with equal numbers of insects with 0 and 1 spot. This population remained stable in spot-frequency distribution from 1954 to 1956 despite its small size. It was estimated to number about 100 to 150 flying insects when it was studied by the method of mark-release-recapture in 1954 and 1955.

E. Natural Selection and Spot-Number

The data collected on spot-number in *Maniola jurtina* are some of the most extensive for any quantitative character in the world. The interpretation of this information is therefore of great interest to population geneticists. E. B. Ford has pointed out that the very constant composition of populations throughout southern England suggests that spot-numbers must be under strong genetic control. If this were not so one would expect very great heterogeneity, not homogeneity, between the various populations since those studied live in extremely diverse habitats as far as geological structure, climate, vegetation, and population density are concerned. Moreover, despite the much more uniform climatic conditions of the Isles of Scilly, the populations are far more diversified but retain their distinct individuality year after year with only minor changes in a few of them. Such changes as have been noted

appear to be associated with particular changes in the ecology of the habitat, as that produced by the removal from Tean of a small herd of cattle. Ford has also pointed out that the situation found on the Devon-Cornwall border argues strongly in favor of the genetic control of spot-number. It would be difficult to conceive a set of environmental conditions which alone would maintain such a sharp discontinuity as that observed, but maintain a constant spot-frequency distribution over a wide area each side of the border. It becomes even more difficult to invoke purely environmental factors when it is remembered that although the discontinuity has remained sharp it has moved 3 miles eastward in but a single generation.

Ford and his co-workers therefore conclude that spot-number is mainly under strict genetic control and that the genes concerned (though not spot-number itself) are subject to very strong selection. They also point out that the situation in the Isles of Scilly is in complete agreement with this view. The main populations on the three large islands have spot-frequency distributions which are remarkably similar to one another, and the habitats occupied by the butterflies are very diverse. The populations on the small islands and on the small isolated areas of the large islands are markedly different from one another and each, owing to the small area involved, inhabits a more uniform environment. They therefore conclude that the main populations on the large islands are genetically adjusted to the average of very diverse conditions encountered by each population and are thus much alike. On the other hand, natural selection has very clearly adjusted each population in the more uniform habitats to the particular conditions prevailing in its area and therefore these populations are very dissimilar.

Dowdeswell *et al.* (1960) have estimated the order of magnitude of the change in selection in those instances where a change in spot distribution has been recorded. Thus, from the change in Tresco, observed in 1957, they deduce that about 60% of the females with 2 or more spots were eliminated. The subsequent fate of this population will be of considerable interest since strong selection in one generation should have effects detectable over several generations. Should the selection be maintained, one would expect the rapid elimination of high-spotted females unless they are mainly produced by the additive effect of recessives.

The difference between the spot-distribution in the two sexes as well as the other evidence accumulated makes it certain that spot-number is affected by genetic factors and that populations differ in their genetic make-up with respect to spotting. It therefore seems likely that

the differences between populations on the Isles of Scilly and on the Devon-Cornwall border reflect accurately the genetic adjustment of the gene-frequencies of the populations as the result of natural selection. Nevertheless, it does not seem possible, at this time, to judge the magnitude of the selection from observed changes in spot-frequencies from year to year. Genetically controlled characters, particularly quantitative ones, seem almost always to be directly affected to varying degrees by the environment. Consequently, any environmental change large enough to alter so drastically the selective value of high-spotted females might also be drastic enough to alter the phenotype directly. Moreover, the fact that females which emerge early may have a higher mean spot-number in some populations makes it difficult to compare different populations, or even the same population from year to year, unless the colonies are sampled throughout the season, since mean date of emergence can vary from population to population and year to year. Only after the heritability of spot-number has been accurately ascertained for the populations in question can the observed changes (or absence of changes) in spot-frequency from generation to generation be interpreted in terms of gene-frequency change or can the intensity of natural selection be estimated.

XI. Polygenically Controlled Characters in *Panaxia dominula*

Kettlewell has performed an experiment (see Sheppard, 1954, 1956) which demonstrates how very powerful can be the selection operating in small populations and how this can override any effects of genetic drift, or the founder principle, which after all is only a special case of genetic drift. Kettlewell worked with the scarlet tiger moth, *Panaxia dominula*. In a stock of this moth he selected for a reduction in the size of the black marks on the hind wing, and for an increase in the pale buff and white spots on the forewing.

After about ten generations of careful selection Kettlewell had developed a stock with an extremely abnormal pattern. There was still considerable variability, but the appearance of the majority of the insects departed more from the English forms than do most of the European subspecies of this moth. A large number of the insects are preserved in the Rothschild-Cockayne-Kettlewell collection at Tring.

Kettlewell selected in the main for a number of characters of variable expression. He showed (1942) that one of these was an incomplete dominant controlled by a single gene. On going to South Africa he decided to preserve the stock by starting an artificial colony at Tring Museum. This was done by planting the larval food plant in a suitable place in the grounds of the museum. There was no chance of the stock

becoming contaminated when released since the moth is a colony-forming species and no local populations were available to contribute individuals to the artificial colony. It is known (Sheppard, 1951, 1953a) that populations even 1½ miles apart are effectively isolated.

Kettlewell released a small number of broods of larvae, from the most extreme parents, in the area prepared for them. No direct observation was kept on the colony but the museum staff reported that the moths were very scarce the first year (1949) but increased steadily in numbers from year to year. By 1953 the larvae were so abundant that most starved to death after decimating their food plant, and few moths appeared. In 1954 only one dead specimen was seen, where several thousand were flying in 1952, and since then none has been found.

The appearance of the moths was not studied for the first two generations but by the third, when the moth was beginning to become common, it was noticed that most insects were quite unlike the original stock and were close to the normal English form in pattern. Moreover, not a single specimen as extreme as the least extreme of the founder parents was to be seen. By 1953, when adequate samples were taken by collecting and rearing the starving larvae, it was observed that the stock had almost, but not quite, reverted to the normal English pattern. Moreover, it could be shown that this evolutionary change was not caused by contamination with other stocks or wild individuals since the insects all carried the semidominant gene, although by now its effect on the wing pattern had been very much reduced.

It is clear that for this very great change to have occurred in so few generations, very extreme selection must have been operating. It is almost certain that the selection was not acting directly on the color pattern. The moth does not gain protection from its vertebrate enemies by having a very cryptic pattern but is highly distasteful to birds (see Rothschild and Lane, 1960, for references) and, although not very conspicuous at rest, it exhibits warning coloration if disturbed (Sheppard, 1951). Not only did Kettlewell's selection enhance this warning pattern but predator selection is not likely to have been of much importance, particularly since in the grounds of Tring the moths would have been in some measure protected from attack. Furthermore, since olfactory, not visual, stimuli are important in courtship in *Panaxia dominula* (Sheppard, 1952), sexual selection is not likely to have been acting directly on wing pattern. The selection was almost certainly for a balanced gene-complex previously disturbed by the artificial selection used in the production of the stock. Mather and Harrison (1949) have shown, in laboratory stocks of *D. melanogaster*, the same type of reversion to an original form after artificial selection is relaxed.

XII. Summary

(1) Some recent results of evolutionary interest, obtained by population genetics studies in the Lepidoptera, are reviewed. Information previously given by Ford (1953) and Remington (1954) is not discussed in detail, except where important results have been obtained since their papers were published.

(2) It has been demonstrated by observations on natural and experimental populations that major genes can have very large selective values in the wild, and that these can fluctuate violently in direction and intensity from year to year, and place to place.

(3) Major genes which are responsible for the presence of a polymorphism frequently affect more than one character, and selection acts on both the visual and physiological characters controlled by them.

(4) Not all polymorphisms in the Lepidoptera are maintained by the advantage of the heterozygote over both homozygotes. There is reason for believing, however, that such a situation may evolve if two or more allelomorphs are maintained at high frequency for a prolonged period.

(5) The spread of a major gene in a population often results in a reconstruction of the gene-complex due to changes in selection consequent upon the presence of the major gene. Thus, the viability of industrial melanics, and their resemblance to the soot-covered surfaces on which they rest, is improved after the melanics have become well established. Similarly, the presence of genes controlling mimetic resemblances to distasteful models leads to a reconstruction of the rest of the gene-complex, which improves the resemblance between mimic and model.

(6) Experiments in the laboratory and field observations have shown that various protective devices such as cryptic coloration, the presence of eye spots on the wings, warning coloration, Müllerian mimicry, and Batesian mimicry, can be effective in reducing predation, and that powerful selection must be operating to perfect and maintain them.

(7) It is suggested that the presence of polymorphisms confined to the female sex in butterflies, but not in moths, depends on the nature of courtship in the Lepidoptera. In butterflies visual stimuli, as well as olfactory ones, are important in courtship, but in moths olfactory stimuli are mainly utilized. Consequently, a novel color pattern in a male butterfly may be disadvantageous, since the female may reject the male, whereas in a moth the color pattern as such will not affect the female's choice.

(8) Disruptive selection can lead to the evolution of a polymorphism

controlled by a switch-gene. There is evidence that in the course of time more and more loci can become incorporated in a switch-gene, as the result of selection for increased linkage between the loci.

(9) Disruptive selection can be effective in nature as well as in the laboratory in producing bimodality for a quantitative character within the population.

(10) Evidence is available which strongly suggests that dominance, if absent initially, can be evolved in a polymorphic character. Moreover, if there are two such populations different modifiers of dominance may be utilized in each.

(11) Quantitative variation in wing patterns is often under polygenic control. Although the pattern itself may be unimportant, so far as selection is concerned, the physiological effects of the polygenes may be subject to powerful selection. Consequently, each population becomes highly adapted to the environment in which it lives.

(12) In one experiment it has been possible to show that when the genetic balance has been disturbed by artificial selection natural selection can quickly restore this balance.

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References

Acton, A. B., 1957a. Chromosome inversions in natural populations of *Chironomus tentans*. *J. Genet.* **55,** 61–94.

Acton, A. B., 1957b. Chromosome inversions in natural populations of *Chironomus dorsalis*. *J. Genet.* **55,** 261–275.

Aé, A. S., 1959. A study of hybrids in *Colias* (Lepidoptera, Pieridae). *Evolution* **13,** 64–88.

Aé, A. S., 1960. A study of hybrids between *Papilio xuthus* and the *P. polyxenes-machaon* group. *J. Lepidopterists' Soc.* **14,** 5–18.

Blest, A. D., 1957a. The function of eyespot patterns in the Lepidoptera. *Behaviour* **11,** 209–255.

Blest, A. D., 1957b. The evolution of protective displays in the Saturnioidea and Sphingidae (Lepidoptera). *Behaviour* **11,** 257–310.

Blest, A. D., 1960. The resting position of *Cerodirphia speciosa* (Cramer), (Lepidoptera, Saturniidae): The ritualization of a conflict posture. *Zoologica* **45,** 81–90.

Bowater, W., 1914. Heredity of melanism in Lepidoptera. *J. Genet.* **3,** 299–315.

Bowater, W., 1955. Private communication.

Brower, J. V., 1958a. Experimental studies of mimicry in some North American butterflies. I. *Danaus plexippus* and *Limenitis archippus archippus*. *Evolution* **12,** 32–47.

Brower, J. V., 1958b. Experimental studies of mimicry in some North American butterflies. II. *Battus philenor* and *Papilio troilus, P. polyxenes* and *P. glaucus*. *Evolution* **12,** 123–136.

Brower, J. V., 1958c. Experimental studies of mimicry in some North American butterflies. III. *Danaus gilippus berenice* and *Limenitis archippus floridensis*. *Evolution* **12,** 273–285.

Brower, J. V., 1960. Experimental studies of mimicry. IV. The reactions of starlings to different proportions of models and mimics. *Am. Naturalist* **94,** 271–282.

Brower, L. P., 1959. Speciation in butterflies of the *Papilio glaucus* group. II. Ecological relationships and interspecific sexual behavior. *Evolution* **13,** 212–228.

Brower, L. P., and Brower, J. V., 1956. Cryptic coloration in the anthophilous moth *Rhododipsa masoni*. *Am. Naturalist*. **90,** 177–182.

Carpenter, G. D. H., and Ford, E. B., 1933. "Mimicry." Methuen, London.

Caspari, E., 1950. On the selective value of alleles *Rt* and *rt* in *Ephestia kühniella*. *Am. Naturalist* **84,** 367–380.

Clarke, C. A., and Sheppard, P. M., 1955a. A preliminary report on the genetics of the machaon group of swallowtail butterflies. *Evolution* **9,** 182–201.

Clarke, C. A., and Sheppard, P. M., 1955b. The breeding in captivity of the hybrid *Papilio rutulus* female × *Papilio glaucus* male. *Lepidopterists' News* **9,** 46–48.

Clarke, C. A., and Sheppard, P. M., 1955c. The breeding in captivity of the hybrid swallowtail *Papilio machaon gorganus* Fruhstorfer ♀ × *Papilio hospiton* Gene ♂. *Entomologist* **88,** 265–270.

Clarke, C. A., and Sheppard, P. M., 1956. A further report on the genetics of the machaon group of swallowtail butterflies. *Evolution* **10,** 66–73.

Clarke, C. A., and Sheppard, P. M., 1957. The breeding in captivity of the hybrid *Papilio glaucus* female × *Papilio eurymedon* male. *Lepidopterists' News* **11,** 201–205.

Clarke, C. A., and Sheppard, P. M., 1959a. The genetics of some mimetic forms of *Papilio dardanus,* Brown, and *Papilio glaucus,* Linn. *J. Genet.* **56,** 236–259.

Clarke, C. A., and Sheppard, P. M., 1959b. The genetics of *Papilio dardanus,* Brown. I. Race *cenea* from South Africa. *Genetics* **44,** 1347–1358.

Clarke, C. A., and Sheppard, P. M., 1960a. The genetics of *Papilio dardanus,* Brown. II. Races *dardanus, polytrophus, meseres* and *tibullus*. *Genetics* **45,** 439–457.

Clarke, C. A., and Sheppard, P. M., 1960b. The genetics of *Papilio dardanus,* Brown. III. Race *antinorii* from Abyssinia and race *meriones* from Madagascar. *Genetics* **45,** 683–698.

Clarke, C. A., and Sheppard, P. M., 1960c. The evolution of dominance under disruptive selection. *Heredity* **14,** 73–87.

Clarke, C. A., and Sheppard, P. M., 1960d. The evolution of mimicry in the butterfly *Papilio dardanus*. *Heredity* **14,** 163–173.

Clarke, C. A., and Sheppard, P. M., 1960e. Super-genes and mimicry. *Heredity* **14,** 175–185.

Cockayne, E. A., 1938. The genetics of sex in Lepidoptera. *Biol. Revs. Cambridge Phil. Soc.* **13,** 107–132.

Cott, H. B., 1940. "Adaptive Coloration in Animals." Methuen, London.

Creed, E. R., Dowdeswell, W. H., Ford, E. B., and McWhirter, K. G., 1959. Evolutionary studies on *Maniola jurtina*: the English mainland 1956–57. *Heredity* **13,** 363–391.

de Ruiter, L., 1952. Some experiments on the camouflage of stick caterpillars. *Behaviour* **4,** 222–232.

de Ruiter, L., 1956. Countershading in caterpillars. An analysis of its adaptive significance. *Arch. néerl. zool.* **11,** 285–341.

Dobzhansky, Th., 1959. Evolution of genes and genes in evolution. *Cold Spring Harbor Symposia Quant. Biol.* **24,** 15–30.

Dobzhansky, Th., and Pavlovsky, O., 1957. An experimental study of interaction between genetic drift and natural selection. *Evolution* **11,** 311–319.

Dowdeswell, W. H., and Ford, E. B., 1952. The distribution of spot-numbers as an index of geographical variation in the butterfly *Maniola jurtina* L. (Lepidoptera: Satyridae). *Heredity* **6,** 99–109.

Dowdeswell, W. H., and Ford, E. B., 1953. The influence of isolation on variability in the butterfly *Maniola jurtina* L. *Symposia Soc. Exptl. Biol. No.* **7,** 254–273.

Dowdeswell, W. H., and Ford, E. B., 1955. Ecological genetics of *Maniola jurtina* L. on the Isles of Scilly. *Heredity* **9,** 265–272.

Dowdeswell, W. H., Fisher, R. A., and Ford, E. B., 1949. The quantitative study of populations in the Lepidoptera 2. *Maniola jurtina* L. *Heredity* **3,** 67–84.

Dowdeswell, W. H., Ford, E. B., and McWhirter, K. G., 1957. Further studies on isolation in the butterfly *Maniola jurtina* L. *Heredity* **11,** 51–65.

Dowdeswell, W. H., Ford, E. B., and McWhirter, K. G., 1960. Further studies on the evolution of *Maniola jurtina* in the Isles of Scilly. *Heredity* **14,** 333–364.

Fisher, R. A., 1930. "The Genetical Theory of Natural Selection." Clarendon Press, Oxford.

Fisher, R. A., 1958. Polymorphism and natural selection. *J. Ecol.* **46,** 289–293.

Fisher, R. A., and Ford, E. B., 1947. The spread of a gene in natural conditions in a colony of the moth *Panaxia dominula* L. *Heredity* **1,** 143–174.

Ford, E. B., 1937. Problems of heredity in the Lepidoptera. *Biol. Revs. Cambridge Phil. Soc.* **12,** 461–503.

Ford, E. B., 1940. Genetic research in the Lepidoptera. *Ann. Eugen.* **10,** 227–252.

Ford, E. B., 1945. "Butterflies." Collins, London.

Ford, E. B., 1953. The genetics of polymorphism in the Lepidoptera. *Advances in Genet.* **5,** 43–87.

Ford, E. B., 1955a. "Moths." Collins, London.

Ford, E. B., 1955b. Polymorphism and taxonomy. *Heredity* **9,** 255–264.

Ford, H. D., and Ford, E. B., 1930. Fluctuation in numbers and its influence on variation in *Melitaea aurinia*. *Trans. Roy. Entomol. Soc.* (*London*) **78,** 345–351.

Forman, B., Ford, E. B., and McWhirter, K. G., 1959. An evolutionary study of the butterfly *Maniola jurtina* in the north of Scotland. *Heredity* **13,** 353–361.

Glass, B., 1959. In discussion of "The evolution of mimicry; a problem in ecology and genetics." *Cold Spring Harbor Symposia Quant. Biol.* **24,** 140.

Goldschmidt, R. B., 1934. *Lymantria. Bibliogr. Genet.* **11,** 1–186.

Goldschmidt, R. B., 1945. Mimetic polymorphism, a controversial chapter of Darwinism. *Quart. Rev. Biol.* **20,** 147–164, 205–230.

Howarth, T. G., 1960. Two examples of protective resemblance in Lepidoptera. *Entomologists' Gaz.* **11,** 184.

Kettlewell, H. B. D., 1942. A survey of the insect *Panaxia* (*Callimorpha*) *dominula* L. *Proc. S. London Entomol. Nat. Hist. Soc.* pp. 1–49.

Kettlewell, H. B. D., 1955. Selection experiments on industrial melanism in the Lepidoptera. *Heredity* **9,** 323–342.

Kettlewell, H. B. D., 1956. Further selection experiments on industrial melanism in the Lepidoptera. *Heredity* **10,** 287–301.

Kettlewell, H. B. D., 1958. A survey of the frequencies of *Biston betularia* (L.) (Lep.) and its melanic forms in Great Britain. *Heredity* **12,** 51–72.

Kettlewell, H. B. D., 1959a. New aspects of the genetic control of industrial melanism in the Lepidoptera. *Nature* **183,** 918–921.

Kettlewell, H. B. D., 1959b. Brazilian insect adaptations. *Endeavour* **18,** 200–210.

Kettlewell, H. B. D., 1959c. Darwin's missing evidence. *Sci. American* **200,** 48–53.

Levitan, M., 1958. Non-random associations of inversions. *Cold Spring Harbor Symposia Quant. Biol.* **23,** 251–268.

Lorković, Z., 1958. Some peculiarities of spatially and sexually restricted gene exchange in the *Erebia tyndarus* group. *Cold Spring Harbor Symposia Quant. Biol.* **23,** 319–325.

McWhirter, K. G., 1957. A further analysis of variability in *Maniola jurtina* L. *Heredity* **11,** 359–371.

Magnus, D. B. E., 1958. Experimental analysis of some "overoptimal" sign-stimuli in the mating-behaviour of the fritillary butterfly *Argynnis paphia* L. (Lepidoptera. Nymphalidae). *Proc. 10th Intern. Congr. Entomol.* **2,** 405–418.

Mather, K., 1955. Polymorphism as an outcome of disruptive selection. *Evolution* **9,** 52–61.

Mather, K., and Harrison, B. J., 1949. The manifold effects of selection. *Heredity* **3,** 1–52, 131–162.

Mayr, E., 1954. *In* "Evolution as a Process" (J. S. Huxley, A. C. Hardy, and E. B. Ford, eds.). Allen and Unwin, London.

Pontecorvo, G., 1959. "Trends in Genetic Analysis." Oxford Univ. Press, London and New York.

Remington, C. L., 1954. The genetics of *Colias* (Lepidoptera). *Advances in Genet.* **6,** 403–450.

Remington, C. L., 1956. Interspecific relationships of two rare swallowtail butterflies, *Papilio nitra* and *Papilio hudsonianus,* to other members of *Papilio machaon* complex. *Am. Phil. Soc. Year Book 1955* pp. 142–146.

Remington, C. L., 1958. Genetics of populations of Lepidoptera. *Proc. 10th Intern. Congr. Entomol.* **2,** 787–805.

Remington, C. L., 1959. Wide experimental crosses between *Papilio xuthus* and other species. *J. Lepidopterists' Soc.* **13,** 151–164.

Rothschild, M., and Lane, C., 1960. Warning and alarm signals by birds seizing aposematic insects. *Ibis* **102,** 320–330.

Schmidt, R. S., 1958. Behavioural evidence on the evolution of Batesian mimicry. *Animal Behaviour* **6,** 129–138.

Schmidt, R. S., 1960. Predator behaviour and the perfection of incipient mimetic resemblances. *Behaviour* **16,** 149–158.

Sexton, O. J., 1960. Experimental studies of artificial Batesian mimics. *Behaviour* **15,** 244–252.

Sheppard, P. M., 1951. A quantitative study of two populations of the moth *Panaxia dominula* (L.) *Heredity* **5,** 349–378.

Sheppard, P. M., 1952. A note on non-random mating in the moth *P. dominula*. *Heredity* **6,** 239–241.

Sheppard, P. M., 1953a. Polymorphism and population studies. *Symposia Soc. Exptl. Biol. No.* **7,** 274–289.

Sheppard, P. M., 1953b. Polymorphism, linkage and the blood groups. *Am. Naturalist* **87,** 283–294.

Sheppard, P. M., 1954. *In* "Evolution as a Process" (J. S. Huxley, A. C. Hardy, and E. B. Ford, eds.). Allen and Unwin, London.

Sheppard, P. M., 1956. Ecology and its bearing on population genetics. *Proc. Roy. Soc.* **B145,** 308–315.

Sheppard, P. M., 1959a. "Natural Selection and Heredity." Hutchinsons, London.
Sheppard, P. M., 1959b. The evolution of mimicry; a problem in ecology and genetics. *Cold Spring Harbor Symposia Quant. Biol.* **24,** 131–140.
Sheppard, P. M., 1961. Some aspects of the geography, genetics and taxonomy of a butterfly. *The Systematics Assoc. London Publ.* No. **4,** 79–96.
Stehr, G., 1959. Hemolymph polymorphism in a moth and the nature of sex-controlled inheritance. *Evolution* **13,** 537–560.
Stride, G. O., 1958. On the courtship behaviour of a tropical mimetic butterfly *Hypolimnas misippus* L. (Nymphalidae). *Proc. 10th Intern. Congr. Entomol.* **2,** 419–424.
Thoday, J. M., 1953. Components of fitness. *Symposia Soc. Exptl. Biol.* No. **7,** 96–113.
Thoday, J. M., 1959. Effects of disruptive selection. I. Genetic flexibility. *Heredity* **13,** 187–218.
Thoday, J. M., 1960. Effects of disruptive selection. III. Coupling and repulsion. *Heredity* **14,** 35–49.
Thoday, J. M., and Boam, T. B., 1959. Effects of disruptive selection. II. Polymorphism and divergence without isolation. *Heredity* **13,** 205–218.
Tinbergen, L., 1960. The dynamics of insect and bird populations in pine woods. *Arch. néerl. zool.* **13,** 259–473.
Van Someren, V. G. L., and Jackson, T. H. E., 1959. Some comments on protective resemblance amongst African Lepidoptera (Rhopalocera). *J. Lepidopterists' Soc.* **13,** 121–150.
Williamson, M. H., 1961. On the polymorphism of the moth *Panaxia dominula* (L.). *Heredity* **5,** 139–151.
Windecker, W., 1939. *Euchelia* (*Hypocrita*) *jacobaeae* L. und das Schutztrachten-problem. *Z. Morphol. Ökol. Tiere.* **35,** 84–138.
Wright, S., 1931. Evolution in mendelian populations. *Genetics* **16,** 97–159.
Wright, S., 1932. The roles of mutation, inbreeding, cross-breeding and selection in evolution. *Proc. 6th Intern. Congr. Genet.* **1,** 356–366.
Wright, S., 1948. On the roles of directed and random changes in gene frequency in the genetics of populations. *Evolution* **2,** 279–294.

ORIGIN AND CYTOGENETICS OF THE COMMERCIAL POTATO

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	Page
I. Introduction	217
II. The Relationship between *S. tuberosum* L. and *S. andigena* Juz. et Buk.	219
A. Historical Position	221
B. Botanical Characters	221
C. Photoperiodic Response	222
D. Cytogenetic Behavior	223
III. The Nature of Polyploidy in *S. tuberosum*	224
A. Basic Chromosome Number	224
B. Nature of Tetraploidy	230
IV. Probable Ancestor of *S. tuberosum*	240
A. Center of Origin of Cultivated Potatoes	240
B. Tetraploid Species Related to *S. tuberosum*	242
C. Diploid Ancestor of *S. tuberosum*	243
V. Conclusion	247
References	249

I. Introduction

The potato varieties cultivated commercially in most countries outside South America belong to the species *Solanum tuberosum* L. The genus *Solanum* to which this species belongs is a large one comprising some 2000 species, but only about 100 of these are tuber-bearing. While members of this genus occur in all parts of the world, the tuber-bearing species are found only in the American continent. Dunal (in de Candolle, 1852) divided the genus into two sections, *Pachystemonum* and *Leptostemonum,* the former including species with thick anthers and no thorns, and the latter those with thorns and long and narrow anthers. Dunal divided *Pachystemonum* into five subsections of which the subsection *Tuberarium* includes the tuber-bearing species. Bitter (1912–1914) divided *Tuberarium* into two subsections, *Basarthrum* and *Hyperbasarthrum,* using the position of pedicel articulation and structure of cell hairs as the criteria for classification. Most of the species in *Hyperbasarthrum* are tuber-bearing; some, however, do not possess stolons or tubers but have apparently been included in this section by Bitter on the basis of morphological resemblances. No crosses have so far been

made between tuber-bearing and nontuber-bearing *Solanum* species. *S. lycopersicoides* and *S. pennellii*, two nontuberous species belonging to the section *Hyperbasarthrum*, have, however, been successfully crossed with *Lycopersicon esculentum* (Rick, 1951, 1960).

The species of the subsection *Hyperbasarthrum* form an euploid series with 24, 36, 48, 60, and 72 somatic chromosomes. Hawkes (1956a) has recognized 17 distinct taxonomic series in this subsection. The commercial potato, *S. tuberosum*, belongs to the series *Tuberosa* first described by Rydberg in 1924. Both wild and cultivated species with $2n = 24$, 36, 48, and 60 occur in this series and they are found in a wide area of South America. Following the visit of various plant collecting expeditions to Mexico and South America, starting with the one sent by the Russian Government under the leadership of N. I. Vavilov in 1925, the existence of an enormous variability in morphological, physiological, and biochemical characters among species of the section *Tuberarium* came to light. As a result, this group attracted the interest of potato breeders and extensive collections of tuber-bearing *Solanum* species were built up at potato breeding centers in several countries.

The commercial potato, *S. tuberosum*, is a tetraploid with $2n = 48$. A classic study of the history and social influence of this crop plant has been carried out by Salaman (1949). Taxonomists like Bitter (1912–14), Rydberg (1924), Bukasov (1930, 1933), Juzepczuk (1937), Hawkes (1944, 1956a,b), and Correll (1952) have gathered extensive data on the morphological characters and geographical distribution of wild and cultivated potato species. In contrast, cytological and genetic studies in potato species have been relatively few and less intensive. The small size of the chromosomes and the absence of any evidence of distinct genomic differentiation in interspecific hybrids except in a few species (Swaminathan and Hougas, 1954; Marks, 1955) render cytological studies difficult and inconclusive in revealing the phylogenetic history. Vegetative propagation permits the accumulation of chromosome structural changes, and varying degrees of meiotic abnormalities occur in many species and species hybrids. The task of assessing the adaptive and evolutionary significance of chromosome aberrations is further complicated by the high susceptibility of most potato species to virus diseases and the consequent possibility of the observed meiotic abnormalities arising from disturbed metabolism (Kostoff, 1933; Swaminathan *et al.*, 1959). Genetic studies are also difficult owing to various reasons among which the following are some of the more important. First, all potato varieties are heterozygous for many of the characters studied, and homozygous plants can only be obtained, if at all, by several years of selfing. Secondly, among the seedlings derived from selfing a variety,

or from crossing two varieties, there is usually a varying percentage of degenerate individuals which may affect the segregation ratio for the character being studied. Thirdly, there is the difficulty that among the offspring many may not flower, or if they do, may have pollen and ovule sterility. Fourthly, segregations are often complex owing to the polyploid constitution of the potato. Finally, bud mutations and chimeras occur frequently. However, the improvement in cytological and breeding techniques effected during recent years has led to the accumulation of much valuable data regarding the cytogenetics of tuber-bearing *Solanum* species. In the present review the available cytogenetic and other evidence pertaining to the origin of *S. tuberosum* is examined. Though in South America several species belonging to the series *Tuberosa* are cultivated, and some species of the series *Commersoniana* are grown by farmers in tropical countries like Ceylon owing to their ability to tuberize under high air temperature conditions, *S. tuberosum* is the potato of commerce in most parts of the world. Besides *S. tuberosum* another cultivated tetraploid species, *S. andigena*, has been recognized by Juzepczuk and Bukasov (1929) from their study of South American potatoes. The relationship between the two cultivated tetraploid species, the nature of tetraploidy in them, and the probable mode of origin of *S. tuberosum* are considered in this paper.

II. The Relationship between *S. tuberosum* L. and *S. andigena* Juz. et Buk.

To Caspar Bauhin goes the honor of having published the first botanical description of the potato and of giving it the name *Solanum tuberosum*. Bauhin described the potato variety grown in Europe at that time in his Phytopinax, published in 1596. The herbarium specimen of the potato made by him is still preserved in excellent condition at the Basle Herbarium in Switzerland. Linnaeus adopted in his "Species Plantarum" Bauhin's name, *Solanum tuberosum*, for the potato plants cultivated in Europe up to 1753. Linnaeus' description of *S. tuberosum* appears to have been based not only on living plants seen by him in Botanical Gardens in Sweden and Holland but also on the descriptions and figures of Bauhin and Clusius (Hawkes, 1956b). It was so constructed as to enable the botanist to distinguish the species of *Solanum* known in Europe at that time and is obviously of no value in distinguishing the many tuber-bearing *Solanum* species brought to Europe subsequently.

The Russian expedition which visited Mexico and South America during the years 1925–1932 found that cultivated tetraploid potato strains grew over a wide geographical range, from Mexico and Guatemala in the north, southward through the mountains of Colombia,

Ecuador, Peru, Bolivia, and northern Argentina and also on the seacoast of southern Chile in the region of the island of Chilóé. On the basis of the similarity between the Chilean tetraploids and the European cultivated potatoes in morphological characters and photoperiodic response, Juzepczuk and Bukasov (1929) suggested that the potatoes originally introduced into Europe were from the Chilóé region in Chile. In the coastal regions of southern Chile (41° to 43° S latitude) the length of day is 15–17 hours during the growing season in contrast to a day length of 12 hours found in the Andean region. The tetraploid potatoes found in these two regions hence tuberize best under long- and short-day length conditions respectively. In addition, these two types of cultivated tetraploids differ from each other in some morphological characters. Thus, the potatoes from the Andes have narrower leaflets, with leaves set at an acute angle from the stem and generally more dissected. In the Chilean and European potatoes, on the other hand, the leaves are less dissected, have wider leaflets, and are generally arched and set at wide angle to the stem. Also the pedicels are thickened at the apex while this is not the case in the Andean strains. From a consideration of these differences, Juzepczuk and Bukasov (1929) assigned the name *S. tuberosum* L. *sensu stricto* to the European and Chilean potatoes and proposed the name *S. andigenum* Juz. et Buk. for the tetraploids from the Andes. [Hawkes (1956b) has adopted the name *S. andigena* in accordance with the revised Kew orthography, and this revised nomenclature is also used in the present paper.] Bukasov (1930) classified the varieties of *S. andigena* into seven main groups based on the region of their occurrence and regarded the central Peruvian forms as typical for the whole species. He pointed out that in comparison with the range of diversity exhibited by *S. andigena*, there is much less variability in the *S. tuberosum* forms occurring in Chile. Bukasov (1930) concluded that "From the mountainous center in the Andes, the Chiloean center on the south coast where *S. tuberosum sensu stricto* the ancestor of all our commercial varieties took its origin, must be separated."

Bukasov and Kameraz (1959) have recently again stressed that on physiological and morphological grounds, *S. andigena* should be considered as a species distinct from *S. tuberosum*. Salaman (1937, 1946, 1954) and Salaman and Hawkes (1949) have, however, disagreed with the view of the Russian workers and suggested that there is only a single cultivated tetraploid *Solanum* species and that this arose in the Andean region. Also, the European potato is a derivative of the original introductions made in the sixteenth century from Colombia, Ecuador, or Bolivia. Subsequent research by Van der Plank (1946), Hawkes

(1956a,b), Swaminathan (1958), and several other workers, summarized below, supports the conclusions of Salaman.

A. Historical Position

Salaman (1937) pointed out that in about 1570, when potatoes were being grown in Spain, the Spanish hold on Chile was tenuous. They had, however, conquered the Andean region some fifty years earlier. Further, no journey from Chile was made to Europe via the Straits of Magellan until 1579, and it seems unlikely that the potatoes from Chile could have survived the long overland journey back to Europe. On the other hand, every ship that sailed to Spain stopped at Cartagena on the coast of Colombia, and a regular trade route to the potato-growing regions of the interior had been opened up by 1549. Thus, there is a greater likelihood that the Andean potatoes were the first to be brought to Europe.

B. Botanical Characters

Salaman (1937) and Hawkes (1956b) have shown that the morphological differences that separate *andigena* and *tuberosum* are of value only when taken as a whole or when extreme or "typical" examples from each group are considered. Otherwise, the range of variability occurring in the two groups for characters of diagnostic value appears to be a continuous one. Also, a study of a series of some twenty herbarium specimens of the potato cultivated in England and western Europe during the seventeenth and eighteenth centuries starting with a preparation left by Caspar Bauhin himself (made in about 1620 or perhaps earlier) showed that the earliest types of potato grown in these countries were typical of *S. andigena* (Salaman and Hawkes, 1949). The study further revealed that new varieties were continually being raised from true seed and that by selection forms with a larger and more condensed type of leaf mounted on a stouter stem were evolved. In other words, *S. andigena* has by selection been converted into *S. tuberosum*. According to Salaman and Hawkes (1949), this process seems to have proceeded rather more rapidly and at an earlier date in England than in western Europe. A study of the woodcuts of the potato found in contemporary Herbals and an aquatint depicting the type of potato plant received by Clusius and distributed by him throughout western and central Europe, preserved in the Plantin Museum at Antwerp, provided collateral support for the views formulated by examining herbarium specimens.

Since leaf shape is a key character in distinguishing *andigena* from

tuberosum, Salaman (1946, 1954) studied the leaf indices in several European, Peruvian, and Chilean potato varieties. The varieties from the Chilóé island or from the province of La Serena had the same leaf index as varieties of *S. andigena.* In the northern area of South America, types of *S. andigena* occurred which were akin in leaf character and habit of growth to the European potato varieties. Salaman (1954) hence concluded that there would appear to be no reason based on morphology why Chile rather than Colombia should be looked upon as the original home of the European potato. Correll (1952) has pointed out that though seed potatoes of *S. tuberosum* are imported into Mexico and Central America from the United States and elsewhere, and are grown in those countries where the native species occur, very few specimens of the broad leaflet *S. tuberosum* type are seen in Mexico. Nearly all of the specimens fall into the *S. andigena* category. It would thus appear that when *S. tuberosum* is taken into short-day regions a change develops in its habit and that the distinction between these two species will consequently be artificial.

Support for Salaman's views was obtained by Van der Plank (1946) from his study of the morphological characters of the potato varieties grown in Basutoland in South Africa. These varieties had been introduced in 1833, i.e., before the blight epidemics of 1845 had destroyed most European varieties and led to the extensive breeding of new varieties. These Basutoland varieties resembled *S. andigena* in morphological characters and photoperiodic behavior. Similarly, the "indigenous" potato varieties of India, which appear to be relics of strains introduced into Europe during the late sixteenth century, possess all the characters normally attributed to *S. andigena* (Swaminathan, 1958).

C. Photoperiodic Response

Hawkes (1944, 1956b) has suggested that the first European potatoes may have been long-day or day-neutral types since such strains do occur occasionally among Andean varieties and that, even if they were short-day types, they may have grown fairly well in the low latitudes of southern Europe (36° to 45° N) to which they were first introduced. The short-day character might have been subsequently lost when selection for high yield took place in progenies grown from true seed. It is also probable that along with tubers, berries containing true seeds were also brought into Europe from South America, and thus conscious or unconscious selection for the desired day-length response might have been initiated in the plants raised from them.

There is also evidence to suggest that the genes governing photoperiodic reaction in *S. tuberosum* are relatively unstable. In many

cultivated varieties, a small frequency of "bolter" or "giant-hill" plants (plants with greater height, stiffer foliage, smaller leaflets, later maturity, greater capacity for flower-bearing, higher pigmentation, longer stolons, and coarse tubers) occurs. Hawkes (1947) showed that "bolters," when grown under short-day conditions, are indistinguishable in most of their features from normal plants, and thus confirmed the suggestion of Carson and Howard (1944) that the change from normal to "bolter" is due to a mutation of one or more unstable genes controlling response to day length. Hawkes (1947) also suggested that the "bolter" is a reversion toward the ancestral type of short-day adapted potato found in the Andean region. Swaminathan (1958) recorded the occurrence of long-day adapted mutants in the *andigena* type of potatoes grown during the winter months in northern India and thus demonstrated that the reverse mutation, i.e., from a short-day or day-neutral type to a long-day adapted condition, also occurs in the potato. The conversion of *andigena* strains into long-day adapted *tuberosum* (*sensu stricto*) varieties could have taken place by some such process.

D. Cytogenetic Behavior

Multivalent configurations, chiefly trivalents and quadrivalents, occur in varieties of both *andigena* and *tuberosum* (*sensu stricto*). The number of quadrivalents per cell ranges from 0 to 9 (Lamm, 1945; Swaminathan, 1954a,b, 1958). Gottschalk and Peters (1954) studied the configuration of the macrochromomeres in the heterochromatic regions of the chromosomes at pachytene in varieties of *andigena* and *tuberosum* and found that the chromosome sets of the two species were identical. Genetically, both *andigena* and *tuberosum* varieties show tetrasomic inheritance (see Section III for details). Bukasov (1933), Hawkes (1944), Swaminathan (1954a), and various other workers have found that *S. tuberosum* from Chile and *S. andigena* form perfectly viable hybrids.

The historical, botanical, genetic, and cytological data thus all indicate that *S. andigena* Juz. et Buk. and *S. tuberosum* L. should be regarded as conspecific. Though it is clear that the *tuberosum* type of potato is an evolutionary by-product of the *andigena* type, the law of priority in nomenclature necessitates the retention of the name *S. tuberosum* to indicate both the *andigena* and *tuberosum* varieties (Hawkes, 1944, 1956b). Thus, *S. tuberosum* L. (*sensu latiore*) may be regarded as a collective species, consisting of two subspecies, namely, (a) subspecies *andigena* (Juz. et Buk.) Hawkes, occurring in the mountains of Venezuela, Colombia, Ecuador, Peru, Bolivia, and northern Argentina and (b) subspecies *tuberosum* found in Europe and southern

Chile. Although the European and Chilean potatoes are included under the same subspecific category, they appear to have evolved independently from original Andean stocks. Hawkes (1956b) has given a detailed description of these two subspecies, taking into account the complete range of variability occurring in them. As far as the first European potatoes are concerned, there is little doubt that they came from the Andes. But it is possible that other varieties may have been brought from Chile at a later date. If this were so, several of the European potato varieties would have originated from both Andean and Chilean sources. According to Hawkes (1956b), the conversion of *andigena* forms into *tuberosum* types by selection and breeding under European conditions must have taken place by 1753, since Linnaeus' potato specimen appears to belong to the *tuberosum* (*sensu stricto*) type.

III. The Nature of Polyploidy in *S. tuberosum*

A. Basic Chromosome Number

It was pointed out earlier that in the section *Tuberarium* of the genus *Solanum*, an euploid series with $2n = 24$, 36, 48, 60, and 72 exists, and that *S. tuberosum* has the somatic chromosome number 48. Though the lowest gametic chromosome number found in the present-day species of *Solanum* is 12, some authors have suggested that this is a derived number, the true basic number being $x = 6$. If this view is correct, *S. tuberosum* will be an octoploid. Black (1943) interpreted some genetic data relating to resistance to late blight on the assumption that *S. tuberosum* is an octoploid. From a critical consideration of the available data, which is briefly summarized below, Prakken and Swaminathan (1952) and Swaminathan and Howard (1953) have concluded that the basic chromosome number in the genus can be considered for most purposes to be 12, though this number might have been derived from a lower one in the remote past. Recently, Magoon *et al.* (1958a, 1959) and Magoon and Ramanujam (1960) have discussed the nature of pairing at different ploidy levels and have suggested that the possibility of the basic number in the genus *Solanum* being lower than 12 will have to be seriously considered.

1. *Meiosis in Haploids*

The only record of occurrence of a haploid plant ($2n = 12$)in a diploid *Solanum* species is that of Gilles (1955), who found a haploid in *S. polyadenium*. The cytological behavior of this haploid has unfortunately not been reported. Among allied genera, haploids with $2n = 12$ have been reported in *Datura stramonium* (Belling and

Blakeslee, 1923), *Capsicum annum* (Christensen and Bamford, 1943; Toole and Bamford, 1945; Campos and Morgan, 1958), and *Lycopersicon esculentum* (Rick and Butler, 1956). At meiosis all these haploids showed mostly 12 univalents. The doubled haploids had 12 bivalents and a regular meiosis.

2. *Meiosis and Primary Pairing of Chromosomes in Species and Species Hybrids*

a. *Diploids* ($2n = 24$). Gottschalk and Peters (1954) have presented extensive data on pachytene analysis in several diploid and tetraploid species (Gottschalk, 1954a,b, 1955; Gottschalk and Peters, 1954, 1955; Peters, 1954). Using the number and position of the macrochromomeres in the heterochromatic regions these workers identified the different types of chromosomes in the haploid set and also traced the same chromosome from species to species. Many diploid species possessed fewer than twelve distinct chromosome types, and they postulated that these species are still in part phylogenetic tetraploids whose original ancestor possessed a basic number of 6. These authors also suggested from their data that an interesting positive correlation exists between the average number of macrochromomeres per nucleus and the degree of evolutionary advancement of the species studied by them. Von Wangenheim *et al.* (1957) have, however, criticized the conclusions of Gottschalk and Peters on the grounds that first, the squash methods used in the pachytene preparations alter the relative position of the chromosomes, thus rendering observations on secondary pairing and the occurrence of multivalents of little critical value; secondly, not all the chromosomes of the haploid set can be positively traced from one species to another, and thirdly, the structural differences between species reported by Gottschalk (1954a) could not be verified. Hence, in their view such observations cannot offer decisive evidence on the "phylogenetic tetraploid" nature of certain diploids (see also von Wangenheim, 1954, 1957).

Unpaired chromosomes have been noted in various frequencies in some diploid *Solanum* species at metaphase I and have been attributed to precocious separation (Swaminathan and Howard, 1953). On the other hand, Magoon *et al.* (1958a,d, 1960) have suggested, from the large number of such univalents, the consistency of their occurrence at MI, and also the fact that unpaired chromosomes were noted by them at diakinesis, that precocious separation of bivalents alone cannot fully account for these univalents and that a certain amount of primary nonpairing may also be occurring.

Choudhuri (1943) recorded the occurrence of a trivalent in the diploid species *S. stenotomum*. In clones of *S. rybinii* and *S. stenotomum*,

Lamm (1945) observed a quadrivalent in several cells at diakinesis and MI. Gottschalk and Peters (1954) noted the occurrence of "partner exchanges" between two bivalents at late diakinesis in some diploids. Magoon *et al.* (1958a) found multivalents both at diakinesis and MI in some diploids. Gilles (1955) observed as many as four quadrivalents at diplotene in the diploid species *S. polyadenium.* He also noted a definite reduction in the number of quadrivalents from diplotene to MI, presumably due to the slipping off of the chiasmata.

Several workers have reported that meiosis is generally regular in diploid species and species hybrids whereas some authors have shown that such regularity is not always present (Swaminathan and Howard, 1953). Magoon *et al.* (1958a, 1960) observed irregularities such as chromatin bridges and fragments, laggards, delayed separation of bivalents, nonorientation and noncongression of bivalents, irregular nucleoli formation, restitution nuclei, micronuclei, and T-chromosomes in a few cells in some diploids. They attributed the occurrence of meiotic irregularities in apparently pure diploid species to the possible hybrid origin of these clones.

The data from meiotic studies in diploid species thus indicate that whole or partial chromosome duplications and structural changes like translocations and inversions may exist in some of them. However, in view of the several limitations conditioning cytological studies in potatoes, it may be misleading to draw definite conclusions regarding the basic chromosome number from the occurrence of meiotic irregularities in a small percentage of microsporocytes.

b. *Triploids* ($2n = 36$). Meiosis in triploids is very irregular, the naturally occurring triploids being maintained mostly by vegetative propagation. Study of meiosis in triploids is of interest for understanding whether any autosyndetic pairing occurs within a haploid set of 12 chromosomes (autosyndetic pairing is indicated when the number of bivalents plus trivalents exceeds 12). Von Olah (1938) observed a mean frequency of 5.76 trivalents per cell in *S. commersonii,* a naturally occurring triploid species, while Lamm (1945) found an average of 7.9 trivalents per cell in another natural triploid species, *S. chaucha.* Propach (1937b) observed a slight degree of autosyndesis in the triploid interspecific hybrid *S. acaule* ($2n = 48$) $\times$ *S. chacoense* ($2n = 24$), the mean number of bivalents plus trivalents per cell being 12.82. Emme (1936a) reported that in F_1 *S. ajuscoense* $\times$ *S. rybinii,* 18 "units" were formed at MI. Likewise, Koopmans (1951) found 18 "units" in polar view observations of MI in six triploid interspecific hybrids, particularly in the F_1 of *S. tuberosum* $\times$ *S. rybinii.* On the assumption that these 18 "units" are all bivalents, these two authors considered their observations

as evidence for a basic chromosome number of 6. Schwartz (1937) in F_1 (*S. phureja* × *S. rybinii*) × *S. acaule* and *S. tuberosum* × *S. phureja,* and Ivanovskaja (1941) in *S. rybinii* × *S. tuberosum,* also found considerable autosyndetic pairing.

Prakken and Swaminathan (1952) analyzed MI of meiosis in a triploid hybrid between *S. tuberosum* ($2n = 48$) and *S. chacoense* ($2n = 24$) and reported a mean bivalent plus trivalent frequency of 12.46 per cell. This closely agrees with the frequency recorded by Propach (1937b) in the triploid hybrid, *S. acaule* × *S. chacoense.* Matsubayashi (1955a,b) observed that hybrids between *S. longipedicellatum* ($2n = 48$) and *S. schickii* ($2n = 24$) and *S. longipedicellatum* and *S. chacoense* ($2n = 24$) showed respectively an average of 12.10 and 12.50 bivalents plus trivalents per cell and he concluded that most of the bivalents are derived from allosyndetic pairing between the genome from diploid species and one of the two genomes from the tetraploid species.

Meiosis in three triploid hybrids derived from crosses between *S. polytrichon* ($2n = 48$) × *S. phureja* ($2n = 24$), *S. stoloniferum* ($2n = 48$) × *S. neohawkesii* ($2n = 24$), and an induced tetraploid of *S. chacoense* ($2n = 48$) × *S. neohawkesii* ($2n = 24$) were analyzed by Magoon *et al.* (1960). The mean number of bivalents plus trivalents recorded in these three hybrids were 13.3, 14.2, and 13.5, respectively. The maximum number of bivalents plus trivalents found was 15 in one hybrid (*S. polytrichon* × *S. phureja*) and 16 in the other two hybrids. This would appear to indicate that at least as many as three or four autosyndetic associations should be present. The triploid hybrid obtained by crossing an induced tetraploid *S. chacoense* with diploid *S. neohawkesii* is of special interest because the two diploid species are combined in such a way that a diploid complement of chromosomes from one parent and a haploid complement from the other parent are present in this hybrid, and hence the chromosomes from the former may pair more or less regularly as they do in the naturally occurring *S. chacoense.* Such pairing would then produce a maximum of 12 bivalents. The haploid complement derived from the other diploid species involved could remain unpaired or pair allosyndetically with the bivalents formed by *S. chacoense* chromosomes to give a maximum of 12 trivalents or pair autosyndetically within itself. That autosyndetic pairing also occurs is shown by the presence of more than 12 bivalents plus trivalents. A maximum of 16 such configurations (i.e., $2_{III} + 14_{II} + 2_{I}$) occurred in one cell. They therefore inferred that in this cell at least 8 out of 12 *neohawkesii* chromosomes paired autosyndetically. This high degree of autosyndetic pairing as well as the fact that, in a large number of the cells analyzed in this hybrid, at least 2 or more such autosyndetic

bivalents occurred led them to conclude that these bivalents cannot entirely be explained on the basis of structural changes or nonhomologous pairing. (For detailed alternative explanations see Magoon *et al.*, 1960.)

Meiosis has also been studied by several authors in polyhaploid plants with $2n = 36$ derived from *S. demissum* ($2n = 72$). One to 12 bivalents and very occasional trivalents occurred and the mean frequency of bivalents per cell in different polyhaploid plants varied from 4.7 to 9.8 (Dodds, 1950; Bains and Howard, 1950; Howard and Swaminathan, 1953; Marks, 1955). There was no evidence of autosyndesis in this material and these authors hence concluded that two sets of 12 chromosomes in the haploid complement of *S. demissum* are somewhat related, the third set being quite distinct.

Finally, it should be noted that triploids, which are largely pollen and seed sterile are not found in the nontuber-bearing species of *Solanum*. In the tuber-bearing potatoes they owe their continued existence to vegetative reproduction.

c. *Pentaploids* ($2n = 60$). A few pentaploid species and species hybrids have been studied cytologically by Rybin (1933), Lamm (1945), Schnell (1948), Koopmans (1951), Howard and Swaminathan (1952), and J. P. Cooper and Howard (1952). The results indicate that very little autosyndesis occurs in this material.

3. *Secondary Association of Chromosomes*

Secondary association of chromosomes at meiosis has been used as a tool for determining the polyploid origin of several supposedly diploid plants (Darlington, 1937). Differences of opinion, however, exist regarding the real significance of secondary pairing of chromosomes and from the available evidence, Stebbins (1950) has concluded that "at present, secondary association can be considered an actual phenomenon and one which in many instances suggests the polyploid nature of a species or genus, but one which may be considerably modified by segmental interchange, duplication of chromosome segments and other phenomena not at all related to polyploidy; it is, therefore not a reliable index of the exact basic haploid number possessed by the original ancestors of a group."

For potatoes, Lawrence (1931) found evidence of secondary associations in the drawings of Longley and Clark (1930) and was led to suggest from this that 6 was the original basic chromosome number of the genus *Solanum*. Müntzing (1933), Ellison (1936), Emme (1937), Choudhuri (1943 and 1944), Okuno (1951), and Gilles (1955) have all supported the hypothesis of 6 being the basic chromosome number in

the genus on the basis of their analysis of secondary pairing in diploid and tetraploid species. On the other hand, Meurman and Rancken (1932), who observed secondary associations in *S. tuberosum*, did not find them in diploid species. Bleier (1931) and Propach (1937a) have attributed such associations to bad fixation, resulting in the clumping of chromosomes. Lamm (1945) found a lower degree of secondary association in diploid *S. stenotomum* and a higher degree in triploid *S. chaucha* than was found by Müntzing (1933) and further observed a marked heterogeneity between the results for different diploid species, for some triploids, and for tetraploids. Magoon *et al.* (1958a) also noted that the frequency of various types of groupings differed from species to species and even varied between different clones of the same species.

4. *Secondary Balance in Progenies from Triploids and Pentaploids*

Darlington (1937) has shown that one effect of secondary polyploidy would be the occurrence of a balance between the primary and secondary basic chromosome numbers in the progenies of crosses like triploids × diploids, and triploids × tetraploids. On the assumption of a basic number of 6 in the genus *Solanum*, a secondary balance involving this number would be expected in progenies of crosses of triploid × diploid, of triploid × tetraploid, and of pentaploid × tetraploid. No such balance was found by von Olah (1938) in *S. commersonii* ($2n = 36$) × *S. henryi* ($2n = 24$), and by Lamm (1945) in *S. chaucha* ($2n = 36$) × *S. stenotomum* ($2n = 24$). On the other hand, Lamm (1945) observed chromosome numbers, approaching the expected mean number of $2n = 54$ in *S. curtilobum* ($2n = 60$) × *S. tuberosum*, and in *S. curtilobum* × *S. andigena*.

5. *Genetic Studies in Diploid Species*

Typical disomic ratios have been obtained by Emme (1936b, 1937), Propach (1940), Choudhuri (1944), Dodds (1955), Dodds and Long (1955, 1956), and Swaminathan (1956) in genetic studies in diploid *Solanum* species. Koopmans (1951, 1952) found in three crosses between diploid species a clear monofactorial (1:1 and 3:1) segregation for colored against white flowers. However, she found a suspected 35:1 segregation for the recessive character "dark heart of the leaves" (observed 484 normal:13 dark heart) in the F_2 of *S. rybinii* × *S. commersonii*. Prakken and Swaminathan (1952), from a detailed analysis of Koopmans' (1951) data, concluded that owing to the variability and high deviations in the values obtained by her, further studies are necessary before the "dark heart" character can be considered as a certain case of tetrasomic inheritance in diploid potatoes.

Many diploid potato species are self-incompatible and the genetics of self-incompatibility has been found to be simple in several intervarietal and interspecific crosses (Pal and Pushkarnath, 1942; Pushkarnath, 1942; Lewis, 1949). More than four groups of self-incompatible but cross-compatible plants have, however, been observed among the F_1 plants of some crosses (Carson and Howard, 1942; Bains, 1951). Choudhuri (1948) concluded from his data on the inheritance of self-incompatibility in reciprocal crosses between *S. simplicifolium* and *S. rybinii* that the results are best explained on the assumption that the diploids are really tetraploids. Pandey (1960) found that while several South American diploid potato species possess a one-gene, multi-allelic, gametophytic system of self-incompatibility, two Mexican species, *S. ehrenbergii* and *S. pinnatisectum,* have a two-gene system. To explain this he suggested two possibilities. First, if the basic chromosome number is 6, the two *S* loci in the Mexican species may be the result of duplication of the original *S* gene. Secondly, a segmental interchange could have brought about the duplicate *S* loci.

Livermore and Johnstone (1940), Swaminathan (1951), and Magoon *et al.* (1958b) found that following chromosome doubling, some self-incompatible diploid species become self-compatible. There are, however, other diploid species in which induced polyploidy has not affected the self-incompatibility mechanism (Swaminathan, 1951; Magoon *et al.*, 1958b). Since heterozygosity for structural changes like inversions and translocations is common among diploid potatoes and may affect the study of the genetics of self-incompatibility, it will be desirable that such studies are carried out in conjunction with cytological examination.

From the foregoing it appears that while a definite decision as to the basic chromosome number in *Solanum* will have to await further extensive studies of the whole genus, *Solanum tuberosum* can be considered as a tetraploid for the purpose of tracing its immediate ancestry.

B. Nature of Tetraploidy

Stebbins (1947, 1950) has considered in detail the problems involved in the classification of polyploids and has proposed the following four major categories of polyploids, stressing at the same time that these represent only modal classes and may be connected by intermediate types.

(1) Autopolyploid: The constituent genomes are completely homologous, the progenitor being a fertile species.

(2) Segmental allopolyploid: Contains two pairs of genomes which possess in common a considerable number of homologous chromosomal

segments or even whole chromosomes, but differ from each other in respect to a sufficiently large number of chromosome segments, so that the different genomes produce sterility when present together at the diploid level.

(3) Genomic allopolyploid: Contains two or more sets of very different genomes, and the only type of pairing which normally occurs is that between similar chromosomes of the same genome.

(4) Autoallopolyploid: Combines the characteristics of the preceding types. Plants belonging to this group will have the constitution AAAABB etc. These terms are used in this paper in accordance with the above definitions.

Recent studies have indicated that criteria such as formation of multivalent chromosome associations at meiosis or the inheritance pattern at a few loci cannot provide conclusive evidence with regard to the nature of polyploidy in a given plant. Thus, Gilles and Randolph (1951) and Swaminathan and Sulbha (1959) have shown that with "evolution," a gradual shift from a multivalent to a bivalent type of synapsis occurs in colchicine induced autotetraploids of maize and *Brassica campestris* var. *toria*, respectively. Müntzing and Prakken (1940) found in an autotriploid *Phleum pratense* a genotypically controlled tendency to form only bivalents. Of still greater interest is the recent finding of Riley and Chapman (1958) and Sears and Okamoto (1958) that the regular bivalent formation normally found in *Triticum aestivum* is under the control of a single gene or a block of genes situated in one chromosome pair. There is thus evidence now to suggest that autopolyploids—both naturally occurring and induced—may acquire either gradually or suddenly the cytological characteristics of a diploid.

Analysis of chromosome associations in a vegetatively propagated plant like potato presents both some special problems and possibilities. Clonal propagation affords an opportunity for the accumulation of chromosome structural changes, and thus it may be difficult to distinguish between multivalent associations arising from whole chromosome duplications and from reciprocal translocations. Also, much hybridization, selection, and introduction into new environments have gone into the evolution of the commercial potato varieties, and as a result, alterations in karyotype are possible. On the other hand, a shift from a multivalent to a bivalent type of synapsis, which may be favored both by natural and human selection in autopolyploids in which the plant part of economic value is the seed, may be of relatively little importance in potato, particularly since there appears to exist a negative correlation between pollen and seed fertility and tuber yield. This inference

receives support from the finding of Armstrong and Robertson (1956) that in autotetraploids of alsike clover meiotic abnormalities persist after many years of selection for fodder yield. Hence the chromosome associations in current varieties of *S. tuberosum* may be more representative of the situation that existed in this species at the time of its origin than similar associations observed in varieties of a seed-propagated polyploid species.

The occurrence of polysomic ratios of inheritance (particularly those arising from random chromatid segregation) have been considered as evidence of autopolyploidy in several plants. Genetic ratios again may not provide absolute proof of the nature of origin of long established plants since the initially homologous genes lying in the different chromosome sets of a polyploid may mutate in different directions and gradually become so distinct as to be no longer allelic. Such a differentiation may eventually transform a polyploid into a species that has most genes represented only once in the gametes. Further, tetrasomic inheritance at one locus and disomic inheritance at two others have been observed by Swaminathan (1956) in the progeny of an induced tetraploid of a hybrid between two diploid *Solanum* species. Studies on the inheritance of several unlinked loci will hence be necessary before any inference on the nature of ploidy in a plant is drawn from genetic data.

These limitations will have to be borne in mind while discussing the mode of origin of *S. tuberosum* from evidence obtained in present day studies. However, from a critical consideration of all the available data, it should be possible to trace at least in outline its phylogenetic history. The relevant data are summarized below.

1. *Chromosome Morphology*

a. *Somatic Chromosomes.* The small size of the chromosomes and their relatively large numbers render critical analysis of somatic plates difficult. In his study of the somatic complements of forty-two British potato varieties, Ellison (1935) neglected constrictions in chromosomes as a means of identification since reliance could not be placed upon their position and number, and based his identification solely upon chromosome length. He reported that the somatic chromosomes of *S. tuberosum* could not be classified into groups of four by their size. His results are misleading since he found a difference between the chromosomes of Langworthy and Golden Wonder. These two varieties should have the same chromosomes in their root tips since Golden Wonder arose as a periclinal chimera with an inner core of Langworthy (Crane, 1936). In fact, Upcott in Crane (1936) found that the two varieties have similar chromosome complements. Sepeleva (1937) made a detailed

study of the somatic chromosomes of *S. tuberosum* and of six diploid species, and found that *S. tuberosum* had the same types of chromosomes as the diploid species but more of each. Lamm (1945), who observed that *S. tuberosum* had only two chromosomes with satellites while autotetraploid *S. rybinii* had four, suggested the probable occurrence of amphiplasty (Navashin, 1928) during the evolution of *S. tuberosum.* Swaminathan (1954a) classified the chromosomes of *S. tuberosum* using over-all length, position of primary and secondary constrictions, and presence of satellites as diagnostic characters. He found two chromosomes bearing satellites in their short arms and two chromosomes with secondary constrictions in their long arms.

b. *Pachytene Analysis.* Gottschalk and Peters (1954) studied the configuration of the macrochromomeres in the heterochromatic regions of the chromosomes at pachytene in varieties of *S. tuberosum* and found that each haploid set consisted of pairs of identical chromosomes. They considered this as evidence of the autopolyploid nature of this species.

2. *Chromosome Pairing at Meiosis*

a. *Current Varieties.* Multivalent associations of chromosomes (chiefly quadrivalents and trivalents) have been found in all the potato varieties, in which pairing at diakinesis and MI of meiosis has been examined critically (Table 1; Fig. 1). It would appear from the data in Table 1 that the frequency of multivalent associations found in some varieties is nearly similar to that recorded in induced autotetraploids of diploid species. *S. polyadenium,* for which data are given in Table 1, is a self-fertile species with an average chiasma frequency of two per bivalent, the maximum found in any diploid species. The occurrence of quadrivalents and trivalents at meiosis in commercial potato varieties has also been reported by Thomas (1946), Schnell (1948), Bains (1951), and Gilles (1955), but since detailed data are not given in their reports it has not been possible to include their results in Table 1. In addition to quadrivalents, Longley and Clark (1930), Meurman and Rancken (1932), Müntzing (1933), Ellison (1936), and Cadman (1943) found either hexavalents or octavalents in some varieties. Longley and Clark (1930), Meurman and Rancken (1932), and Ellison (1936) suggested that the meiotic irregularities frequently observed in *S. tuberosum* could be explained if it was assumed that the species had arisen as a hybrid between two species whose chromosomes are not wholly homologous. However, Lamm (1945), Swaminathan (1953), Beamish *et al.* (1957), and Magoon *et al.* (1958b) have all observed similar irregularities in synthetic autotetraploids.

b. *Polyhaploids.* Ivanovskaja (1939) obtained a single polyhaploid

TABLE 1

Frequency of Multivalents at Diakinesis and MI in *S. tuberosum* (subsp. *andigena* and *tuberosum*) and in an Induced Autotetraploid

Subspecies and variety	No. of cells with a quadrivalent + trivalent frequency of										Multivalents		Reference
	0	1	2	3	4	5	6	7	8	9	Mean per nucleus	Coefficient of realization	
Subspecies *andigena*													
C.P.C. 1384	8	8	4	4	3	0	0	0	0	0	1.48	0.12	Swaminathan (1954a)
Phulwa	0	1	6	13	15	22	27	6	0	0	4.36	0.36	Swaminathan (1958)
Subspecies *tuberosum*													
Flourball	1	0	4	5	13	15	15	11	4	2	5.24	0.436	Cadman (1943)
Deodara	1	1	3	5	7	3	0	0	0	0	3.25	0.27	Lamm (1945)
36/200	1	8	8	2	1	0	0	0	0	0	1.70	0.14	Lamm (1945)
Clark B. 72	1	5	6	3	2	1	0	0	0	0	2.16	0.18	Swaminathan (1954a)
Chippewa	0	0	3	10	12	8	8	5	3	1	4.80	0.40	Swaminathan (1954b)
Cobbler	0	3	7	14	1	0	0	0	0	0	2.52	0.21	Swaminathan (1954b)
Earlaine	0	1	7	16	5	4	1	1	0	0	3.31	0.28	Swaminathan (1954b)
Green Mountain	0	9	16	7	7	1	0	0	0	0	2.38	0.20	Swaminathan (1954b)
Houma	0	0	6	6	1	1	0	0	0	0	2.79	0.23	Swaminathan (1954b)
Katahdin	1	6	15	13	11	3	1	0	0	0	2.80	0.23	Swaminathan (1954b)
Kennebec	9	11	22	8	0	0	0	0	0	0	1.58	0.13	Swaminathan (1954b)
Red Pontiac	1	5	15	15	7	6	1	0	0	0	2.88	0.24	Swaminathan (1954b)
Russet Burbank	0	0	15	11	5	2	2	0	0	0	3.00	0.25	Swaminathan (1954b)
Russet Rural	0	2	11	12	10	5	0	0	0	0	3.20	0.27	Swaminathan (1954b)
Sebago	5	10	12	20	6	6	0	1	0	0	2.58	0.22	Swaminathan (1954b)
Triumph	0	2	1	5	4	2	1	0	0	0	3.40	0.28	Swaminathan (1954b)
S. polyadenium (4*x*)	0	0	0	3	4	5	5	4	4	0	5.56	0.46	Swaminathan (1954b)

($2n = 24$) plant of *S. tuberosum* var. *Aurora*. Usually 12 bivalents were present at MI in this plant but occasionally 11 bivalents and 2 univalents occurred. Bridges and fragments were observed in 7% of the sporocytes at anaphase, indicating some differentiation of the two genomes. Hougas and Peloquin (1957), Hougas *et al.* (1958), and Cooper and Rieman (1958) have recently isolated several polyhaploid plants in the F_1 progeny of crosses between varieties of *S. tuberosum* and several diploid South American species. Meiosis was studied in two polyhaploids,

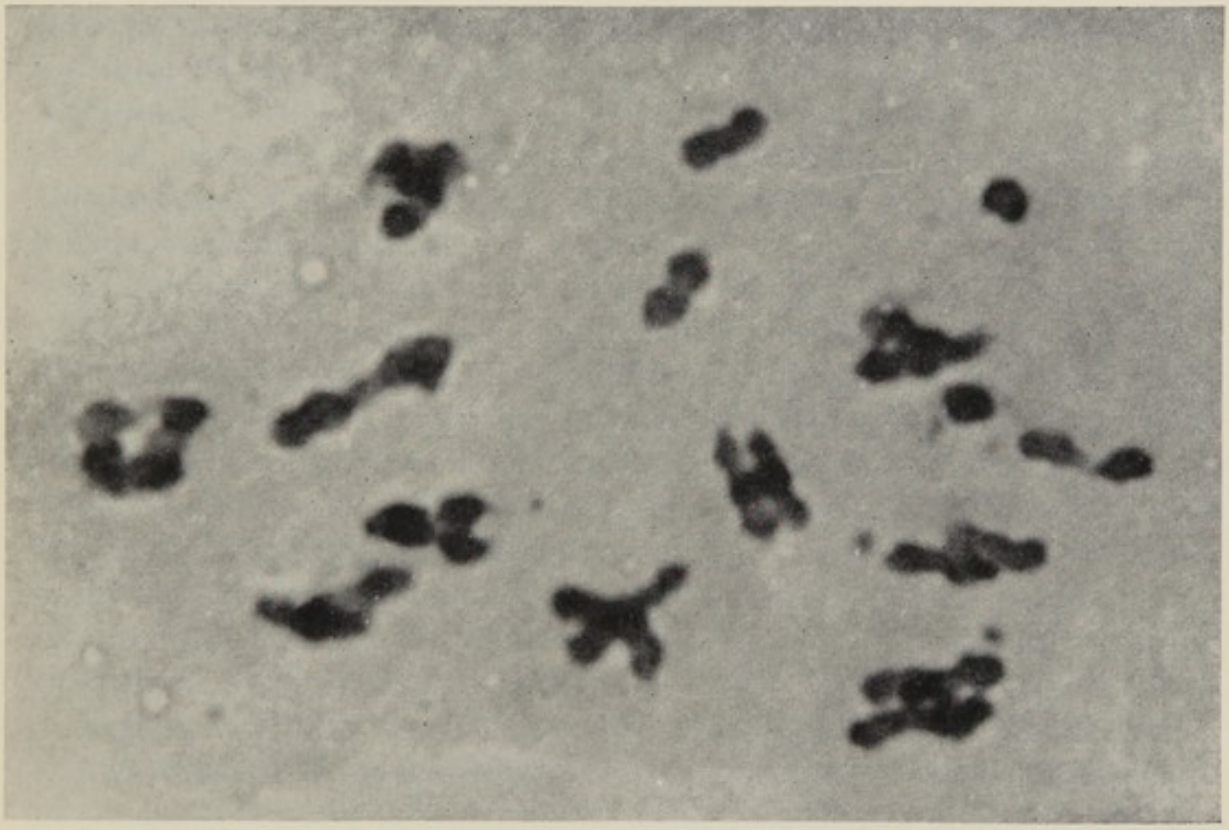


FIG. 1. Metaphase I of meiosis in the potato variety *Chippewa*. 9 quadrivalents +5 bivalents +2 univalents (× 3600). (From Swaminathan, 1954b; courtesy of *J. Heredity*.)

one with 75% and the other with 5 to 10% pollen fertility. In the former, meiosis was regular with 12 bivalents at MI, while in the latter only about 10% of the microsporocytes had 12 bivalents. In the rest, there were several univalents and also multivalents, especially quadrivalents (Peloquin and Hougas, 1958). These polyhaploids appear to arise from the parthenogenetic development of the egg cell. Since quadrivalents and disjunctional abnormalities normally occur in the commercial potato varieties, there is the possibility that some of the polyhaploid plants may arise from egg cells whose chromosome complements consist of duplications for some chromosomes and deficiencies for some

others. The observation of Okuno (1952) that plants with $2n = 46$, 47, and 49 occur in cultivated varieties suggests that gametes with whole chromosome deficiencies and duplications may be viable.

c. *Interspecific Hybrids.* The pairing behavior of the two genomes in the gametic set of *S. tuberosum* can be inferred from hybrids such as the tetraploids obtained from the cross *S. tuberosum* × diploid species (due either to the functioning of unreduced gametes in the diploid parent or the use of autotetraploids of diploids), and hexaploids derived from crosses between induced octoploids of tetraploid species and *S. tuberosum*. Most of the reported results agree in showing that the chromosomes of a gametic set of *S. tuberosum* may pair to form approximately 12 bivalents (Swaminathan and Howard, 1953). Kawakami and Matsubayashi (1957) inferred from the study of chromosome pairing in F_1 *S. tuberosum* var. *Deodara* × autotetraploid *S. saltense* that the 24 chromosomes of *S. tuberosum* may form 10 bivalents and 4 univalents. From the occurrence of univalents and rod bivalents in this hybrid they concluded that although the two genomes in the gametic set of *S. tuberosum* are capable of forming 12 bivalents by preferential pairing, true affinity between them is not great. In the hexaploid F_1 plants between octoploid *S. stoloniferum* and *S. tuberosum* and octoploid *S. acaule* and *S. tuberosum*, Swaminathan (1954a) observed 36 bivalents in a majority of cells, which indicates that the chromosomes of the parents involved in these crosses may pair among themselves. Swaminathan (1954a) also studied meiosis in a plant with $2n = 60$ from the cross (octoploid *S. acaule* × *S. tuberosum*) × *S. tuberosum*. Thirty-six chromosomes of this hybrid are from *S. tuberosum*, and an analysis of the pairing behavior suggested that 8 chromosomes of the three sets of 12 can associate together to form trivalents. The interpretation of the exact genomic ancestry of the chromosomes which pair with each other in the interspecific hybrids has to be largely extrapolative since no morphological or other criteria exist for identifying individual chromosomes (see Magoon *et al.*, 1958c,d, 1959). However, the general pattern of pairing observed in several hybrids in which 24 or 36 chromosomes are drawn from *S. tuberosum* suggests that, as in polyhaploids, there could be pairing between the basic sets.

3. *Pollen Fertility and Seed Setting*

a. *Commercial Varieties.* Many of the commercial potato varieties are pollen-sterile but readily set seed on being crossed with a variety producing plenty of good pollen. Such pollen sterility has been attributed by various authors to cytological, genetic, physiological, and environmental factors acting either singly or in combination. The stage at which

the abortion of pollen takes place varies in different varieties, and differences among reciprocal crosses in the extent of pollen sterility suggest that cytoplasmic factors may also be involved. Komarov (1931) suggested that the sterility of many cultivated varieties as contrasted with the fertility of many of the wild species, indicates a hybrid origin of the former. Stow (1927), Fukuda (1927), Ellison (1936), and Williams (1955) have, however, demonstrated that the sterility of many varieties is largely conditioned by environmental factors. Williams (1955) found that while no seed development beyond a many-celled endosperm usually occurs in the variety *Chippewa,* an abundant seed-set is obtained under certain greenhouse conditions. Male sterility is not so marked in subsp. *andigena,* though a few varieties seem to be unable to set seed (Hawkes, 1958). The probable influence of selection for higher cropping and early maturity on pollen fertility and seed setting has already been mentioned.

b. *Polyhaploids.* Ivanovskaja (1939) observed that the single polyhaploid *S. tuberosum* plant obtained by her was highly sterile and she hence concluded that *S. tuberosum* is an allotetraploid. Lewis (1949) pointed out that it is to be expected that haploid *S. tuberosum* plants will be self-incompatible like the majority of diploid *Solanum* species. Hougas and Peloquin (1957), Hougas *et al.* (1958), Peloquin and Hougas (1958), and D. C. Cooper and Rieman (1958) have isolated several polyhaploids of commercial potato varieties, and their observations indicate that while pollen fertility varies in the different polyhaploid plants from high fertility to high sterility, there is no evidence of ovule sterility in any of them. Hougas and Peloquin (1958) have hence stressed the potential value of these polyhaploids in potato breeding.

4. *Behavior of Polyploids*

Colchicine-induced octoploid plants ($2n = 96$) of *S. tuberosum* were dwarf and weak in contrast to such plants of *S. acaule* and *S. longipedicellatum* which were large and vigorous. Also, decaploid (*S. demissum* $\times$ *S. tuberosum,* $2n = 120$) plants had more vigor than octoploid *S. tuberosum* (Swaminathan, 1954a). Since species hybrids generally appear to suffer less from chromosome doubling than true species, the response of *S. tuberosum* to polyploidy is similar to that of a species and not of a species hybrid (Lamm, 1945; Swaminathan, 1954a).

5. *Morphological Characters*

Fukuda (1927) suggested that *S. tuberosum* arose from a single species since the floral characters in the various varieties have remained

constant during the past three hundred years. Longley and Clark (1930), however, pointed out that there was no need to suppose that more than one type of calyx and corolla were involved in the early ancestry and that it would be possible for a dominant type of flower to be carried unchanged through several generations of inbreeding, especially if it was linked with desirable economic characters. All the varieties investigated by Lunden (1937) segregated for flower color, and this was considered by him to point to the autotetraploid nature of the cultivated potato.

6. *Genetic Results*

Müller (1930) was the first to record that *S. tuberosum,* owing to its tetraploid nature, does not behave genetically in the same way as a diploid. Prior to that time, all genetic results had been interpreted on a simple disomic basis. Assejeva and Nikolaeva (1935) obtained evidence for the occurrence of several duplicate factors controlling plant and tuber color, some of which appeared to be allelomorphic. The occurrence of tetrasomic inheritance in the commercial potato was clearly established by Lunden (1937). In addition to typical tetrasomic ratios of inheritance at five unlinked loci, he found double reductional segregation at the following loci. First, in reciprocal crosses of the type $Pp_3 \times p_4$ and $Dd_3 \times d_4$ (P is a factor for purple pigmentation and D a factor needed for the development of plant color), P segregated as $1P:1p$ while D segregated as $13D:15d$. The $13D:15d$ segregation implies that there must be a 100% quadrivalent formation for the chromosomes in which the D gene is situated. Secondly, duplex R_2r_2 individuals were found in backcross progenies of the type $Rr_3 \times r_4$ (R is a factor for red color). Thirdly, nulliplex d_4 offspring occurred in $D_3d \times d_4$ crosses. Segregation for gene N_x, which controls the top necrotic reaction to virus X, and for genes X, Y, and Z, which confer resistance to the wart disease, has also been found to conform clearly to the tetrasomic pattern (Cadman, 1942 and Cockerham, 1943a,b for gene N_x; and Lunden, 1950 for genes X, Y, and Z). Since the publication of Lunden's (1937) paper, segregations for several characters in commercial potato varieties have been explained on a tetrasomic basis (see Swaminathan and Howard, 1953, for review). Genetic studies in varieties of subsp. *andigena* have been few. However, in recent years, the inheritance of resistance to the potato root nematode (*Heterodera rostochiensis*) has been followed in some *andigena* clones. The genes conferring resistance have been found to be inherited tetrasomically (Toxopeus and Huijsman, 1953; Huijsman, 1955; Cole and Howard, 1957).

The effect of inbreeding on the yield of tubers has been critically

studied by Krantz (1946) in some commercial potato varieties. He compared the actual yields obtained in the different inbred generations with those calculated on allotetraploid inheritance. The actual yields were significantly higher than those calculated, and Krantz (1946) suggested that this was evidence for the autotetraploid nature of *S. tuberosum,* since, as pointed out by Haldane (1930) and Fisher (1949), the rate of progress towards homozygosity is slower in tetrasomics.

To summarize the results of the cytogenetic studies in *S. tuberosum* outlined so far: (a) Cytological results show that first, 12 bivalents can be formed in polyhaploids; secondly, a triploid complement may form up to 8 trivalents; and thirdly, a maximum frequency of 9 quadrivalents and a mean number of 2 to 4 quadrivalents per metaphase I plate occur in several current commercial potato varieties. (b) Genetic results suggest that first, 7 presumably unlinked loci show tetrasomic inheritance including both random chromosome and chromatid segregation, and secondly, potato varieties respond to inbreeding in an autotetraploid pattern.

The findings of Müntzing and Prakken (1940), Gilles and Randolph (1951), Riley and Chapman (1958), Sears and Okamoto (1958), and Swaminathan and Sulbha (1959) that the cytological and genetic characteristics of a polyploid may be considerably modified subsequent to its evolution necessitate a dual approach to the consideration of the nature of polyploidy in a crop plant. First, the data relating to the cytogenetics, taxonomy, geographical distribution, and history of the species will have to be considered in their broad aspects with a view to infer the nature of ploidy from a phylogenetic standpoint. Secondly, the nature of ploidy as prevalent in the present day varieties will have to be judged by examining critically the detailed cytological and genetic behavior of each variety. From the latter viewpoint, which is naturally of greater interest to plant breeders, the nature of polyploidy in the current commercial potato varieties can be considered to vary from autotetraploidy toward segmental allotetraploidy (Stebbins, 1957). Stebbins' (1957) conclusion derives support from the occurrence of a considerable amount of variability in the cytological behavior, particularly with reference to the extent of multivalent associations formed in different varieties. A similar variability is found in the cytological behavior and fertility characteristics of the polyhaploids derived from different varieties. Also, following Lunden's (1937) genetic findings, tetrasomic inheritance has been assumed for all the loci studied by different workers. Simplex genotypes have been assigned in many instances without any other evidence of polysomic inheritance, and in such cases, it is equally possible that the inheritance is disomic (Swaminathan, 1954a).

Stebbins' (1957) term "intervarietal autopolyploid" may be the most suitable to indicate the nature of polyploidy in *S. tuberosum* in an evolutionary sense. This term is ideal since, while implying that hybridization is involved in the ancestry, it at the same time underlines the fact that differences in the homology of the chromosome sets of the parents, if present, are not of such a magnitude as to bring about sterility in the diploid hybrid. This term seems to be the most compatible with the general cytogenetic behavior exhibited by the *andigena* and *tuberosum* varieties and the polyhaploids derived from them, and with the taxonomic and distribution data discussed in Section IV.

IV. Probable Ancestor of *S. tuberosum*

From the data already discussed, it appears likely that the cultivated tetraploid potatoes have had a common origin, belong to the single species *S. tuberosum*, and are intervarietal autotetraploids. In tracing the ancestry of this tetraploid species it has to be ascertained whether it arose directly through chromosome doubling in a cultivated diploid species or secondarily from one or more wild tetraploid species. The diploid species most likely to have been the progenitor of *S. tuberosum* has also to be identified. Before these questions are discussed, it may be useful to recapitulate the position concerning the geographical distribution of tuber-bearing *Solanum* species.

A. Center of Origin of Cultivated Potatoes

Most of the tuber-bearing *Solanum* species occur in two well-defined geographical regions, namely, (1) central Mexico and (2) the Andes of southern Peru, Bolivia and northwestern Argentina. Juzepczuk (in Emme, 1938) considered that the area around the Bolivian-Argentina frontier might be the primary center of origin of tuber-bearing potatoes since forms with a "semi-stellate" corolla, intermediate between the truly stellate *Commersoniana* and *Circaeifolia* on the one hand, and the typically rotate *Tuberosa* and *Acaulia* on the other, occur in this region. However, according to Hawkes (1958), the taxonomic and crossability data render it likely that the tuber-bearing species originated somewhere in the region of Mexico during late Cretaceous to early Tertiary times. The most primitive series *Morelliformia, Bulbocastana, Cardiophylla,* and *Pinnatisecta* occur in this region at the present day. Before mid-Eocene certain species probably migrated to South America, where secondary centers of diversity developed in the newly formed Andean Cordilleras. Thus, the differentiation of South American species probably progressed from mid-Eocene to Pliocene

times. Following the restoration of the Central American land bridge, hybridization between Mexican and South American species followed by chromosome doubling could have taken place, giving rise to the polyploid species in the Mexican series *Demissa* and *Longipedicellata*. This general hypothesis is supported by the findings of Swaminathan and Hougas (1954) and of Marks (1955) that a marked degree of genome differentiation exists in the polyploid species belonging to the series *Demissa* and *Longipedicellata* occurring in Mexico.

For the following reasons Hawkes (1956b) has suggested that *S. tuberosum* originated in the central Andes of Peru and Bolivia and spread north and south to Colombia and Chile, respectively. First, there exists in this region a wide genetic variability of an extent not found in other regions. Secondly, several closely related diploid cultivated species occur in this region. Many of these hybridize freely with *S. tuberosum,* giving rise to triploids, which have in several instances been recognized as distinct species. Thirdly, many related wild and weed diploid species occur in this area. Finally, this region is also the center of the more highly developed Andean civilizations such as Tiahuanaco and Inca.

Genetic studies on cultivated potatoes indicate a wide range of genetic diversity in the plateaus and valleys of southern Peru-northern Bolivia (Swaminathan and Howard, 1953). A large number of distinct potato varieties occur in these regions, and Vavilov (1926), Juzepczuk and Bukasov (1929), and Bukasov (1933) have all concluded that potato cultivation probably first took place in the high mountain valleys and plateaus of the Andes of southern Peru and northern Bolivia between 12 and 18° south latitude.

Though the Mexican species *S. stoloniferum* Schlechtd., (= *S. longipedicellatum* Bitt.) has been suggested as a probable ancestor of *S. tuberosum* (Bitter, 1912–14), the taxonomic, geographical, historical, and cytogenetic data are in accord with the view that the cultivated species had their origin only in South America. It is of interest that when a wide range of Mexican and South American potatoes were tested against *S. tuberosum* antiserum using an immuno-electrophoresis technique, all the South American species showed a four-line spectrum similar to that of *S. tuberosum* itself, while some of the Mexican species showed a different type of reaction (Gell, Hawkes, and Wright, 1960). Also, the diploid tuber-bearing *Solanum* species occurring in Mexico possess a two-gene gametophytic system of self-incompatibility while the South American species have a one-locus system (Pandey, 1960). In view of the above, a Mexican origin of cultivated potatoes seems unlikely.

B. Tetraploid Species Related to *S. tuberosum*

As mentioned earlier, the Mexican tetraploid species *S. stoloniferum* was considered by Bitter (1911) to be the ancestral potato. Apart from the fact that species of the series *Tuberosa* have their center of maximum diversity in the Peru-Bolivian region, it is unlikely that *S. stoloniferum* could have been the ancestor of *S. tuberosum* since it behaves cytogenetically as a typical allopolyploid (Swaminathan, 1954a; see also Figs. 3 and 4). The tetraploid species belonging to series other than *Tuberosa* studied so far also exhibit an allopolyploid behavior (Swaminathan, 1954a). In the series *Tuberosa,* Hawkes (1956a) has recognized 47 species, out of which 8 are cultivated species and the rest, wild ones. Among the wild species, *S. sucrense* Hawkes and *S. wittmackii* Bitt. possess the chromosome number $2n = 48$. While Bukasov (1938, 1939) reported that *S. wittmackii* is a tetraploid with $2n = 48$, Hawkes (1956b) found that all the forms collected by him had $2n = 24$. This species hence needs to be investigated further. *S. sucrense* is a weed species which differs from subsp. *andigena* chiefly in the substellate corolla. It occurs mostly in central Bolivia. Hawkes (1956b) found about 50% inviable types in one F_2 family of a cross between *S. sucrense* and a variety of subsp. *andigena*. He therefore considers that *S. sucrense* may be a distinct species. Swaminathan (unpublished) observed regular bivalent pairing in *S. sucrense*. This species hence does not seem to have played any direct role in the evolution of *S. tuberosum*.

Bukasov (1933), who presumed that *S. tuberosum* and *S. andigena* arose independently of each other, suggested that *S. leptostigma* Juz., *S. fonckii* Juz., and *S. molinae* Juz. are the wild progenitors of *S. tuberosum,* and *S. herrerae* Juz., of *S. andigena*. All are tetraploids, with the former occurring in Chile and *S. herrerae* in Peru. A tetraploid weed species, *S. subandigena* occurs in Bolivia. *S. herrerae* and *S. subandigena* differ from the cultivated varieties of subsp. *andigena* only in the longer stolons and unpigmented tubers. Hawkes (1956b, 1958) considers that the Chilean weed species *S. leptostigma, S. molinae,* and *S. fonckii,* far from being progenitors of *S. tuberosum,* can only have been derived by segregation from cultivated forms since no truly wild diploids or tetraploids occur in that region. These species have the red tuber pigmentation characteristic of cultivated potato varieties and are probably escapes from cultivation. Similar "wild" forms of *S. tuberosum* occur in widely separated localities, thus lending support to the view that they should be regarded as escapes from a widespread cultivated species and not as relics of a wild tetraploid species. In contrast to Chile, where no diploid wild or cultivated potatoes occur, there are hundreds of

different forms of wild and cultivated diploid potatoes in Peru and Bolivia. This is further evidence for the single origin of the cultivated tetraploids.

Hawkes (1956b) studied the F_2 families raised from crosses between *S. andigena* (from Peru) and *S. leptostigma* (from Chilóé, Chile) and *S. subandigena* (from Bolivia) and *S. andigena*. The F_2 families were composed of uniform thrifty plants. Since neither crossability barriers nor restrictions to recombination arising from chromosomal differentiation exist in these species, they are best regarded as varieties of the same species. Hawkes (1956a) has therefore placed all these weed species under *S. tuberosum*. It is thus clear that no related wild tetraploid species exists in the series *Tuberosa* from which the cultivated forms could have evolved, thereby suggesting that *S. tuberosum* never existed in the wild but most probably arose directly by chromosome doubling from an already existing diploid cultigen.

S. maglia Schlechtd. (series *Tuberosa* Rydb.) and *S. commersonii* Dun. (series *Commersoniana* Buk.) have also been suggested as probable ancestors of the cultivated potato. In both these species, forms with $2n = 24$ and 36 occur. *S. maglia* occurs in Chile and western Argentina while strains of *S. commersonii* are found in east-central Argentina, Uruguay, and southern Brazil. Neither the geographical distribution nor the cytological data lend any support to the view that *S. tuberosum* is derived from these species.

C. Diploid Ancestor of *S. tuberosum*

During a study of the types of anthocyanidins and their genetic loci in wild and cultivated diploid *Solanum* species, Dodds and Long (1955) found that types of anthocyanidins other than petunidin occur only in cultivated potatoes and that the pigments occurring in the cultivated diploids and tetraploids are similar. As it is unlikely that the same series of pigment mutations has occurred twice in the evolutionary history of domestic potatoes, it may be assumed that the *R* alleles of domestic tetraploids are derived from the cultivated diploids. Also, the type of genetic variability found for most morphological characters in *S. tuberosum* is similar to that occurring in the primitive diploid cultivated species. Finally, there exists at present no wild diploid or tetraploid potato species from which *S. tuberosum* could have arisen directly. Thus, the search for the diploid ancestor of *S. tuberosum* has to be directed largely towards the cultivated diploid species of Bolivia and Peru. Hawkes (1956b) has recognized four major diploid cultivated species. They are *S. stenotomum* Juz. et Buk., *S. goniocalyx* Juz. et Buk., *S. ajanhuiri* Juz. et Buk., and *S. phureja* Juz. et Buk. All these diploid

cultivated species when intercrossed with each other produce fertile hybrids showing regular meiosis. They are thus genetically closely related. Their role in the origin of the cultivated tetraploid forms can hence be assessed only in relative terms.

(1) *S. ajanhuiri:* This species is restricted in its distribution to the high altitude regions in northern Bolivia and has limited variability. It resembles *S. stenotomum* in the decurrent bases of the uppermost leaflet pair and in the form of corolla but differs from it in the small regular calyx, smaller blue flower, and very high pedicel articulation. Gottschalk and Peters (1954) analyzed the pachytene chromosomes of this species and found that certain chromosomes were very different from those present in *tuberosum* or *andigena*. According to Hawkes (1956b), it could have arisen fairly recently as a result of natural crosses between the frost-resistant tetraploid species *S. acaule* and *S. stenotomum*.

(2) *S. goniocalyx:* This species occurs in central Peru and its distribution does not coincide with the region of the greatest diversity of *S. tuberosum*. In view of its limited morphological and genetic variability, Emme (1937) and Hawkes (1956b) have suggested that it may be a northern derivative of the widespread species *S. stenotomum*. On the basis of pachytene analysis, Gottschalk and Peters (1954) also do not consider this species to have been involved in the ancestry of *S. tuberosum*.

(3) *S. phureja* (synonyms: *S. rybinii, S. kesselbrenneri, S. cardenasii, S. boyacense*, and *S. ascasalii*): This has a wide distribution ranging from Venezuela and Colombia southward to central Bolivia. It is, however, very distinct from *S. tuberosum* in its ecological requirements, since it is confined to the wetter lower altitude zones of the eastern flanks of the Andes in Bolivia and Peru and the high-rainfall mountain forests of Ecuador and Colombia (Hawkes, 1956b). Under its natural growing conditions in South America it matures in 3 months and totally lacks tuber dormancy. These characters are usually not found in *S. tuberosum*. Pachytene analysis also suggests that this species may not have been the progenitor of *S. tuberosum* (Gottschalk and Peters, 1954). The same conclusion emerges from Swaminathan's (1953) study of the extent of homology existing between the chromosomes of several diploid *Solanum* species and *S. tuberosum* (see Table 2).

(4) *S. stenotomum:* This species is widely distributed in southern Peru and northern Bolivia and is also cultivated on a large scale in these countries by the native farmers. According to Hawkes (1944, 1956b), the genetic variability in this species is immense and is quite comparable with that of *S. tuberosum* itself. Further, *S. stenotomum*

is grown in the same regions (often in the same fields) as *S. tuberosum* and is adapted to the same ecological conditions. Brücher (1960) found that the diploid potatoes in cultivation in Chilóé also belong to *S. stenotomum,* thus lending further support to the view that a potato migration took place from the north to the south and not from a supposed "Chilotan gene center" across the Cordillera towards the Altiplano. It differs from *S. tuberosum* mostly in quantitative characters.

Swaminathan (1953) studied the types of chromosome associations found in hybrids between *S. tuberosum* and certain autotetraploids and allotetraploids produced by colchicine treatment from diploid species belonging to the taxonomic series *Tuberosa* and *Commersoniana.* His data are given in Table 2.

TABLE 2
Multivalent Frequency at MI in Hybrids between Induced Polyploids and *S. tuberosum* *

Hybrid		Chromosome constitution of hybrid †	Frequency of			
			Trivalents		Quadrivalents	
♀ Parent	♂ Parent		Range	Mean	Range	Mean
4*x* *S. phureja*	*S. tuberosum*	TTTT	0–1	0.32	1–5	2.50
4*x* *S. stenotomum*	*S. tuberosum*	TTTT	0–1	0.06	1–7	3.29
4*x* *S. chacoense*	*S. tuberosum*	CCTT	0	0	0–2	0.55
4*x* (*S. kurtzianum* × *S. simplicifolium*)	*S. tuberosum*	TTTT	0–1	0.12	0–3	1.02
4*x* (*S. chacoense* × *S. phureja*)	*S. tuberosum*	CTTT	0–5	1.86	0–2	0.26
4*x* (*S. stenotomum* × *S. chacoense*)	*S. tuberosum*	CTTT	0–6	2.44	0–1	0.26

* From Swaminathan, 1953.

† T = 1 set (12) of *Tuberosa* chromosomes. C = 1 set (12) of *Commersoniana* chromosomes.

It is obvious from the data that the pairing behavior observed in the hybrids is what would be expected if some degree of preferential pairing exists among the chromosome sets of related species grouped under the same taxonomic series. Thus, the presence of several quadrivalents in 4*x* *stenotomum* × *tuberosum,* the absence of trivalents and the rarity of quadrivalents in 4*x* *chacoense* × *tuberosum* and the formation of as many as 6 trivalents in 4*x* (*stenotomum-chacoense*) × *tuberosum* clearly indicate the greater affinity between *stenotomum* and *tuberosum* chromosomes in comparison with the complements of *chacoense.* The occurrence of fewer quadrivalents in the hybrid between 4*x* (*kurtzianum* ×

simplicifolium) × *tuberosum* in comparison with the frequency in other TTTT hybrids would suggest that cultivated diploid *Tuberosa* species like *S. stenotomum* and *S. phureja* are genetically more closely related to *S. tuberosum* than the wild *Tuberosa* species like *S. kurtzianum* and *S. simplicifolium*. The observation that more quadrivalents and trivalents are formed in crosses involving *stenotomum* than in those with *phureja* lends support to the view of Hawkes (1944) that *S. stenotomum* is the most important diploid species from the point of view of the evolution of *S. tuberosum*. The autotetraploids *S. stenotomum* bear close morphological resemblance to varieties of subsp. *andigena*.

Gottschalk (1954a) and Gottschalk and Peters (1954) observed that among 11 diploid species studied by them, *S. stenotomum* was nearest to the cultivated tetraploid potato with regard to the morphology of the pachytene chromosomes (Fig. 2). Of the 11 chromosomes identi-

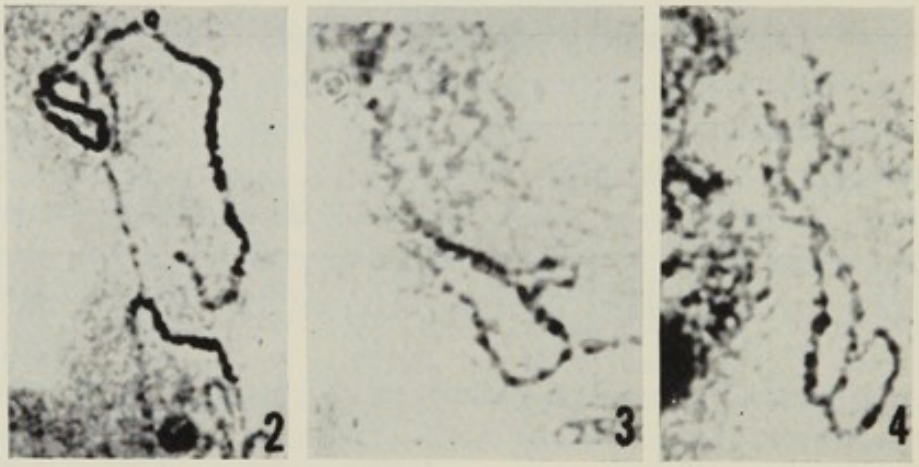


FIG. 2. Pachytene chromosomes of *S. stenotomum* showing prominent heterochromatic regions. These chromosomes are similar to those found in *S. tuberosum*.

FIGS. 3 and 4. Pachytene in F_1 *S. stoloniferum* × *S. stenotomum*. Large differential segments and synapsis in heterochromatic regions are seen. (Courtesy of Prof. W. Gottschalk.)

fied in *S. stenotomum*, 10 were wholly or largely identical with the chromosomes of *S. tuberosum*. Most chromosomes of *S. tuberosum* itself were replicated four times and hence they concluded that *S. tuberosum* is an autotetraploid derived from either *S. stenotomum* or its immediate ancestor.

The cytogenetic, taxonomic, and distribution data thus all agree in indicating *S. stenotomum* as the likely progenitor of the cultivated tetraploid species. It should, however, be again emphasized that all the four major diploid cultivated species are capable of crossing with each other and giving rise to fertile hybrid progenies characterized by regular meiotic behavior (Swaminathan and Howard, 1953). They are thus genetically closely related and probably all arose from an ancestral

form of *stenotomum* through evolutionary divergence. While *S. stenotomum* appears to have played a relatively more dominant role in the evolution of the cultivated tetraploid potato, *S. ajanhuiri, S. goniocalyx,* and *S. phureja* might also have contributed to the genetic variability found in *S. tuberosum* through crosses between them and *S. tuberosum,* in which unreduced gametes of the diploid parents function (unreduced gamete formation in $2x \times 4x$ crosses is relatively frequent in this material—see Prakken and Swaminathan, 1952). This process could have been followed by varying degrees of introgressive hybridization. Brücher (1954, 1956, 1959) has suggested that *S. vernei,* a diploid species occurring in northwestern Argentina, and some other species allied to it have played an important part in the evolutionary history of potato. However, neither the cytological data of Gottschalk and Peters (1954) nor the taxonomic studies of Hawkes (1956b) and Bukasov and Kameraz (1959) support this view. Hawkes (1956a) has pointed out that while *S. tuberosum* might not have arisen through chromosome doubling in a hybrid between *S. stenotomum* and *S. phureja* or any other cultivated diploid, the diploid weed species *S. sparsipilum* (Bitt.) Juz. et Buk., may be a second ancestor of *S. tuberosum. S. sparsipilum* is a very polymorphic species and occurs as a weed of cultivated fields and waste places in the same ecological regions as *S. tuberosum* and *S. stenotomum.* It is morphologically very similar to subsp. *andigena* and its wide distribution may be due to the fact that it was carried by man as a weed with the cultivated species all through Bolivia and Peru. Hawkes (1956b) found that in the F_1 hybrids between *S. stenotomum* and *S. sparsipilum* the distinctive bilabiate calyx of *S. stenotomum* was recessive, thereby giving the F_1 plants a close resemblance to *S. tuberosum.* The cytology of these hybrids and the amphidiploids produced from them is yet to be studied and as such, it is difficult at present to define precisely the role of *S. sparsipilum* in the origin of *S. tuberosum.*

V. Conclusion

Discussing the origin of the early European potato, Salaman (1954) concludes: "The domestic potato of Europe, though given the specific name of *S. tuberosum,* is in fact a variety of the species *S. andigena* which has acquired its present range of characteristic forms of leaf and habit as a result of prolonged selection for higher cropping varieties on the one hand and early or late maturity on the other. In short, man has created the various domestic types of *S. tuberosum* by planned breeding and selection from the original *S. andigena* of South America in much the same manner as he has won for his use the widely divergent types of the domestic horse from the shaggy, sturdy pony of paleolithic times."

The data presented in this paper, as well as the fact that plants of *S. tuberosum* (*sensu stricto*) have never been found in a wild state, support this conclusion. Nomenclatural rules, however, necessitate the retention of the name *S. tuberosum* to indicate forms belonging to both *andigena* and *tuberosum* (*sensu stricto*) although phylogenetic considerations would suggest that this name should be *S. andigena*. While the potatoes introduced into Europe in the latter half of the sixteenth century possessed the morphological features characteristic of *S. andigena*, it can be inferred from Linnaeus' description of the potato that *tuberosum* types as known today were under cultivation in Europe by the middle of the eighteenth century. This rapid evolution of *S. tuberosum* seems to have been facilitated by the following factors: First, the habit and form of a potato plant varies depending on whether it is grown under short-day or long-day conditions (Correll, 1952). Secondly, the genetic factors controlling photoperiodic behavior are relatively unstable with the result that it is easy to isolate short-day-requiring mutants in long-day-adapted ones and vice versa (Swaminathan, 1958). Thirdly, characteristics of commercial value such as yield and size of tubers seem to be very much influenced by the temperature conditions under which the potato plant is grown. Thus, Went (1959) has shown that when potato plants are grown at cool temperatures the tuber yield not only of these plants but also of the following generations was much higher than when they were grown under warm conditions. The temperature under which the parent generation had been grown also influenced the subsequent tuber generations in other characters such as tuber shape, number of eyes in the tuber, taste, and flavor. Fourthly, while many of the *S. tuberosum* (*sensu stricto*) varieties are characterized by varying degrees of pollen and ovule sterility as well as a shy or nonflowering nature, varieties of *andigena* usually show a high pollen fertility and form in abundant quantity berries with viable seeds. Because of their profuse flowering nature, *andigena* strains were grown for ornamental purposes soon after their introduction into Europe. Hence, there is sufficient scope for breeding and selection to have been practiced in the sexual progenies of varieties of *S. andigena*. In the current commercial potato varieties pollen sterility and a shy-flowering habit appear to have been introduced by human selection, since there is evidence to suggest that pollen and seed fertility are negatively correlated with tuber yield in *S. tuberosum* (Krantz, 1946). Finally, Brücher (1960) has recently shown that varieties which have a neutral reaction as regards day length requirement occur in the short day zone of the Andean region. Such varieties would be capable of yielding well under the European and Chilean long days.

The *andigena* strains from which the commercial *S. tuberosum* has been developed by man appear to have arisen directly through chromosome doubling in the cultivated diploid species, *S. stenotomum*. This process might have occurred several times, the parent diploid involved being different strains or intervarietal hybrids of *S. stenotomum* or hybrids between this species and its close relatives. Though it is obvious that *S. stenotomum* itself is a product of evolution among wild species of the series *Tuberosa*, the available cytogenetic and taxonomic data are not adequate to name the wild species most directly involved in this process.

References

Armstrong, J. M., and Robertson, R. W., 1956. Studies of colchicine induced tetraploids of *Trifolium hybridum* L. I. Cross- and self-fertility and cytological observations. *Can. J. Agr. Sci.* **36,** 255–266.

Assejeva, T., and Nikolaeva, N., 1935. Genetic nature of the colouring of potato tubers, sprouts and flowers. *Works Potato Research Inst. Moscow* **9,** 107.

Bains, G. S., 1951. Cytogenetical studies in the genus *Solanum*, Sect. *Tuberarium*. M.Sc. Dissertation, University of Cambridge, England.

Bains, G. S., and Howard, H. W., 1950. Haploid plants in *Solanum demissum*. *Nature* **166,** 795.

Beamish, K. I., Cooper, D. C., and Hougas, R. W., 1957. Induced tetraploid *Solanum* species and species hybrids:meiosis and use in breeding. *Am. J. Botany* **44,** 305–310.

Belling, J., and Blakeslee, A. F., 1923. The reduction division in haploid, diploid, triploid and tetraploid *Daturas*. *Proc. Natl. Acad. Sci. U.S.* **9,** 106–111.

Bitter, G., 1911. Solana nova vel minus cognita. *Rep. Spec. Nov. Reg. Veg.* **10,** 529–565.

Bitter, G., 1912. Solana nova vel minus cognita. *Rep. Spec. Nov. Reg. Veg.* **11,** 1–18, 202–237, 241–260, 349–394, 431–473, 481–491, 561–566.

Bitter, G., 1913. Solana nova vel minus cognita. *Rep. Spec. Nov. Reg. Veg.* **12,** 1–10, 49–90, 136–162, 433–467, 542–545.

Bitter, G., 1914. Solana nova vel minus cognita. *Rep. Spec. Nov. Reg. Veg.* **13,** 88–103, 169–173.

Black, W., 1943. Inheritance of resistance to 2 strains of blight (*P. infestans* de Bary) in potatoes. *Trans. Roy. Soc. Edinburgh* **61,** 137–147.

Bleier, H., 1931. Untersuchungen über die Sterilität der Kartoffel. *Arch. Pflanzenbau* **5,** 545–560.

Brücher, H., 1954. Cytologische und ökologische Beobachtungen an nordargentinischen *Solanum*-arten der Sektion *Tuberarium*. I. Die Wildarten des Aconquija-Gebirges. *Züchter* **24,** 281–294.

Brücher, H., 1956. Kritische Betrachtungen zur Nomenklatur argentinischer Wildkartoffeln. I. Serie *Commersoniana*. *Züchter* **26,** 97–106.

Brücher, H., 1959. Kritische Betrachtungen zur Nomenklatur argentinischer Wildkartoffeln. V. Die Serie *Acaulia*. *Züchter* **29,** 149–155.

Brücher, H., 1960. Problematisches zum Ursprung der Kulturkartoffel aus Chiloé. *Z. Pflanzenzücht.* **43,** 241–265.

Bukasov, S. M., 1930. The cultivated plants of Mexico, Guatemala and Colombia.

Bull. Appl. Botany Genet. Plant Breeding (Leningrad), Suppl. No. **47,** Chap. 14, 191–226.

Bukasov, S. M., 1933. The potatoes of South America and their breeding possibilities. *Bull. Appl. Botany Genet. Plant Breeding (Leningrad), Suppl. No.* **58,** 192 pp.

Bukasov, S. M., 1938. Interspecific hybridization in potato. *Bull. acad. sci. U.R.S.S., Sér. biol.,* pp. 711–732.

Bukasov, S. M., 1939. The origin of potato species. *Physis, Buenos Aires* **18,** 41–46.

Bukasov, S. M., Kameraz, A. Ja., 1959. ("Bases of Potato Breeding"), pp. 528. Gosudarstvennoe Izdatel'stvo Sel'skohozjajstvennoj Literatuny, Moskva, Leningrad.

Cadman, C. H., 1942. Autotetraploid inheritance in the potato. Some new evidence. *J. Genet.* **44,** 33–52.

Cadman, C. H., 1943. Nature of tetraploidy in cultivated European potatoes. *Nature,* **152,** 103–104.

Campos, F. F., and Morgan, D. T., Jr., 1958. Haploid pepper from a sperm. An androgenetic haploid of *Capsicum frutescens. J. Heredity* **49,** 135–137.

Carson, G. P., and Howard, H. W., 1942. Self-incompatibility in certain diploid potato species. *Nature* **150,** 290.

Carson, G. P., and Howard, H. W., 1944. Inheritance of the bolter condition in the potato. *Nature* **154,** 829.

Choudhuri, H. C., 1943. Cytological studies in the genus *Solanum*. I. Wild and native cultivated "diploid" potatoes. *Trans. Roy. Soc. Edinburgh* **61,** 113–135.

Choudhuri, H. C., 1944. Cytological and genetical studies in the genus *Solanum*. II. Wild and cultivated "diploid" potatoes. *Trans. Roy. Soc. Edinburgh* **61,** 199–219.

Choudhuri, H. C., 1948. Cytological and genetical studies in the genus *Solanum*. III. Genetical studies in wild and native cultivated potatoes. *Botan. Soc. Bengal* **2,** 186–200.

Christensen, H. M., and Bamford, R., 1943. Haploids in twin seedlings of pepper. *J. Heredity* **34,** 98–104.

Cockerham, G., 1943a. Potato breeding for virus resistance. *Ann. Appl. Biol.* **30,** 105–108.

Cockerham, G., 1943b. The reaction of potato varieties to viruses X, A, B and C. *Ann. Appl. Biol.* **30,** 338–344.

Cole, C. S. and Howard, H. W., 1957. The genetics of resistance to potato root eelworm of *Solanum tuberosum* Subsp. *andigenum,* Clone C.P.C. 1690. *Euphytica* **6,** 242–246.

Cooper, D. C., and Rieman, G. H., 1958. Diploid plants in a seedling population of the cultivated potato. *Am. Potato J.* **35,** 642.

Cooper, J. P., and Howard, H. W., 1952. The chromosome numbers of seedlings from the cross *Solanum demissum* × *S. tuberosum* backcrossed by *S. tuberosum*. *J. Genet.* **50,** 511–521.

Correll, D. S., 1952. Section *Tuberarium* of the Genus *Solanum* of North America and Central America. *U.S. Dept. Agr. Monograph,* No. 1, 1–243.

Crane, M. B., 1936. Note on a periclinal chimera in the potato. *J. Genet.* **32,** 73–77.

Darlington, C. D., 1937. "Recent Advances in Cytology," 2nd ed., 671 pp. Churchill, London.

Dodds, K. S., 1950. Polyhaploids of *S. demissum*. *Nature* **166,** 795.

Dodds, K. S., 1955. Positional pseudo-alleles in diploid potatoes. *Nature* **175,** 394–395.

Dodds, K. S., and Long, D. H., 1955. The inheritance of colour in diploid potatoes. I. Types of anthocyanidins and their genetic loci. *J. Genet.* **53,** 136–149.

Dodds, K. S., and Long, D. H., 1956. The inheritance of colour of diploid potatoes. II. A three-factor linkage group. *J. Genet.* **54,** 27–41.

Dunal (*in* De Candolle), 1852. Prodromus (Solanaceae), Vol. **13,** pp. 27–387, Paris.

Ellison, W., 1935. A study of the chromosome number and morphology in certain British varieties of the common cultivated potatoes (*Solanum tuberosum* L.). *Genetica* **17,** 1–26.

Ellison, W., 1936. Meiosis and fertility in certain British varieties of the cultivated potato (*S. tuberosum*). *Genetica* **18,** 217–254.

Emme, E. K., 1936a. Triploide Bastarde der gegen *Phytophthora infestans* Arten von *S. antipoviczii* Buk. Sp. Coll. *Biol. Zhur.* **5,** 901–914.

Emme, E. K., 1936b. Genetik der Kartoffel. I. Vererbung der Blütenfärbung bei 24-chromosomigen Kartoffelarten. *Biol. Zhur.* **5,** 977–1000.

Emme, E. K., 1937. Genetik der Kartoffel III. *S. rybinii* Juz. et Buk; *S. stenotomum* Juz. et Buk. und *S. goniocalyx*—ihre Bedeutung für die Züchtung und phylogenetische Beziehungen. *Biol. Zhur.* **6,** 787–796.

Emme, E. K., 1938. Studies on interspecific hybridization of tuber-bearing potatoes, Section *Tuberarium* Bitter, genus *Solanum* L. *Biol. Zhur.* **7,** 1093–1104.

Fisher, R. A., 1949. "The Theory of Inbreeding," 120 pp. Oliver & Boyd, London.

Fukuda, Y., 1927. Cytological studies on the development of the pollen grains in different races of *Solanum tuberosum* L. with special reference to sterility. *Botan. Mag. (Tokyo)* **41,** 459–474.

Gell, P. G. H., Hawkes, J. G., and Wright, S. T. C., 1960. The application of immunological methods to the taxonomy of species within the genus *Solanum*. *Proc. Roy. Soc.* **B151,** 364–383.

Gilles, A., 1955. Recherches cytogénétiques sur les *Solanum*, Sect. *Tuberarium*. I. Nombres chromosomiques et association méiotiques. *La Cellule* **57,** 7–31.

Gilles, A. and Randolph, L. F., 1951. Reduction of quadrivalent frequency in autotetraploid maize during a period of 10 years. *Am. J. Bot.* **38,** 12–17.

Gottschalk, W., 1954a. Die Chromosomenstruktur der Solanaceen unter Berücksichtigung phylogenetischer Fragestellungen. *Chromosoma* **6,** 539–626.

Gottschalk, W., 1954b. Die Grundzahl der Gattung *Solanum* and einiger *Nicotiana* Arten. *Ber. deut. botan. Ges.* **67,** 369–376.

Gottschalk, W., 1955. Die Paarung homologer Bivalente und der Ablauf von Partnerwechseln in den frühen Stadien der Meiosis autopolyploider Pflanzen. *Z. induktive Abstammungs- u. Vererbungslehre* **87,** 1–24.

Gottschalk, W., and Peters, N., 1954. Die Chromosomenstruktur als Kriterium für Abstammungsfragen bei Tomate und Kartoffel. *Z. Pflanzenzücht.* **34,** 71–84.

Gottschalk, W., and Peters, N., 1955. Die Chromosomenstruktur diploider Wildkartoffel-Arten und ihr Vergleich mit der Kulturkartoffel. *Z. Pflanzenzücht.* **34,** 351–374.

Haldane, J. B. S., 1930. Theoretical genetics of autopolyploids. *J. Genet.* **22,** 359–372.

Hawkes, J. G., 1944. Potato collecting expeditions in Mexico and South America. II. Systematic classification of the collections *Imperial Agricultural Bureau Bull.*, 142 pp.

Hawkes, J. G., 1947. The photoperiodic reactions of potato bolters. *Empire J. Exptl. Agr.* **15,** 216–226.

Hawkes, J. G., 1956a. A revision of the tuber-bearing *Solanums*. *Ann. Rept. Scottish Plant Breeding Station*, pp. 37–109.

Hawkes, J. G., 1956b. Taxonomic studies on the tuber-bearing *Solanums*. 1. *Solanum tuberosum* and the tetraploid species complex. *Proc. Linnean Soc. London* **166,** 97–144.

Hawkes, J. G., 1958. Kartoffel. I. Taxonomy, cytology and crossability. *Handbuch Pflanzenzücht.* **3,** 1–43.

Hougas, R. W., and Peloquin, S. J., 1957. A haploid plant of potato variety Katadhin. *Nature* **180,** 1209–1210.

Hougas, R. W., and Peloquin, S. J., 1958. The potential of potato haploids in breeding and genetic research. *Am. Potato J.* **35,** 701–707.

Hougas, R. W., Peloquin, S. J., and Ross, R. W., 1958. Haploids of the common potato. *J. Heredity* **49,** 103–107.

Howard, H. W., and Swaminathan, M. S., 1952. Species differentiation in the genus *Solanum,* Sect. *Tuberarium* with particular reference to the use of interspecific hybridization in breeding. *Euphytica* **1,** 20–28.

Howard, H. W., and Swaminathan, M. S., 1953. The cytology of haploid plants of *Solanum demissum*. *Genetica* **26,** 381–391.

Huijsman, C. A., 1955. Breeding for resistance to potato root eelworm. II. Data on the inheritance in *andigenum-tuberosum* crosses obtained in 1954. *Euphytica* **4,** 133–140.

Ivanovskaja, E. V., 1939. A haploid plant of *S. tuberosum* L. *Compt. rend. acad. sci. U.R.S.S.* **24,** 517–520.

Ivanovskaja, E. V., 1941. A cytological analysis of hybrids between diploid and tetraploid species of potatoes. *Bull. acad. sci. U.R.S.S. Classe sci. math. nat. sér. biol.* **1,** 21–33.

Juzepczuk, S. W., 1937. New species of the genus *Solanum* L., in the group *Tuberarium* Dun. *Bull. acad. sci. U.S.S.R.* **2,** 295–331.

Juzepczuk, S. W., and Bukasov, S. M., 1929. A contribution to the question of the origin of the potato. *Proc. U.S.S.R. Congr. Genet. Plant & Animal Breeding* **3,** 593–611.

Kawakami, K., and Matsubayashi, M., 1957. Studies on species differentiation in the section *tuberarium* of *Solanum*. IV. Cytological behaviour of F_1 hybrids from *S. tuberosum* × *S. saltense* (4n) with special remarks on the polyploid nature of *S. tuberosum*. *Japan. J. Botan.* **16,** 128–134.

Komarov, V. L., 1931. "Origin of Cultivated Plants," 240 pp. States Press of Agricultural and Co-operative literature, Moscow & Leningrad.

Koopmans, A., 1951. Cytogenetic studies on *Solanum tuberosum* L. and some of its relatives. *Genetica* **25,** 193–337.

Koopmans, A., 1952. Changes in sex in the flowers of the hybrid *Solanum rybinii* × *S. chacoense*. *Genetica* **26,** 359–380.

Kostoff, D., 1933. A contribution to the sterility and irregularities in the meiotic process caused by virus diseases. *Genetica* **15,** 103–114.

Krantz, F. A., 1946. Potato breeding methods. III. A suggested procedure for potato breeding. *Tech. Bull. Minn. Agr. Exptl. Sta. No.* **173,** 24 pp.

Lamm, R., 1945. Cytogenetic studies in *Solanum,* Sect. *Tuberarium*. *Hereditas* **31,** 1–128.

Lawrence, W. J. C., 1931. The secondary association of chromosomes. *Cytologia* **2,** 352–384.

Lewis, D., 1949. Incompatibility in flowering plants. *Biol. Revs. Cambridge Phil. Soc.* **24,** 472–496.

Livermore, J. R., and Johnstone, F. E., 1940. The effect of chromosome doubling on the crossability of *S. chacoense, S. jamesii* and *S. bulbocastanum* with *S. tuberosum*. *Am. Potato J.* **17,** 169–173.

Longley, A. E., and Clark, C. F., 1930. Chromosome behaviour and pollen production in the potato. *J. Agr. Research* **41,** 867–888.

Lunden, A. P., 1937. Arvelighetsundersokelser i Potet. *Meldinger. Norg. Landbrukshøgskole* **20,** 1–156.

Lunden, A. P., 1950. Undersokelser over reaksjon mot kreft (*Synchytrium endobioticum*) hos Potet. *Meldinger Norg. Landbrukshøgskole* **137,** 48 pp.

Magoon, M. L., and Ramanujam, S., 1960. Nature of chromosome pairing in the genus *Solanum,* Section *Tuberarium*. *Indian J. Genet. & Plant Breeding* **20,** 21–44.

Magoon, M. L., Cooper, D. C., and Hougas, R. W., 1958a. Cytogenetic studies of some diploid *Solanums* Section *Tuberarium*. *Am. J. Botany* **45,** 207–222.

Magoon, M. L., Cooper, D. C., and Hougas, R. W., 1958b. Induced polyploids of *Solanums* and their crossability with *S. tuberosum*. *Botan. Gaz.* **119,** 224–233.

Magoon, M. L., Hougas, R. W., and Cooper, D. C., 1958c. Cytogenetical studies of tetraploid hybrids in the genus *Solanum*. *J. Heredity* **49,** 171–178.

Magoon, M. L., Hougas, R. W., Cooper, D. C., 1958d. Cytogenetic studies of South American diploid *Solanums,* Section *Tuberarium*. *Am. Potato J.* **35,** 375–394.

Magoon, M. L., Hougas, R. W., and Cooper, D. C., 1959. Cytogenetical studies of complex hybrids in the genus *Solanum* Section *Tuberarium*. *J. Heredity* **49,** 285–293.

Magoon, M. L., Hougas, R. W., and Cooper, D. C., 1960. Chromosome pairing at different ploidy levels in the tuber bearing *Solanums*. *J. Genet.* **57,** 279–298.

Marks, G. E., 1955. Cytogenetic studies in the tuberous *Solanum* species. I. Genomic differentiation in the group *Demissa*. *J. Genet.* **53,** 262–269.

Matsubayashi, M., 1955a. Studies on species differentiation in the section *Tuberarium* of *Solanum*. *Sci. Repts. Hyogo Univ. Agr.* **2,** 25–31.

Matsubayashi, M., 1955b. Chromosome affinity between *Solanum longipedicellatum* and *S. chacoense* based on the meiotic behaviour in the induced amphidiploids. Address given at 27th Ann. Meeting of the Genetics Society of Japan in Okayama.

Meurman, O., and Rancken, G., 1932. Untersuchungen über die Chromosomenverhältnisse bei kultivierten Kartoffelsorten (*Solanum tuberosum*). *Soc. Sci. Fennica Commentationes Biol.* **3,** 20, 1–28.

Müller, K. O., 1930. Über die Phytophthora-Resistenz der Kartoffel und ihre Vererbung. *Angew. Botan.* **12,** 299–324.

Müntzing, A., 1933. Studies on meiosis in diploid and tetraploid *S. tuberosum* L. *Hereditas* **17,** 223–245.

Müntzing, A., and Prakken, R., 1940. The mode of chromosome pairing in *Phleum* twins with 63 chromosomes and its cytogenetic consequences. *Hereditas* **26,** 463–501.

Navashin, M., 1928. "Amphiplastie"—eine neue karyologische Erscheinung. *Z. induktive Abstammungs- u. Vererbungslehre Suppl.* **2,** 1148–1152.

Okuno, S., 1951. Cytological studies on potatoes with some remarks on genetical experiments. Part I. *Idengaku Zasshi* **26,** 79–103.

Okuno, S., 1952. Cytological studies on potatoes with some remarks on genetical experiments. Part II. *Idengaku Zasshi* **27,** 3–21.

Pal, B. P., and Pushkarnath, 1942. Genetic nature of self- and cross-incompatibility in potatoes. *Nature* **149,** 246–247.

Pandey, K. K., 1960. Self incompatibility system in two Mexican species of *Solanum*. *Nature* **185,** 483–484.

Peloquin, S. J., and Hougas, R. W., 1958. Fertility in two haploids of *Solanum tuberosum*. *Science* **128,** 1340–1341.

Peters, N., 1954. Zytologische Untersuchungen an *Solanum tuberosum* und polyploiden Wildkartoffel-Arten. *Z. induktive Abstammungs- u. Vererbungslehre* **86,** 373–398.

Prakken, R., and Swaminathan, M. S., 1952. Cytological behavior of some interspecific hybrids in the genus *Solanum* section *Tuberarium*. *Genetica* **26,** 77–101.

Propach, H., 1937a. Cytogenetische Untersuchungen in der Gattung *Solanum* Sekt. *Tuberarium*. I. Die Sekundär-Paarung. *Z. induktive Abstammungs- u. Vererbungslehre* **72,** 555–563.

Propach, H., 1937b. Cytogenetische Untersuchungen in der Gattung *Solanum* Sekt. *Tuberarium*. II. Triploide und tetraploide Artbastarde. *Z. induktive Abstammungs- u. Vererbungslehre* **74,** 376–387.

Propach, H., 1940. Cytogenetische Untersuchungen in der Gattung *Solanum* sect. *Tuberarium*. V. Diploide Artbastarde. *Z. induktive Abstammungs- u. Vererbungslehre* **78,** 115–128.

Pushkarnath, 1942. Studies on sterility in potatoes. 1. The genetics of self and cross incompatibility allelomorphs. *Indian J. Genet. & Plant Breeding* **5,** 92–105.

Rick, C. M., 1951. Hybrids between *Lycopersicon esculentum* Mill. and *Solanum lycopersicoides* Dun. *Proc. Natl. Acad. Sci. U.S.* **37,** 741–744.

Rick, C. M., 1960. Hybridization between *Lycopersicon esculentum* and *Solanum pennellii*: phylogenetic and cytogenetic significance. *Proc. Natl. Acad. Sci. U.S.* **46,** 78–82.

Rick, C. M., and Butler, L., 1956. Cytogenetics of the tomato. *Advances in Genet.* **8,** 267–382.

Riley, R., and Chapman, V., 1958. Genetic control of the cytologically diploid behaviour of hexaploid wheat. *Nature* **182,** 713–715.

Rybin, V. A., 1933. Cytological investigations of the South American cultivated and wild potatoes and its significance for plant breeding. *Bull. Appl. Botany, Genet. Plant Breeding (Leningrad) Ser. II*(2), 3–100.

Rydberg, P. A., 1924. The section *tuberarium* of the genus *Solanum* in Mexico and Central America. *Bull. Torrey Botan. Club* **51,** 145–154; 167–176.

Salaman, R. N., 1937. The potato in its early home and its introduction to Europe. *J. Roy. Hort. Soc.* **62,** 61–77, 112–123, 153–162, 253–266.

Salaman, R. N., 1946. The early European potato: its characters and place of its origin. *J. Linnean Soc., London, Botany* **53,** 1–27.

Salaman, R. N., 1949. "The History and Social Influence of Potato," 685 pp. Cambridge Univ. Press, London and New York.

Salaman, R. N., 1954. The origin of the early European potato. *J. Linnean Soc. London, Botany* **55,** 185–190.

Salaman, R. N., and Hawkes, J. G., 1949. The character of the early European potato. *Proc. Linnean Soc. London* **161,** 71–84.

Schnell, L. O., 1948. A study of meiosis in the microsporocytes of interspecific hybrids of *Solanum demissum* × *S. tuberosum*, carried through four back crosses. *J. Agr. Research* **76,** 185–212.

Schwartz, P. A., 1937. Cytogenetic investigation of the potato. I. Interspecific hybrids (*S. phureja* × *S. rybinii*) × *S. acaule*. *Bull. Acad. Sci. U.S.S.R. Ser. Biol.* **1,** 59–67.

Sears, E. R., and Okamoto, M., 1958. Intergenomic chromosome relationships in hexaploid wheat. *Proc. 10th Intern. Congr. Genet.* **2,** 258–259.

Sepeleva, E. M., 1937. Morphology of the chromosomes of some species of potato. *Compt. rend. acad. sci. U.S.S.R.,* **15,** 207–209.

Stebbins, G. L., 1947. Types of polyploids: their classification and significance. *Advances in Genet.* **1,** 403–429.

Stebbins, G. L., 1950. "Variation and Evolution in Plants," 643 pp. Oxford Univ. Press, London and New York.

Stebbins, G. L., 1957. Genetics, evolution and plant breeding. *Indian J. Genet. & Plant Breeding* **17,** 129–141.

Stow, I., 1927. A cytological study on the pollen sterility in *S. tuberosum* L. *Japan J. Botany* **3,** 217–238.

Swaminathan, M. S., 1951. Notes on induced polyploids in the tuber-bearing *Solanum* species and their crossability with *S. tuberosum. Am. Potato J.* **28,** 472–489.

Swaminathan, M. S., 1953. Studies on the inter-relationships between taxonomic series in the section *Tuberarium, Genus Solanum.* I. *Commersoniana* and *Tuberosa. Am. Potato J.* **30,** 271–281.

Swaminathan, M. S., 1954a. Nature of polyploidy in some 48-chromosome species of the genus *Solanum* sect. *Tuberarium. Genetics* **39,** 59–76.

Swaminathan, M. S., 1954b. Microsporogenesis in some commercial potato varieties. *J. Heredity* **45,** 265–272.

Swaminathan, M. S., 1956. Disomic and tetrasomic inheritance in a *Solanum* hybrid. *Nature* **178,** 599–600.

Swaminathan, M. S., 1958. The origin of the early European potato–evidence from Indian varieties. *Indian J. Genet. & Plant Breeding* **18,** 8–15.

Swaminathan, M. S., and Hougas, R. W., 1954. Cytogenetic studies in *Solanum verrucosum* variety *Spectabilis. Am. J. Botany* **41,** 645–651.

Swaminathan, M. S., and Howard, H. W., 1953. The cytology and genetics of the potato (*Solanum tuberosum*) and related species. *Bibliographia genet.* **16,** 1–192.

Swaminathan, M. S., and Sulbha, K., 1959. Multivalent frequency and seed fertility in raw and evolved tetraploids of *Brassica campestris* var. *toria. Z. Vererbungslehre* **90,** 385–392.

Swaminathan, M. S., Ninan, T., and Magoon, M. L., 1959. Effect of virus infection on microsporogenesis and seed fertility in *Capsicum. Genetica* **30,** 63–69.

Thomas, P. T., 1946. *36th Ann. Rept. John Innes Hort. Inst.,* 11 pp.

Toole, M. G., and Bamford, R., 1945. The formation of diploid plants from haploid peppers. *J. Heredity* **36,** 66–70.

Toxopeus, H. J., and Huijsman, C. A., 1953. Breeding for resistance to potato root eelworm. 1. Preliminary data concerning the inheritance and the nature of resistance. *Euphytica* **2,** 180–186.

Van der Plank, J. E., 1946. Origin of the first European potatoes and their reaction to length of day. *Nature* **157,** 503–505.

Vavilov, N. I., 1926. Studies on the origin of the cultivated plants. *Bull. Appl. Botany Plant Breeding (Leningrad)* **16,** 1–245.

von Olah, L., 1938. Cytogenetische Untersuchungen in der Gattung *Solanum* Sekt. *Tuberarium* III. *S. commersonii* Dun. und einige seiner Bastarde. *Z. induktive Abstammungs-u. Vererbungslehre* **74,** 228–241.

von Wangenheim, K. H., Frh., 1954. Zur Ursache der Kreuzungsschwierigkeiten zwischen *Solanum tuberosum* L. und *S. acaule* Bitt. bzw. *S. stoloniferum* Schlechtd. et Bouche. *Z. Pflanzenzücht.* **34,** 7–48.

von Wangenheim, K. H., Frh., 1957. Das Pachytän und der weitere Ablauf der Meiose in diploiden *Solanum*-Arten und Bastarden. *Chromosoma* **8,** 671–690.

von Wangenheim, K. H., Frh., Frandsen, N. O., and Ross, H., 1957. Über neue Ergebnisse zur Cytologie und verwandte Fragen bei *Solanum*. *Z. Pflanzenzücht.* **37,** 41–76.

Went, F. W., 1959. Effects of environment of parent and grandparent generations on tuber production by potatoes. *Am. J. Botany* **46,** 277–282.

Williams, E. J., 1955. Seed failure in the Chippewa variety of *Solanum tuberosum*. *Botan. Gaz.* **117,** 10–15.

GENETIC ASSIMILATION

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	Page
I. Introduction	257
II. Definitions	258
A. Acquired and Inherited Characters	258
B. Genetic Assimilation	259
III. Experimental Evidence for Genetic Assimilation	260
A. Characters Involving a Developmental Threshold	260
B. Characters Not Involving a Threshold	262
IV. The Genetic Mechanisms of Assimilation	264
A. Evidence that Genetic Assimilation Depends on Selection	264
B. Analysis of the Genotypes of Assimilated Strains	264
C. Origin of the Genetic Variation Utilized in Genetic Assimilation	268
V. Genetic Assimilation and Developmental Canalization	269
A. The Canalization of Development	269
B. Genetic Assimilation as a Consequence of Canalization	271
C. Some Concepts Allied to Canalization	273
D. The Genetic Basis of Canalization	279
VI. The Baldwin Effect	287
VII. Summary and General Conclusions	289
References	290

I. Introduction

"Genetic assimilation" is a name which has been proposed (Waddington, 1953a) for a process by which characters which were originally "acquired characters," in the conventional sense, may become converted, by a process of selection acting for several or many generations on the population concerned, into "inherited characters."

Since the earliest days of evolutionary thought, the problem of "the inheritance of acquired characters" has been a central subject of debate. With the rise of Mendelian genetics in this century, it has often been considered that the problem has finally been resolved, in the sense that acquired characters are not inherited, and that therefore, the fact that characters may be acquired has no direct influence on the course of evolutionary change. This view has been rather generally accepted among geneticists, but has still found some opponents among naturalists, some of whom have felt that the phenomena of adaptation seen in the

organic world force them to conclude that the acquirement of characters must have evolutionary consequences, presumably through some unknown process by which these characters are in fact inherited by later generations. The phenomena revealed by the experiments on genetic assimilation cast no doubt on the thesis, generally accepted by geneticists, that acquired characters are not inherited (except in very special circumstances), but lead to the conclusion that there is no justification for arguing from this that they have no effect on the course of evolution. On the contrary, it becomes apparent that the conventional and accepted facts and theories of genetics provide a mechanism by which "acquired characters" must exert some influence—and probably a rather important one—on the direction in which evolutionary change proceeds.

This article, therefore, will not be concerned with revealing new genetic principles, but with describing a type of process which, although it is new in the sense that it had not been contemplated until a few years ago, derives its flavor of novelty or unexpectedness only from the fact that normal genetic ideas have very often been misinterpreted or incompletely thought out in this context. In order to clarify these misinterpretations, it will be necessary to pay a good deal of attention to the definitions and content of various genetic notions. The general plan of the article will be, first, to provide some of the necessary definitions, then to illustrate the application of these by reference to the experiments which have been conducted on genetic assimilation, and finally to consider some of the wider repercussions of the ideas which have been developed.

II. Definitions

A. Acquired and Inherited Characters

The notions of "acquired" and "inherited" characters, as they are employed in evolutionary theory, are not entirely straightforward. As many authors, e.g., Goodrich (1934), have remarked, all characters of all organisms are to some extent "inherited," in the sense that they can only be developed if the organism contains the hereditary potentialities for developing them, and are also to some extent "acquired," since all development involves some participation of the environment (cf. Begg, 1952; Waddington, 1952c). Such statements have often been dismissed as quibbling truisms, but the train of thought which led to the realization that genetic assimilation may occur arose from taking seriously the fact that "characters" are produced by the joint action of genotype and environment.

The definition of acquired and inherited characters, in the sense in

which those phrases are normally used, may be put as follows. If we have reason to believe that an organism, if reared in environment E, would exhibit the phenotype P, while if reared in environment E′ its phenotype would be P′, then those features in which P′ differs from P are considered as acquired characters, and those features in which P′ resembles P are considered as inherited characters, with reference to this particular change of environment.

Clearly, one of the difficulties in the application in practice of this definition turns on the phrase "have reason to believe." We cannot rear one and the same organism in two different environments. The best we can do is to place in the two environments two organisms, or populations of organisms, which are thought to have effectively similar genotypes. It is only when the two test individuals or groups can be taken from one inbred or clonally propagated population that we can be confident of their genetic identity. When we are dealing with individuals from a population which is crossbred, and therefore somewhat heterogeneous genetically, we must expect to find that the difference between P and P′ will not be exactly the same for all pairs tested. That is to say, there will be some variation in the degree to which any given character is an acquired or an inherited one, according to the particular test-pair used.

B. Genetic Assimilation

The notion of genetic assimilation involves both a phenomenon, and a mechanism by which this phenomenon is brought about.

The phenomenon may be described as the conversion of an acquired character into an inherited one; or better, as a shift (towards a greater importance of heredity) in the degree to which the character is acquired or inherited. Consider a population living in environment E and exhibiting phenotype P. Let a subpopulation be placed in environment E′, where it exhibits a phenotype P′. Then P–P′ is an acquired character in this subpopulation. Now, after the subpopulation has continued in E′ for a greater or lesser number of generations, let it be returned to environment E; and suppose that it there exhibits phenotype P″. Then the degree to which P″ resembles P′ is a measure of the extent to which the original acquired character P–P′ has been converted into an inherited character. The name "genetic assimilation" is given to such processes of conversion when they are brought about by selection acting on the genotypes in the subpopulation which was transferred from E to E′.

As we shall see, other mechanisms of conversion have been suggested (e.g., the "Baldwin Effect"), but there is considerable doubt whether they could possibly occur.

III. Experimental Evidence for Genetic Assimilation

After these abstract definitions, it will be as well to consider next the experimental evidence for the reality of the process of genetic assimilation. The process was first envisaged as a theoretical possibility nearly twenty years ago (Waddington, 1942) and reference was made to it in a number of later general discussions of evolutionary problems (Waddington, 1951, 1953c, 1954, 1957a, 1958a,b, 1959a,c).

A. Characters Involving a Developmental Threshold

The first experimental demonstration of the process was not published till some years later (Waddington, 1952b, 1953a). These experiments started with a wild type population of *Drosophila melanogaster* which, when reared in the normal laboratory culture environment, exhibited the well-known normal phenotype. A subpopulation was placed in another environment E′, which differed from the normal in that a high-temperature shock (4 hours at 40°C) was given at 17–23 hours after puparium formation. It was found that some of the subpopulation treated in this way exhibited an abnormal phenotype P′, which took the form of the breaking of the posterior crossvein on the wings. This "acquired character" was exhibited by only a proportion of the subpopulation. When selection was applied within the subpopulation, by taking only those individuals with broken crossveins for further breeding, the frequency of the crossvein defect increased from generation to generation; similarly, with selection against the appearance of the abnormal phenotype, it decreased in frequency. Thus the subpopulation must have contained genetic variation concerned with the development of the crossveinless phenotype in the environment which included the temperature shock.

After a few generations of selection, it was discovered that the most effective period for the administration of the temperature treatment was between 21 and 23 hours after puparium formation. Using this treatment as the abnormal environment E′, selection proceeded rapidly, so that, after about a dozen further generations of upward selection, well over 90% of the individuals developed with broken crossveins following temperature treatment. In each generation a fair number of flies were examined which had developed from untreated pupae, that is to say, which had been kept all the time in the original normal environment. No crossveinless individuals were found among these until generation 14, when a few isolated cases appeared in the strains being subjected to upward selection. In generation 16 of upward selection there

were 1–2% crossveinless individuals among the flies developing in the normal environment without heat shock, and from these a number of pair matings were set up. By selection over a few generations among the progeny of these pair matings, a number of different strains were derived, some of which produced high frequencies of crossveinless individuals when reared in normal environments lacking any heat shock treatment. In some of the "best" of these lines, in fact, the frequency of crossveinless individuals was 100% at 18°, though somewhat lower at 25°.

In these lines, therefore, the character of crossveinlessness, which had originally been an acquired character only exhibited (at any rate, at noticeable frequencies) in the abnormal environment provided by the heat shock, had been converted into an inherited character, exhibited in the normal environment in the absence of the heat shock. It was clear that this conversion had been brought about by the selection applied, and the process was therefore considered to be one of genetic assimilation, as defined above.

In these first experiments, the relatively complete assimilation finally achieved was obtained by selection operated in two different phases. In the first phase, selection was exerted on the subpopulation submitted to the abnormal environment of the temperature shock. After a certain modest degree of assimilation had been achieved in this way, the second phase of selection was carried out, in the normal environment, on the strains in which this mild assimilation was exhibited. This was done because a greater intensity of selection could be achieved by carrying out the second phase in this way, so that progress toward complete assimilation would be more rapid.

A series of very similar experiments were then made by Bateman (1956a,b, 1959a,b). She also employed a temperature treatment of the pupae as the abnormal environment. A number of different developmental abnormalities were chosen as the acquired characters to be favored by selection. One of these was the breakage or absence of the posterior crossvein, which had been studied by Waddington. Others were (1) the absence of the anterior crossvein, (2) the appearance of an extra crossvein in the first posterior cell, (3) the presence of an extra crossvein in the submarginal cell, (4) the appearance of a distorted wing resembling dumpy. Similar experiments involving temperature treatment of the pupae and selection for absence of posterior crossvein were also made by Milkman (1955). Waddington (1956, 1957b) used a very different abnormal environment (ether treatment of newly laid eggs) to elicit a different type of developmental abnormality—the con-

version of the metathorax into a structure resembling the mesothorax, as in the well-known bithorax phenotype.

In all these cases, essentially similar results were obtained. Selection for a particular acquired character within a population subjected to an abnormal environment increased the frequency with which that character appeared in later generations. After a varied number of generations of such selection, individuals exhibiting the phenotype began to appear in samples of the selected stock which had not been subjected to the unusual environment, and from these individuals strains exhibiting high degrees of genetic assimilation could rapidly be established. Even without resorting to this second stage of selection, Bateman was able, by selection only within the abnormal environment, to raise the percentage of individuals which showed the character concerned, when grown in the normal environment, to the level of 12–24%, and in one case as much as 46%. This provides strong grounds for believing that the use of second phase selection is not essential for the success of genetic assimilation, but is, as had previously been thought, merely a way of speeding it up.

B. Characters Not Involving a Threshold

In all the above experiments, the acquired character was one whose development involves something in the nature of a threshold; that is to say, in many individuals in the treated populations it is not exhibited at all—the crossvein remains unbroken or the metathorax shows no signs of being modified into a mesothorax. The genetic assimilation of a character not involving a threshold has recently been investigated by Waddington (1959b). This case is further interesting for two other reasons. Firstly, the selection applied was natural selection, brought about by the action of the abnormal environment itself and not by artificial intervention of the experimenter; and, secondly, the character produced is one which appears to be of adaptive value to the organisms when living in the abnormal environment.

The abnormal environment in this case was that produced by adding salt (sodium chloride) to normal *Drosophila* food. This was added in quantities (about 6% to begin with) sufficient to cause considerable mortality to the larvae reared on such media. Strains were carried on with no artificial selection, but simply by breeding from those individuals which had survived this stringent natural selection. The acquired character which was investigated was the size of the anal papillae. These were known to be concerned in the regulation of the osmotic pressure of the hemolymph, although their exact mode of operation is still obscure. It was found, in practice, that *Drosophila* larvae reared in media containing added salt have slightly larger anal papillae

(corrected for variations in total body size) than those reared on normal media.

The degree to which the acquired character had become assimilated after 21 generations of natural selection was tested by allowing eggs from the selected strains to develop on media containing varied proportions of salt and measuring the size of their anal papillae. In all three strains tested the results were essentially similar. In the first place, the selection had resulted in an improvement in the adaptation of the strains to high salt media, as shown by the fact that in such conditions a higher proportion of the eggs from the selected strains developed through to adults than from the unselected strains. Further, in all three strains, the acquired character—enlarged anal papillae—had become to some extent genetically assimilated, since when the selected strains were grown in media with low salt content these organs were larger than in the unselected strains grown in the same media. The selection had also led to an improvement in the capacity of the strains to react to increased salt content of the medium by the development of larger papillae. This is shown by the fact that the curve relating size of papillae to salt content of medium rises more steeply in the selected strains than in the unselected.

The nature of the physiological change involved in this adaptation is little understood. Croghan and Lockwood (1960) showed that the hemolymph of larvae of the selected strain, grown on food containing 7% added salt, is markedly hypotonic to the medium, the osmotic pressure not being much greater than that of unselected larvae on normal food; but since they did not study the blood of unselected larvae on salted food, the significance of their observations is uncertain.

All these experiments demonstrate that if selection takes place for the occurrence of a character acquired in a particular abnormal environment, the resulting selected strains are liable to exhibit that character even when transferred back into the normal environment. That is to say, the process which has been defined as genetic assimilation really occurs. Insofar as this is true, the appearance of acquired characters which are of value to an organism in terms of natural selection will have evolutionary consequences. Natural selection for such characters will lead to the appearance of populations in which the character is an inherited one and will be developed even in environments other than that which originally provoked it and in which it is of adaptive value. We have, therefore, experimental justification for using the notion of genetic assimilation to explain all those evolutionary phenomena which people in the past have been tempted to attribute to the inheritance of acquired characters in the Lamarckian sense.

IV. The Genetic Mechanisms of Assimilation

A. Evidence that Genetic Assimilation Depends on Selection

Since selection, either artificial or natural, was applied during the production of the strains exhibiting genetic assimilation, it is natural to hold it responsible for the occurrence of the phenomenon. The point was, however, specifically tested by Bateman in experiments in which an attempt was made to bring about genetic assimilation in inbred strains. In two such strains, in which genetic variability must have been very small or negligible, selection for the acquired character crossveinless was completely without effect and no trace of genetic assimilation occurred.

Bateman (1959b) also investigated the results of relaxing selection at various stages during the process of genetic assimilation. It was found that when selection was stopped in a strain in which, say, 60 or 80% of the individuals exhibited the acquired phenotype in the normal environment, then over the next few generations the frequency of occurrence of this phenotype declined, eventually settling down, after about a dozen generations, to some level well above that of the unselected stock but far from 100%. However, in strains in which assimilation was complete, the abnormal phenotype appearing in 100% of individuals raised in normal environment, the relaxation of selection was without much effect. It may be concluded from this that the assimilation is due to gradual increase in frequency of appropriate genes, which eventually, in the completely assimilated stocks, reach 100%.

B. Analysis of the Genotypes of Assimilated Strains

Genotypes of assimilated strains have been analyzed by means of crosses and backcrosses between them, or between them and wild types or downward selected strains, and also by the use of chromosome markers (Waddington, 1953a, 1956, 1957b; Bateman, 1959a,b). All the evidence indicates that the differences between the assimilated strains and their foundation stocks always involve all the chromosomes. For instance, in his original experiments, Waddington (1953a) showed that in his assimilated crossveinless stock, the second and the third autosomes and the X-chromosome all had a tendency to produce a crossveinless phenotype; the third chromosome had perhaps the strongest effect. The crossveinless-producing effect of fragments of the X-chromosome was also demonstrated for parts of the chromosome not containing the well-known sex-linked crossveinless locus. In crosses between the assimilated crossveinless stock and strains carrying the sex-linked *cv*

factor, a heterozygous female showed evidence of a summation of the effects of a single dose of *cv* and of an X-chromosome derived from the assimilated stock, but it did not appear that this chromosome contained any simple recessive allele of the *cv* locus. Waddington found further evidence of the participation of many genes in determining the character of the assimilated stock in the fact that the degree of dominance exhibited by the stock differed markedly in different crosses to various other stocks which had been derived during the process of selection. In crosses with downward selected stocks the crossveinless effect of the assimilated stock behaved almost as a complete recessive. On the other hand, when the assimilated stock was crossed to certain lines isolated during the process of assimilation, but themselves showing very low numbers of crossveinless individuals in normal environments, the assimilated genotype acted almost as a dominant.

Bateman (1959a,b) also analyzed the genetic constitution of several assimilated stocks, and again found that, in general, all the major chromosomes had some effect, although the intensity of effect varied from chromosome to chromosome. We may consider first the result of the analysis of stocks in which various venation phenotypes had been assimilated. Bateman analyzed three stocks characterized by absence of the posterior crossvein, one with absence of the anterior crossvein and two with extra crossveins, either in the submarginal or the first posterior cell. In all three in which the posterior crossvein was absent, the third chromosome had the strongest effect, although in one of them the X-chromosome was also rather strongly effective. In the stock with absent anterior crossvein, the second and third chromosomes were of more or less equal, moderately strong, effect, while in the strains with extra crossveins, the second chromosome was the most important in both cases. As Bateman pointed out, a considerable number of loci which produce breakages of crossveins are known on chromosome 3, while on chromosome 2 there are quite a large number of genes which tend to produce extra veins. It still remains very obscure why genes producing these two types of phenotypic effect should be, as it were, sorted out into different chromosomes. Crosses were made between the assimilated crossveinless stocks and a number of laboratory stocks containing third chromosome factors such as *cv-c*, *cv-d*, and *det*. In some cases a fairly high percentage of crossveinless flies appeared in the F_1, but this could not be interpreted unambiguously to indicate that the assimilated stocks contained allelomorphs of the loci concerned, since similar results would be expected if the condition in the assimilated stocks had a multifactorial basis.

The situation was rather different in the stock in which Bateman had

assimilated the dumpy phenotype. In this case the abnormal environment was a treatment at 40° for a period of 4½ hours at 16–18 hours after puparium formation. In the early generations the incidence of the dumpy phenotype was erratic, but after a time the incidence rose considerably and by generation 30 of selection the phenotype occurred in about 90% of the treated flies after only 2 hours treatment. The beginning of genetic assimilation, i.e., the appearance of the phenotype in untreated individuals, did not occur till generation 25, but from these flies a fully assimilated dumpy stock was eventually derived. The genetic analysis of this stock revealed that it contained an allele of the dumpy locus extremely similar to, and perhaps identical with, the allele known as dp^{TP}. When this allele was removed from the assimilated stock, the remainder of the genotype was not able to produce any dumpy phenotypes in the normal environment. It could be shown that the assimilated stock contained other factors influencing the dumpy phenotype, since selection for severity of expression was effective, but, in this case, the assimilation depended on the presence of the particular relatively powerful dp^{TP} allele, and did not occur in its absence.

Genetic analysis of these various stocks derived during the assimilation of the bithorax phenotype also revealed some points of interest (Waddington, 1956, 1957b). In the attempt to assimilate this phenotype two replicate experiments were started from two different Oregon-K foundation stocks. In each experiment both an upward and a downward selected line were carried on. In the 8th and 9th generations of the upward selected line in experiment 2, and in the 29th generation of the up-selected line in experiment 1, flies occurred in which there was a slight enlargement of the halteres (i.e., a slight bithorax phenotype) in the normal environment. In both these cases the very slight degree of assimilation achieved was shown to be due to the presence of a single gene, namely an allele of *Bxl* with a dominant halteres-enlarging, and a recessive lethal, effect. In the 29th generation of upward selection in experiment 1, there also occurred some individuals in which, in the normal environment, the metathorax was more completely converted into a mesothorax. By "second phase" selection among these, stocks were eventually built up in which the bithorax phenotype was very completely assimilated, being strongly expressed in over 80% of individuals in the normal environment.

The analysis of the genotype of this assimilated bithorax stock showed that it contained genes of two rather different types. On the one hand it was shown, by chromosome substitution experiments, that both the second and the third chromosomes contain factors tending to

produce the bithorax phenotype in the individuals homozygous for them. Secondly, it was shown that there was present a sex-linked gene which exerted a recessive maternal effect, such that females homozygous for it tend to lay eggs which develop into bithorax phenotypes. If this gene is removed from the general background of the assimilated bithorax stock and crossed into a normal wild type, homozygous females still give rise to a few bithorax offspring, but the frequency with which the phenotype occurs is very much reduced. A somewhat unexpected finding was that, although females homozygous for the maternally acting gene lay eggs which tend, in the normal environment, to develop into bithoraxes, these eggs are no more sensitive than others containing the same general genotype to the effects of the abnormal environment (ether treatment shortly after laying), by which the abnormal phenotype was originally produced.

Summarizing these results we may say that in all cases in which complete or nearly complete assimilation has been achieved, the process has involved changes at many loci throughout the whole genotype. The only instances in which the genetic change was restricted, as far as is known, to a single locus are the two occurrences of a *Bxl*-like mutant in the bithorax experiments. In all the other experiments many loci must have been involved, but there is a considerable range between cases in which all the involved loci seem to be of relatively similar importance, to others in which one or a few loci are particularly strongly effective, while the others can be considered as mere modifiers or genetic background. Thus, in the dumpy assimilation, a *dp* allele is so important as to be essential, though its action is modified by other parts of the genotype. In the bithorax assimilation a relatively important maternally acting sex-linked genetic factor can be detected, although attempts to determine whether the effect is produced by a single locus, or two or more loci, remained inconclusive; the condition, however, has by no means the same relative importance as the dumpy allele in the dumpy-assimilated stock. In the stocks in which various venation phenotypes were assimilated, it was, in general, impossible to identify any one predominant locus. Bateman made an estimate, for several of her stocks, of the relative importance of a hypothetical major gene as compared with that of the rest of the genotype in producing the assimilated character. For three of the assimilated stocks the estimates were that a major gene might be 1.57, 1.99, or 4.38 times as important as the remainder of the genotype. For a fourth stock the estimate was as high as 6.71, but this stock (known as *WE pcvl O*) had been derived in rather a special manner, and will be mentioned again later.

C. Origin of the Genetic Variation Utilized in Genetic Assimilation

As we have seen, genetic assimilation fails completely, at least over periods of fairly small numbers of generations, in inbred strains lacking genetic variability. It can be successfully carried out when the foundation stock is a normal random-bred population in which considerable genetic variability is present. It is clear that this already-existing variability must provide a great deal of the genetic material which is finally incorporated into the fully assimilated stock. The question arises whether it provides all of it. It is difficult to see how to give, experimentally, a completely positive answer to this question, and we are driven to ask the somewhat less penetrating one, namely, is there any definite evidence that the assimilation process has utilized genetic variation which was not present in the foundation stock?

If the foundation stock contains all the genetic variation necessary for complete genetic assimilation, then we should expect to find, under a system of random mating, that the assimilated genotype would occur in the foundation stock under the normal environment although probably at an excessively low frequency. In Waddington's (1953a) original experiments with absence of posterior crossvein, no thorough search of the foundation stock for this phenotype was made, although it was not noticed in the inspection of a few hundred individuals. In Bateman's experiments the phenotype was found in 0.7% of one of the foundation stocks and 0.25% in another. Of the other phenotypes she investigated, the presence of an extra crossvein in the submarginal cell was found in 0.1% of the foundation, but the absence of the anterior crossvein and the excess crossvein in the first posterior cell were not found at all; neither was the dumpy phenotype. The bithorax phenotype was also not seen in the foundation stock. The failure to find these phenotypes in the foundation stock may, of course, only indicate that they depend on gene-complexes, the elements of which are all very rare so that their combination before selection starts is of excessive rarity.

From the population which showed 0.7% of posterior crossveinlessness before selection started, Bateman selected and bred together the few crossveinless individuals. These responded gradually to selection, in a manner which indicated that many genes were involved, and it was not till generation 10 that the strain was producing 90% of crossveinless flies. This strain, which was produced by straightforward selection from the foundation stock, without the application of any abnormal environmental circumstances, was given the name *WE pcvl O*. It is interesting to note that when Bateman attempted to estimate the relative importance of a major gene as compared with the genetic

background, it was in this strain that the major gene was of most importance, as has been pointed out above. It was presumably only because the foundation flies of this strain contained a comparatively powerfully acting gene that they exhibited the crossveinless phenotype in the background of the unselected foundation stock. The development of the strain must have involved the increase in frequency of the major-acting gene and also a concentration of other genes acting in a similar direction. In the other assimilated stocks which Bateman established from the same initial foundations, a greater part must have been played by the concentration of numerous genes acting in this direction and a lesser by increase in frequency of any single powerfully acting gene.

These results show clearly that, in these cases at least, the foundation stocks contained sufficient genetic variability to produce the strains in which the phenotype was assimilated. In other experiments in the series, however, there is evidence that new genetic variability, contributory to the constitution of the assimilated strain, has arisen during the course of the experiment. It seems that this must certainly have been the case for the two occurrences (the second of which may possibly have been due to a contamination from the first) of the *Bxl*-like allele in the bithorax experiments. Bateman also considered it likely that the dp^{TP} allele, on which the assimilation of the dumpy phenotype was based, arose by mutation during the course of the experiments. The mutation may, in fact, in this case, have been a result of the heat treatment since the first occurrence of a gene of this type was recorded by Plough (cf. Bridges and Brehme, 1944) in experiments on the heat induction of mutations. Finally, it appears rather likely that the maternally acting gene, which plays an important part in the final bithorax-assimilated stock, may also have arisen during the course of the selection, although the possibility cannot be excluded that it was present in very low frequency in the initial foundation stock.

V. Genetic Assimilation and Developmental Canalization

A. The Canalization of Development

Genetic assimilation obviously involves the somewhat paradoxical character of phenotypes to be, on the one hand, to some extent susceptible and, on the other, to some extent resistant, to alteration by environmental agencies. It is only because development can be modified by the environment that an acquired character can appear in the first place, when a population is transferred from environment E to environment E′; but when this character becomes assimilated, that implies that the development of the phenotype is now resistant to the effects of

changing the environment back from E′ to E. The property of a developmental process, of being to some extent modifiable, but to some extent resistant to modification, has been referred to as its "canalization" (Waddington, 1940a). This notion can be applied whether the agents which tend to modify the course of development arise from genetic changes or from changes in the environment.

The fact that phenotypes are somewhat resistant to modification by changes in the genotype is a commonplace of genetics. Its simplest exemplification is perhaps in the phenomenon of dominance. In this, the alteration of one of a pair of dominant alleles to a recessive (from *AA* to *Aa*) does not succeed in bringing about any phenotypic alteration. The way in which geneticists have thought about this phenomenon was, for some years, directed on to rather inadequate lines by the assumption that dominance or recessiveness is a property of particular alleles. The origin of a more adequate view can be found in the work of Muller, Fisher, Stern, Goldschmidt, and others in the years around 1930. Fisher drew attention to the fact that the degree of dominance is subject to control by the rest of the genotype; and Muller discussed the phenomenon of dosage compensation, by which males containing only one dose of sex-linked factors appear very similar to females which contain two doses of the same factors, and emphasized that it is necessary, in such contexts, to consider the whole genotype and not individual alleles. The importance of the total genotype was also exhibited by the phenomenon of epistasis, in which inter-locus interactions lead to the concealing of certain genetic alterations. Again, genes of low penetrance are alleles whose action frequently fails to overtop some threshold set by the remainder of the genotype.

In all these cases, then, we are dealing with an essentially similar phenomenon, namely, a course of development which exhibits some resistance to being modified by genetic changes. It is, of course, also a well-known fact, but one with which in the past embryologists have had more to do than geneticists, that development also tends to resist being modified by environmental agencies. Embryos tend to regulate, that is to say, to produce their normal end-result in spite of external accidents which may occur to them as their development proceeds. Again, the existence of some resistance to modification is shown by the fact that different strains of the same species differ in the extent to which they are modified by a given external stress.

The notion of canalization is, therefore, intended to be a very general summing-up of a large number of well-known facts in genetics and embryology, all of which are summarized in the statement that the development of any particular phenotypic character is to some extent

modifiable, and to some extent resistant to modification, by changes either in the genotype or in the environment. The concept was first formulated on the basis of a detailed study of the developmental effects of some forty genes which cooperate in determining the formation of the *Drosophila* wing; and at the same time it was pointed out that the fairly high degree of resistance to modification which is characteristic of the development of "wild type" genotypes must have been produced by natural selection and is much reduced in many mutant forms (Waddington, 1940a,b, 1941).

B. Genetic Assimilation as a Consequence of Canalization

Since the concept covers both types of possible modifying agent—genetic and environmental—it lends itself to a particularly easy exposition of the process of genetic assimilation, in which both these factors are involved. When a population is taken out of environment E and placed into environment E′, the phenotypes developed by the different individuals will depend on the canalization of their developmental systems with respect to the environmental differences involved. If selection occurs for the production of certain acquired characters, this can be regarded as bringing about a change in the canalization determined by the genotypes that finally result from the selection. The canalization of development in the selected individuals will be such that the characteristic features of environment E′ easily produce the acquired character in question. However, canalization, as we have seen, has two aspects: it involves not only the possibility of modification but also a resistance to modification. If the selected population is now placed back again into environment E, the resistance to modification of its phenotype may be such that this new change of environment does not modify the character back again to the form it had initially.

This way of expressing the situation is a highly general one, which can be applied either to characters whose development exhibits a threshold (such as the wing venation or bithorax phenotypes mentioned above) or those without thresholds (such as the anal papillae). Moreover, it does not attempt to specify by what mechanisms the resistance to genetic or environmental change is brought about. The genes which were present in the initial population, and which become accumulated by selection because they condition the appearance of the acquired character in the new environment, may have been concealed in the old environment either because, under those circumstances, they were of too small effect to be noticeable, or because they were hidden by dominance or epistasy.

Stern (1958, 1959) has offered what he considers to be an alternative

explanation of the process of genetic assimilation, but it is difficult to see that his account differs in any way from that given above, except that it is expressed in slightly less general terms. Stern considers that the process is due to the selection of genetic differences which were "sub-threshold" in the original environment E, but which are supra-threshold in the new environment E′. This is, in effect, merely another way of saying that, in the original population, the canalization of development is such that these genetic differences in the environment E remain concealed owing to phenomena such as dominance, epistasy, etc., while in the environment E′ the environmental stress, added on to the effect of the genetic differences, allows them to come to expression. It is relatively easy to envisage such a situation when one is thinking in terms of canalization, that it to say, in terms of a course of development which has a certain resistance to modification by either genetic or environmental agencies. It is perhaps rather more awkward to do so in Stern's terminology, which leaves out of account the general process of differentiation, and concentrates its attention on the genetic factors, or the environmental factors, as separate agencies.

For instance, in discussing the application of genetic assimilation to the evolution of callosities on parts of the skin which normally are subject to pressure, Stern (1959) expounds his "new hypothesis" as follows: "The hypothesis, in over-simplified form, begins with the fact that many genes have only a slight effect if they are present in single dose but a strong effect in double dose. If, for instance, the gene pair AA did not lead to the spontaneous production of specific callosities while the combination AA' implied a slight tendency towards this trait, then $A'A'$ might well cause its invariable appearance. In a population in which the gene A is highly abundant, and A' correspondingly rare, most individuals would be AA, a few AA' and practically none $A'A'$. Be it assumed that the AA' individuals will under pressure form callosities easier than AA individuals. They will then have been favored by selection and their number will increase. The higher frequency will lead to crosses between AA' males and females, and as a necessary consequence of Mendelian segregation one-quarter of their offspring will be $A'A'$. This latter genotype is the very one which causes the spontaneous appearance of the callosities."

This is, of course, exactly the same explanation (in oversimplified form) as has been offered above; but putting it like this leaves several loose ends. In the first place, it suggests that the concealment of the allele A' in the original population is due only to dominance, whereas it may also be due to inter-locus interactions or epistasy. In the second place, Stern has suddenly to bring into the middle of his argument what

appears to be an extra *ad hoc* hypothesis when he writes: "Be it assumed that the *AA'* will under pressure form callosities easier than *AA* individuals." It is only because, in fact, everyone has at the back of his mind a vague idea, which it is the purpose of the notion of canalization to make explicit, that he accepts it as reasonable to suppose that one and the same gene has the two properties of having a slight tendency to produce callosities in a normal environment and increasing the tendency to react to pressure by the formation of callosities. Finally, the canalization terminology can deal with situations such as that of the anal papillae, in which no thresholds appear to be involved, and where some of the genes selected may very well operate simply to increase the extent to which the size of the papilla reacts to the presence of increased salt, without having any tendency themselves to cause a larger papilla when no salt is present. The existence of such genes, whose action is confined to the control of canalization (or reactivity, if one wishes to express it so), is not allowed for in Stern's scheme. They are, however, certainly a theoretical possibility and, as we shall see, there is considerable evidence for their existence.

C. Some Concepts Allied to Canalization

1. *Autoregulation*

In the late thirties and early forties, Schmalhausen and a group of colleagues in Russia were following a line of thought almost exactly parallel to that which, at about the same time, led to the formulation of the idea of canalization and genetic assimilation. These ideas were brought together in a book published in Russian in 1947 and in American translation in 1949. Schmalhausen (1949) discusses a notion very similar to canalization under the name "auto-regulation," and he points out that the properties of the auto-regulating mechanisms of natural organisms have been brought into being by natural selection. He cites many examples of the apparent "inheritance of acquired characters during evolution" and argues that they should find their explanation in selection for the capacity of organisms to react adaptively with their environment. He did not, however, formulate in any precise form the process which has been referred to here as genetic assimilation. Moreover, such experimental work of his school as is available in English seems to be related to the ideas nowadays referred to as the "Baldwin effect" (see below), rather than to genetic assimilation. In any case, no experiments which were successful in bringing about a result which can be interpreted as genetic assimilation seem to have been reported by him or his students.

2. *Adaptive Modifications and Morphoses*

There are a group of notions connected with the nature of the response of an organism to an environmental stress which require discussion. Schmalhausen attempted to make a distinction between "adaptive modifications" and "morphoses." The former were, as their name implies, reactions to stress which were of adaptive value. Schmalhausen very convincingly pointed out that the capacity of an organism to react in such a way is almost certainly to be attributed to the past actions of natural selection. If, during the course of its evolutionary history, a given type of organism has frequently had to deal with a particular environmental difficulty, it is likely to have acquired, through natural selection, the ability to be modified by this stress in a way which is of use in coping with it. Schmalhausen used the term morphoses to refer to developmental modifications produced by abnormal environments which the organism has not encountered in its evolutionary past (e.g., high doses of ionizing radiation, etc.).

He seemed to imply that there was some real difference in the physiological nature of the developmental reactions involved in the two cases. It is, however, difficult to see why this should be so. If we consider the experiments on wing venation phenotypes described above, the reaction of a *Drosophila* population to a hot shock during the pupal stages must, in the first generation or so, be considered to be the production of a morphosis. However, under the influence of artificial selection, this behaves in exactly the same way as does the production of an undoubted adaptive modification, in the salt-treated larvae, when submitted to natural selection. There is no reason to suppose that, for instance, the adaptive modifications are under proper genetic control while the morphoses, in some way, escape from it; or indeed that the two categories differ in any matter of principle. It would seem likely that there is a continuous range, between developmental modifications in response to environmental stresses which have frequently been met in the past, and for which natural selection has built up a useful reaction, through those in response to circumstances for which natural selection has not been able to find an answer, to modifications produced by rare or unnatural stresses in connection with which previous selection has not been operative.

3. *Phenocopies*

Another term frequently employed in connection with the response of an organism to environmental stress is "phenocopy." This term was

introduced by Goldschmidt (1935), who has the merit of being one of the first to make a serious study of the reaction of organisms to external stresses, particularly those of a rather extreme character. Goldschmidt was primarily interested in throwing light, by such studies, on the mode of action of genetic factors. He felt that this could best be done by the study of situations in which some external factor, applied to a developing wild type individual, caused the appearance of a phenotype which mimicked that produced by some recognized mutant allele. The word phenocopy was indeed coined to imply this copying of a mutant phenotype.

It has, however, gradually become clear that a knowledge of the nature of the environmental stresses which can produce a copy of a given mutant phenotype does not yield much information about the developmental action of the mutant alleles which produce similar phenotypes. The situation is, to employ the canalization terminology, that a particular phenotype is produced by a developmental process whose course is steered by the combined action of the whole genotype and the impinging environment, and that very often there are a considerable variety of genetic changes ("mimic genes") and of environmental stresses, any one of which will steer the development towards the production of a particular phenotypic abnormality. Moreover, when an abnormal environment succeeds in diverting development to some unusual end-result, it is not a matter of major importance whether a mutant allele happens already to be known which produces the same effect. For instance, in the venation experiments mentioned above the flies with broken posterior crossveins are phenocopies in the strict sense of the term, since a crossveinless gene—in fact several quite different crossveinless genes—are already known. The flies with extra crossveins in some of the anterior wing cells are, however, not phenocopies, since no gene producing this abnormality has apparently been described.

An important result of Goldschmidt's work was to show that different strains (in the early work, all wild type strains, but of different origins) differ in the frequency and type of developmental abnormality produced by the same applied environmental stress. This provided one of the bases for the notion that reaction to stress is a genetic property, and can be altered by selection. More recently studies have been made on the modifications caused by a given environmental stress when it is applied to strains which contain, either in homozygous or heterozygous condition, mutant alleles known to produce phenotypic effects similar to those which can be elicited by the environmental stress from wild type strains. For instance, Sang and McDonald (1954) showed that

sodium metaborate, which causes a reduction in eye size when fed to the larvae of wild type *Drosophila* strains at above a certain threshold concentration, gives the same type of effect with a lower threshold when fed to larvae heterozygous for the eyeless gene. These and similar results led Goldschmidt to pose the problem whether the phenocopying effect of a certain environmental stress could, in all cases, be regarded as the unmasking of a sub-threshold gene which was already present in the stock employed.

He devoted a good deal of work to attempting to find out the answer to this problem (Goldschmidt and Piternick, 1957a,b), and showed that in many, though not in all, cases he could isolate some genetic factor tending to produce the phenotype in question. It may, however, be doubted whether the problem is a real one. A phenocopy (or, in general, any abnormal phenotype elicited by an environmental stress) must result from the combined action of the environment and the genotype of the organism. That is to say, genes must be involved in the production of phenocopies, and *ex hypothesi* they must be sub-threshold genes, at least as regards the abnormal phenotype. The only question with which Goldschmidt was really concerned was whether, in the cases he investigated, there was any one gene which was of sufficiently great importance to be identifiable as *the* sub-threshold gene. The situation is strictly comparable to that in the assimilation experiments described above, in which, as we saw, it was sometimes possible to identify a single relatively important locus, and sometimes not. As Landauer, (1957, 1958) has put the matter, in discussing rare developmental abnormalities ("pheno-deviants"): "Our evidence leads us to conclude that sporadic defects, as well as experimental phenocopies, are the results of events through which ordinarily hidden weaknesses of developmental equilibria become manifest, and that these weaknesses have a definite, if complex, genetic basis. If the phenocopy concept in its narrow meaning of a purely environmental interference with developmental processes must be abandoned, it is clear that the existence of crypto-genes and their spontaneous or experimental aberration confront us with many new problems."

Landauer's phrase "developmental equilibria" is, of course, a shorthand form of referring to the notion of canalization, but the word "equilibrium" is not quite satisfactory in this context for two reasons (cf. Waddington, 1948). Firstly, the idea of equilibrium tends to be an absolute one, whereas we need to discuss just how well equilibrated, or stable, developmental processes are (cf. Landauer's reference to "weaknesses" of equilibria). And secondly, developmental processes are not in equilibrium in time, but essentially change as time passes. Both these points are easily dealt with in the canalization terminology.

4. *Homeostasis*

Another word which has been introduced into these discussions, and requires some notice, is "homeostasis." The classic use of this term in biology is by Cannon (1932). He used it to refer to the fact that many physiological systems show a capacity to return, after disturbance, to some standard condition in which they maintain themselves. For instance, if the pH or CO_2 tension in the blood is altered in some way, processes come into operation which tend to annul the alteration and restore the previous condition. Such a situation can be referred to as one of "physiological homeostasis." More recently the word has been used in at least three other different contexts, in addition to that of physiology.

Lerner (1954) has used it to refer to the tendency of a population to maintain, and if necessary restore, a particular distribution of gene-frequencies within it. If, for instance, the gene-frequencies in a population are altered by selection for a few generations, and the population is thereafter left to itself with no further selection, the gene-frequencies are frequently found to return to their original values, the operative machinery being largely that of natural selection. These and other similar phenomena he referred to as "genetic homeostasis."

This usage is rather far from our present context, and need cause little confusion in it. The situation is rather different concerning the other two usages, in which the word homeostasis is used in conjunction either with developmental or with general evolutionary processes. The word presumably always implies that something or other is being held constant, but when it is used in connection with evolution and development it is often difficult to decide what exactly this constant element is supposed to be. The natural interpretation of such a phrase as "developmental canalization" would be that development is being held constant, or to put it more precisely, that development is tending to reach a constant end-result. However, this is not what authors who employ the phrase always have in mind. For instance, Lewontin (1957) uses the phrase "developmental homeostasis" to mean developmental mechanisms (including those which lead to an unusual end-result) which prevent the evolutionary fitness of an organism from being reduced in the particular environment in which it finds itself.

The tendency to keep fitness constant, which Lewontin (1957) and Dobzhansky (1955) have referred to simply as "homeostasis" (without qualification) could perhaps be usefully referred to as "evolutionary homeostasis," although the phenomenon is really a maximization of fitness rather than holding it constant, since if in a given environment fitness increased, there would be no tendency to reduce it. In the con-

text of evolution this "homeostasis" of fitness is of overriding importance, and natural selection will tend to produce some mechanism for bringing it about. Such mechanisms may involve holding the end-product of development constant ("developmental homeostasis") or they may involve the almost precisely opposite gambit of allowing development to be altered in an adaptive way (Thoday, 1953). The use of the word "homeostasis," particularly if not qualified in a way which definitely states what aspect of the animal's existence is being held constant, therefore almost inevitably leads to confusion. The canalization terminology on the other hand is designed precisely to deal with the complementary flexibility and inflexibility of developmental processes which natural selection exploits to ensure that organisms can keep their fitness maximal in different environments. Further discussion of these terminological points will be found in Waddington (1957a).

Dobzhansky and his colleagues have recently made many very important studies on the genetic systems in wild populations and their response to different environments (cf. Dobzhansky, 1955; Beardmore *et al.*, 1960). Some aspects of the phenomena which they have revealed, and have discussed in terms of homeostasis, are certainly related to what we have called developmental canalization, and might perhaps be less ambiguously expressed in that phraseology. This work does not, however, have a sufficiently direct bearing on genetic assimilation for it to be in place to attempt such a discussion here.

Another attack on similar problems, in which the physiological and genetic mechanisms have been, perhaps, more definitely integrated, has recently been started by Forbes Robertson (1959, 1960). He has studied the effect of selecting a strain for a certain character (usually body size) in one environment on its developmental reactions and fitness when taken into some other environment. The environments used were rather precisely known nutritional conditions, and the growth performances of the organisms were analyzed in terms of cell numbers, cell size, and developmental time. The results clearly exhibit both the flexibility and inflexibility of development which constitute canalization, and the effects of previous selection on the ways which the organisms adopt to maximize their fitness under different circumstances. Another study in which the technique of selection has been used to investigate the genetic constitution underlying responsiveness to environmental stresses is that of Falconer (1960). In both these pieces of work, however, the results, although very pertinent to the concept of canalization, are not closely enough related to genetic assimilation to be discussed further here.

D. The Genetic Basis of Canalization

There are a few experiments which have explicitly set out to investigate the genetic basis of canalization and which require at least short discussion.

The idea of canalization involves no more than that the course of development exhibits, in some way, a balance between flexibility and inflexibility. The particular form that this balance takes may vary widely in different cases (Waddington, 1952a). The character of the canalization in a particular case can be expressed by plotting the extent of developmental response against the magnitude of the disturbing stress, either genetic or environmental. We may find cases in which the dose-response curve is linear; others in which variations in dose near the normal range produce considerable effects, but it becomes more and more difficult to alter development as it diverges from the norm (this is probably the case for characters such as body size); or other cases in which considerable variations around the normal dose remain almost ineffective, while with still greater doses the development becomes more easily deformable (this is so when the normal phenotype is protected by thresholds or quasi-thresholds). Most of the experimental analyses of canalization have dealt with cases of the last type.

1. *Variation Around the Wild Type*

The first such study (Waddington, 1955) was designed to discover whether the normal phenotype of the *Drosophila* wing venation pattern is protected by thresholds, i.e., is a case of canalization of the last of the three types mentioned. Four stocks were prepared, in two of which there were absences (either major in one stock, or minor in the other) of the posterior crossvein, while in the other two there were excess pieces of vein (either small or large) attached to the posterior crossvein. A series of crosses, backcrosses, and so on were made between these stocks; and the conclusion clearly emerged that the wild type phenotype can conceal within it a much greater range of dosages of vein-producing genes than can any other phenotype. That implies that the canalization of the normal vein pattern is such that it is highly resistant to the disturbing effects of changes in the dosage of genes tending either to make more or to make less vein. In individuals in which the buffering capacity of the normal developmental course is exceeded, the phenotype does become altered, and in these the pheno-

type which develops reflects not too inaccurately the actual dosage of genes contained in them.

The situation was actually somewhat more complex than this, since the character "quantity of posterior crossvein" turned out not to be "causally homogeneous"; that is to say, the same total quantity of vein could be produced in different ways, for instance, by the presence of a single complete crossvein or by the presence of a broken crossvein plus the addition of an extra fragment attached to its side. A similar complexity arises in some of Forbes Robertson's work, where the same body size can be achieved either by an increase in cell number or an increase in cell size. In such cases the global character which is under investigation is made up of more than one constitutive developmental course, and each of these requires consideration on its own. It remains true, however, in the case of the crossvein experiments under discussion, that each of these developmental courses exhibits canalization of the kind described.

2. *Disruption of Canalization*

When a wild type phenotype is strongly canalized very little of the genetic variation present in a normal population will come to expression and thus be available for selection. A greater amount of the variation can be revealed if some way is found to push the processes of development away from the canalized phenotype, so that they follow a path which is more susceptible to the influence of minor genetic variation. Development can be steered into a less well canalized pathway not only by environmental stresses, as in the experiments on genetic assimilation, but also by the influence of some major gene affecting the character under investigation. One can therefore use such genes to destabilize the development of a phenotypic character which then becomes more responsive to selection.

Dun and Fraser (1959), Fraser *et al.* (1959), and Fraser and Kindred (1960) have used this method to carry out selection on the number of vibrissae in the house mouse. The number of secondary vibrissae in the mouse is normally 19, and in most stocks there is very little variation about this number. The gene Tabby reduces the number to about half, and the Tabby phenotype being less well canalized than the wild type, there is considerable variation. Dun and Fraser kept a line in which Tabby (which is a semidominant) was segregating in each generation. They selected for either increased or lowered number of vibrissae in the Tabby heterozygotes, and found that there was a parallel increase or decrease in numbers in the wild type sibs; the changes produced in the wild type were, however, very much smaller

than those produced in the Tabbies. Thus, selection which changed the vibrissae number in Tabby heterozygotes from about 11 to about 16.5 produced an increase of only about half a vibrissa in the wild type sibs. This result shows clearly that the wild type phenotype is much more resistant to modification, that is, is much more strongly canalized, than is the Tabby phenotype.

Rendel (1959a,b) and Rendel and Sheldon (1960) have made very similar experiments in which they used an allele of scute to force the development of the scutellar bristles in *Drosophila* out of their normal canalized path. In the wild type there are 4 scutellar bristles, and usually very little phenotypic variation away from this number. The scute gene in the original population reduced the mean number to 2, and there was a good deal of variation around this. Selection for scutellar number in lines segregating for scute raised the number gradually to 4, and at the same time the number of bristles in the wild type sibs increased. Again, the change in the wild type was much smaller than that in the destabilized scute stock. Rendel suggested a method of estimating the relative genetic change required to bring about different changes in bristle numbers; and he calculated that to move from 3 bristles through the canalized number of 4 to 5 bristles takes about eight times as much genetic change as it does to move from 1 bristle to 3. This gives an indication of the degree to which the canalized normal phenotype can absorb or conceal genetic variation.

3. *Improvement of Canalization*

Rather similar work has been published by Maynard Smith and Sondhi (1960) concerning the number of ocelli which in *Drosophila* is normally canalized at three. Maynard Smith (1960) has also discussed the degree of precision which must be achieved by the underlying physical processes of the canalized system in order to ensure the constancy in number of organs such as ocelli and bristles.

Another method of demonstrating the reality of canalization is to produce changes in the degree of canalization by means of selection. Wild type phenotypes are already so well canalized that it is not easy to alter their degree of canalization. This can, however, fairly easily be done in the relatively destabilized phenotypes produced by mutant genes. For instance, in *Drosophila melanogaster,* Bar causes a considerable reduction in the size of the eye, and it is well known that the Bar phenotype is sensitive to temperature, the eyes being larger at low temperatures and smaller at high temperatures. Waddington (1960) practiced selection for increased canalization by the following method: a number of pair matings were set up, the progeny being divided into

two lots reared at 18° and at 25°, and selection made by taking those families in which the difference in eye size at the two temperatures was least. After some six generations, families were obtained in which the difference in facet number at the two temperatures had been reduced to between 6 and 15 facets, whereas in the unselected foundation stock, tested at the same time, the difference was about 100 facets. Thus a very considerably increased degree of canalization against the disturbing effects of temperature had been achieved.

4. *The Genetic Structure of Canalized Phenocopies*

Many aspects of the wild type phenocopies are not so well canalized as the number of scutellar bristles or the number of ocelli. The application of the idea of canalization to phenotypes which exhibit continuous variation in normal populations was discussed by Waddington at a conference which took place in 1950 (Waddington, 1952a). A few years later, Lerner (1954) suggested that the homeostasis of gene-frequencies in a population often depends on heterozygotes exhibiting better developmental canalization than homozygotes and therefore being biologically fitter. Several authors have studied Lerner's suggestion that heterozygotes exhibit better developmental canalization. Most of these investigations have used, as a indication of canalization, not the response of the phenotype to some known exterior stress, but the variation, within the body of a single individual, of some phenotypic character which is repeated in a number of different parts of the body which might be expected to be identical.

For instance, Mather (1955), Jinks and Mather (1955), Tebb and Thoday (1954), Thoday (1956, 1958), and Reeve (1960) have studied the degree of asymmetry in numbers of sternopleural bristles on the two sides of a fly, while Reeve and Robertson (1954) have considered the correlation in numbers of abdominal bristles on the various sternites of *Drosophila*. The results make it clear that there is some genetic influence on the degree of asymmetry or repeatability of such characters, but the heritabilities found were in most cases very low. Heterozygotes often exhibited a lower degree of asymmetry, or a higher degree of repeatability, than the corresponding homozygotes from which they were derived, but this rule was by no means always the case.

Doubts have been expressed, however, (e.g., Waddington, 1957a; Reeve and Robertson, 1954) whether the character investigated in these studies, which is the extent of the phenotypic variation produced by intangible alterations of conditions during development, is really an indication of the degree to which the developmental systems are canalized as against more definite environmental changes or alterations in

genotype, and whether they can be taken to throw much light on the genetic make-up of canalization systems in general. It is worthy of note that in Waddington's (1960) experiments on the Bar phenotype, the lines showing a high degree of canalization were considerably more inbred than the foundation population. It is therefore clear that a high degree of heterozygosity is certainly not necessary for strong canalization, which can be achieved in relatively homozygous genotypes if selection is made for it. In these same experiments it was also found that a high degree of canalization against the effects of external temperature was not accompanied by a reduction in the asymmetry of the eyes on the two sides. This strongly suggests that the variation between parts which are repeated within the same individual is not a good indication of canalization in general. Waddington (1957a) suggested that it should be referred to as "developmental noise." Clayton, *et al.* (1957) have spoken of it as "developmental error," and Reeve (1960) refers to it as "chance variance."

In the development of any complex phenotype there will be a large number of developmental pathways leading to the various final adult organs or characters. Mather (1943) at one time attempted to discuss the organization of such a developmental system in terms of a proposed distinction between "oligogenes" and "polygenes." The former are the well-recognized and distinct alleles with which genetics has been mainly concerned, while the latter were supposed to be genes of a special kind, possibly constituted by heterochromatin, which produce only small effects on the phenotype and which were thought of as responsible for the production of continuous variation. Mather suggested that the canalization of the various pathways of development is dependent on polygenes, while oligogenes act mainly by switching parts of the developing system into one or other of the different paths. However, Waddington (1943) argued that the distinction between polygenes and oligogenes is not a real one, since the same allele can appear as a polygene in one genotype but as a well-recognizable oligogene in some other genotype. In this particular context he claimed that there was no reason to suppose that the switching between one path and another could not be done by systems of genes each of small effect (i.e., Mather's "polygenes"), as well as by single identifiable alleles. This has since been shown to be the case, as in the crossveinless experiments described above.

Fraser (1960) has shown how the conditions for the evolution and maintenance of genotypes determining canalized paths of development can be investigated by Monte Carlo methods on a digital computer. This method seems likely to become a powerful tool for studying the

complex theoretical structure of such situations, but so far no very striking results have appeared.

5. *The Evolution of Canalization*

Berg (1959) has given an interesting discussion of the factors which will lead to the evolution of high degrees of developmental canalization. He argues that this will occur when the aspects of the environment which exert natural selective pressure on an organ have, in themselves, no direct effect on the developmental processes by which that organ is produced. It will then be necessary for the developmental system to produce an organ which is precisely tailored to meet the requirements of natural selection, and to do so without being able to use the selective factors to guide the process of development while it is actually going on. As an example, Berg quotes the very low variance (i.e., high degree of canalization) of the dimensions of insect-pollinated flowers which have to deposit their pollen on to particular portions of an insect's anatomy. Here the selective force, i.e., the correct placing of the pollen, certainly plays no part in the developmental processes by which the flower comes into being. It is for this reason, Berg argues, that it is necessary for the development to be very highly canalized, so that flowers of exactly the right dimensions are reliably produced in spite of variations in temperature, moisture, and other climatic conditions during the growth of the plant.

While there are many cases of canalization to which Berg's explanation may be applied, there are probably others which do not so easily fall within its scope. The most difficult fact to understand about the evolution of canalization is its tendency to, as it were, overshoot the mark which would seem to be necessary. Even if one grants that all developmental processes have a certain quality of inflexibility, and that selection for a comparatively extreme type of developmental modification will, owing to this inflexibility, lead to some genetic assimilation of the character in the way described above, it remains rather peculiar that natural evolution so frequently results in a much firmer genetic assimilation than would appear to be demanded. For instance, selection for the ability to form thickened skin on the soles of the feet as a response to pressure might well be expected to lead to some degree of assimilation of the character, so that the foot soles were thick even in people who never walked. Why, however, should this assimilation go so far that the foot soles thicken even *in utero*, before there is any possibility of their being used? One may guess that some sort of "factor of safety" consideration is coming into play, but it is not clear why it should operate in such an apparently exaggerated form.

One possible mode of approach to the topic would be to draw attention to the fact that development is usually easier to modify at early stages than late. It may be, as it were, easier for the genotype to produce an allele which causes thickening of the soles of the feet at an early embryonic stage *in utero*, rather than one that operates exactly at the time when the thickened skin becomes advantageous. We still understand so little about the actual processes involved in development that it is difficult to know how much weight should be lent to such consideration. But we are perhaps too ready to believe that it is quite easy to produce a gene that will do anything required. Actually it may be that mutation plus natural selection should only be expected to produce a system which "gets by," rather than one that does exactly what is required and no more. It is noteworthy that in the bithorax assimilation experiments, one of the important genes involved was a maternal-effect gene which operates on the egg out of which the bithorax individual will develop; that is to say, it acts very much earlier in development than the period at which the selective pressure was exerted. If the species has to use whatever genes it has at its disposal to meet the demands of natural selection, it seems quite likely that it will have to use genes which act earlier than necessary, since it cannot make use of those which act later.

It is presumably mechanisms of this sort which have brought about the great evolutionary divergence in the types of eggs characteristic of different phyla and families of the animal kingdom, since it is very unlikely that these can have been shaped by natural selective forces acting at such early periods of ontogeny. The genes which determine egg structure have probably been fixed by natural selection acting on the adults to which these eggs give rise, rather than on the eggs themselves.

6. *The "Tuning" of Canalized Pathways*

It seems reasonable to expect that the genetic control of canalized processes of development will affect not only the readiness with which a process is modifiable by an environmental stress, but also the precise character of the response, and thus of the end-state to which development eventually leads. Insofar as this is so, natural selection will tend to build up genotypes which not only respond to the environment, but do so in such a way as to produce a phenotype which has positive selective value.

That such theoretical predictions may be realizable in practice is demonstrated by Bateman's experiments on *Drosophila* venation described above. When the environmental stress (heat shock) was applied

to a normal wild type stock, a number of different phenotypic modifications were produced; and when any one of these (e.g., an extra cross-vein in the submarginal cell) was chosen to be favored in selection, genotypes were rapidly produced which gave rise to that modification rather than any of the others. To use somewhat picturesque language, one might say that the selection did not merely lower a threshold, but determined in what direction the developing system would proceed after it had got over the threshold.

The determination of the direction taken by a canalized pathway has been referred to as the "tuning" of it (Waddington, 1957a). The phenomenon is likely to be of particular importance for evolution when one is dealing with highly complex environmental stresses, such as those which might arise from some new manner of bodily movement, such as, to take a relatively simple example, the digging movements practiced by burrowing animals. If the responses to such stresses are to be of adaptive value, it is essential that they shall take place in a whole series of organs in a coordinated way, and this in its turn requires that the modification of each individual organ shall be precisely determined so as to fit in with the over-all pattern. In a normal above-ground mammal which takes to burrowing, the responses of its claws, fingers, arm muscles, shoulder girdle, and so on to the stresses involved will have to be delicately related to one another if the whole system is to be efficient.

In any given species, at a particular time in evolutionary history, much of this correlation is probably brought about by basic developmental mechanisms. In vertebrates at the present day, for instance, there seem to be mechanisms which ensure some basic congruity between the skeleton and muscles of an organ such as the limb, as is shown by the fact that polydactyly genes, for instance, usually produce not only extra digital bones, but the extra muscles to go with them. This is, theoretically, by no means a necessary state of affairs. For instance, in bithorax phenotypes of *Drosophila*, the metathorax which is transformed into a mesothorax does not always contain the appropriate mesothoracic muscles (Shatoury, 1956). These mechanisms, when they exist, must have been brought into being by natural selection acting in the long-past evolutionary history of the organisms, but it is not intended to discuss such "macro-evolutionary" processes in this article. However, even in an organism provided with developmental mechanisms which produce some correlations between systems which must function in harmony, something more will be necessary if the evolving organs are to be maximally appropriate for the tasks they have to perform. For instance, a developmental mechanism which ensured some degree of correlation between a limb and its girdle could hardly

be expected to produce exactly the right modification of the girdle and its musculature to suit a limb strengthened by intensive digging activities. The final "tuning" of the adaptive modification will have to be carried out by selection of genes which control the minor details of the correlations.

VI. The Baldwin Effect

Around the beginning of this century, Baldwin in America and Lloyd Morgan in England made suggestions about a type of evolutionary process which might have results very similar to those of genetic assimilation. They spoke of the process as "organic selection." After the rediscovery of Mendelism and the application of Mendelian ideas to evolutionary theory, the idea of organic selection fell rather into the background, and was scarcely discussed at all until Huxley devoted some ten lines to it in his large work of 1942. It was not until shortly after the first experimental work on genetic assimilation was published that Simpson (1953) brought it to the forefront as an alternative way of accounting for apparently Lamarckian inheritance of acquired characters.

Baldwin's and Lloyd Morgan's discussions were, of course, couched in pre-Mendelian language, and it is not entirely easy to see exactly what their meaning would be when translated into terms of our modern concepts. Most of the authors who have referred to the subject recently, however, seem to understand the theory of organic selection to be that organisms may be able, by nongenetic mechanisms, to adapt themselves to a strange environment, in which they can then persist until such time as random mutation throws up a new allele which will produce the required developmental modification. Natural selection will then increase the frequency of this new allele, so that the developmental modification, which was originally an acquired character, will become an inherited one.

The process, if understood in this sense, differs from the notion of genetic assimilation primarily because it considers the initial adaptation to the new environment to be a nongenetic phenomenon on which selection has no effect. Thus Mayr (1959) speaks of this adaptation as being due to "a nongenetic plasticity of the phenotype," and Huxley and Simpson use essentially similar phrases. This implies that natural selection is having no effect on the system until such time as a new allele appears which causes the production of the acquired developmental modification.

This theory seems to be an impossible one (Waddington, 1953b). The acquirement of an adaptive modification in response to an environ-

mental stress cannot, according to all our basic ideas of genetics, be due simply to a plasticity of the phenotype to which the genotype is quite irrelevant. The adaptive modification, like all other characters of the developed animal, must be an expression of the hereditary potentialities with which the zygote was endowed. Moreover, as we have seen in the genetic assimilation experiments described above, these theoretical considerations are borne out in practice. The ability to acquire a character is a genetic property of a strain, and can be modified by selection. If a population persists in some unusual environment by forming a suitable adaptive modification, natural selection is bound to operate on the genetic factors which control its ability to react in this way. The situation must, in fact, be one of the kind contemplated in the theory of genetic assimilation. The idea of organic selection, at least as it has been interpreted recently, cannot be accepted as a possible alternative to the genetic assimilation mechanism. It is merely an out-of-date speculation which should be allowed to lapse back into the oblivion from which Huxley and Simpson rescued it.

Only very little experimental work has been done in which the notion of organic selection was taken as a guide. However, one series of experiments was carried out by Naumenko (1941), a pupil of Schmalhausen, which should perhaps be considered to fall in this category. Naumenko produced developmental modifications in *Drosophila* by administration of a heavy dose (about 4000 r) of X-rays. He states that he used this agent both because it is known to produce developmental abnormalities and because it is a mutagenic agent which would speed up the production of random mutations. Naumenko continued the treatment for about a dozen generations on each of two lines, in one of which he selected for the development of rough eyes and in the other for that of divergent wings. In both lines there was, according to his figures, a slight increase in the frequency with which these effects were produced.

Naumenko, however, does not appear to have been looking for a gradual response to selection for ability to respond to the environmental stress in these particular ways. He was expecting to find that definite and identifiable mutations would occur which would produce rough eyes or divergent wings, and which would then be fixed by the selection. In point of fact, the result he obtained was that in both lines, after some time, mutations producing divergent wings appeared and could be fixed by selection. This happened in the stock which was being selected for rough eyes as well as in that being selected for divergent wings. Naumenko concluded that the mutations had arisen independently of the selection of the flies for the developmental modifications.

He seems to feel, nevertheless, that the experiments gave grounds for

believing that a "Baldwin effect" is a theoretical possibility. One might perhaps concede that, when one is dealing with such an extremely abnormal environmental stress as high-intensity irradiation, the process of genetic assimilation is likely to be very ineffective; one can scarcely imagine that normal populations carry much genetic variation affecting the way in which an organism's development is modified by such drastic and unnatural agencies. Selection for an appropriate type of response could, therefore, not progress very far, and the only chance for the genetic fixation of the character would be the occurrence of a new mutation. Such types of environmental stress, however, do not provide a very convincing argument for the processes which may be expected to proceed in natural evolution. So far as Naumenko's experiments validate the idea of organic selection at all, they do so only by suggesting that it might be an extreme limiting case of genetic assimilation, occurring when selection among the genes pre-existing in the population is least effective, and almost all the effect has to depend on the occurrence of new mutations.

VII. Summary and General Conclusions

The process of genetic assimilation is one by which a phenotypic character, which initially is produced only in response to some environmental influence, becomes, through a process of selection, taken over by the genotype, so that it is formed even in the absence of the environmental influence which had at first been necessary (see Section II).

The occurrence of such processes has been demonstrated in *Drosophila*, both for characters whose development involves thresholds and for others which do not (see Section III).

Genetic assimilation is brought about by the operation of orthodox genetic and embryological principles. It depends on two main types of fact: (a) that the capacity of an organism to be modified in response to an environmental stress is under genetic control, and can be altered by selection; and (b) that developmental processes exhibit a balance between tendencies to be modified by the environment and tendencies to resist modification (see Section IV).

This balance between flexibility and inflexibility can most easily be expressed in terms of the concept of "the canalization of development." This notion can be considered to follow from general principles; and special investigations of various theorems deduced from it have shown it to be fully justified, but have not shown that it depends on any single type of genetic system, such as, for example, heterozygosity (see Section V).

An alternative mechanism for bringing about similar results is the

"Baldwin effect." This supposes that the responsiveness of the organism to environmental stress is not under genetic control, and it therefore does not invoke the operation of selection in converting an "acquired" character into an "inherited" one, but suggests that this occurs as a consequence of a chance mutation. It is argued that the rejection of the genetic control of responsiveness and of the operation of selection is contrary to general genetic principles and cannot be accepted. The "Baldwin effect" is therefore not a possible general mechanism of evolution; it is, at most, no more than the limiting case toward which genetic assimilation tends when the operation of selection of the genetically controlled capacity to respond is minimally effective (see Section VI).

The main conclusions to be drawn are, not that any new fundamental genetic principles have been disclosed, but rather that it has been shown that well-accepted principles lead to evolutionary consequences quite other than those which have usually been supposed to follow from them. It has been conventional to argue, from the fact that "acquired characters" are not inherited, that the development of adaptive modifications during the lifetime of an organism is irrelevant to the evolution of similar genetically determined phenotypes. The theory of genetic assimilation, and the practical demonstration that the process can occur, shows that this argument is misguided, and provides a new way of accounting for all those evolutionary facts for which, in the past, some authors were tempted to advance a "Lamarckian" explanation. The explanation in terms of genetic assimilation is not alternative to an account in terms of random gene mutations, but is supplementary to it; and what it adds to that theory is the more important, the more one is dealing with evolutionary changes in complex organs or organ systems which must be affected by numerous genes which have to be integrated with one another (see Section V,D,6).

References

Bateman, K. G., 1956a. Studies on genetic assimilation. Ph.D. Thesis, Edinburgh University, Edinburgh, Scotland.

Bateman, K. G., 1956b. Studies on genetic assimilation. *Proc. Roy. Phys. Soc. Edinburgh* **25,** 1–6.

Bateman, K. G., 1959a. Genetic assimilation of the dumpy phenocopy. *J. Genet.* **56,** 341–352.

Bateman, K. G., 1959b. Genetic assimilation of four venation phenocopies. *J. Genet.* **56,** 443–474.

Beardmore, J. A., 1960. Developmental stability in constant and fluctuating temperatures. *Heredity* **14,** 411–422.

Beardmore, J. A., Dobzhansky, Th., and Pavlovsky, O. A., 1960. An attempt to

compare the fitness of polymorphic and monomorphic experimental populations of *Drosophila pseudoobscura*. *Heredity* **14,** 19–33.

Begg, M., 1952. Selection of the genetic basis for an acquired character. *Nature* **169,** 625.

Berg, R. L., 1959. A general evolutionary principle underlying the origin of developmental homeostasis. *Am. Naturalist* **93,** 103–105.

Bridges, C. B., and Brehme, K. S., 1944. The mutants of *Drosophila melanogaster*. *Carnegie Inst. Wash. Publ. No.* **352,** 257 pp.

Cannon, W. B., 1932. "The Wisdom of the Body." Norton, New York.

Clayton, G. A., Robertson, A., and Morris, J. A., 1957. An experimental check on quantitative genetical theory. I. Short-term responses to selection. *J. Genet.* **55,** 131–151.

Croghan, P. C., and Lockwood, L. A. P. M., 1960. The composition of the haemolymph of the larva of *Drosophila melanogaster*. *J. Exptl. Biol.* **37,** 339–343.

Dobzhansky, Th., 1955. A review of some fundamental concepts and problems of population genetics. *Cold Spring Harbor Symposia Quant. Biol.* **20,** 1–10.

Dun, R. B., and Fraser, A. S., 1959. Selection for an invariant character, vibrissa number, in the house mouse. *Australian J. Biol. Sci.* **12,** 506–523.

Falconer, D. S., 1960. Selection of mice for growth on high and low planes of nutrition. *Genet. Research, Cambridge* **1,** 91–113.

Fraser, A. S., 1960. Simulation of genetic systems by automatic digital computers. VI. Epistasis. *Australian J. Biol. Sci.* **13,** 150–162.

Fraser, A. S., 1960. Simulation of genetic systems by automatic digital computers. VII. Effects of reproduction rate and intensity of selection on genetic structure. *Australian J. Biol. Sci.* **13,** 344–350.

Fraser, A. S., and Kindred, B. M., 1960. Selection for an invariant character, vibrissa number, in the house mouse. II. Limits to variability. *Australian J. Biol. Sci.* **13,** 48–58.

Fraser, A. S., Nay, T., and Kindred, B., 1959. Variation of vibrissa number in the mouse. *Australian J. Biol. Sci.* **12,** 331–339.

Goldschmidt, R. B., 1935. Gen und Ausseneigenschaft. *Z. induktive Abstammungs-u. Vererbungslehre* **69,** 38–131.

Goldschmidt, R. B., and Piternick, L. K., 1957a. A genetic background of chemically induced phenocopies in *Drosophila*. *J. Exptl. Zool.* **135,** 127–202.

Goldschmidt, R. B., and Piternick, L. K., 1957b. A genetic background of chemically induced phenocopies in *Drosophila*. 2. *J. Exptl. Zool.* **136,** 201–228.

Goodrich, D. S., 1934. "Living Organisms." Oxford Univ. Press, London and New York.

Huxley, J. S., 1942. "Evolution: The Modern Synthesis," 645 pp. Allen and Unwin, London.

Jinks, J. L., and Mather, K., 1955. Stability of development of heterozygotes and homozygotes. *Proc. Roy. Soc.* **B143,** 561–578.

Landauer, W., 1957. Phenocopies and genotype, with special reference to sporadically-occurring developmental variants. *Am. Naturalist* **91,** 79–90.

Landauer, W., 1958. On phenocopies, their developmental physiology and genetic meaning. *Am. Naturalist* **102,** 201–213.

Lerner, I. M., 1954. "Genetic Homeostasis," 134 pp. Oliver and Boyd, Edinburgh.

Lewontin, R. C., 1957. The adaptations of populations to varying environments. *Cold Spring Harbor Symposia Quant. Biol.* **22,** 395–408.

Mather, K., 1943. Polygenic balance in the canalisation of development. *Nature* **151,** 68–71.

Mather, K., 1955. Genetical control of stability in development. *Heredity* **7,** 297–336.

Maynard Smith, J., 1960. Continuous, quantized and modal variation. *Proc. Roy. Soc.* **B152,** 397–409.

Maynard Smith, J., and Sondhi, K. C., 1960. The genetics of a pattern. *Genetics* **45,** 1039–1050.

Mayr, E., 1959. Where are we? *Cold Spring Harbor Symposia Quant. Biol.* **24,** 1–14.

Milkman, R., 1955. Unpublished Ph.D. thesis, and personal communication.

Milkman, R. D., 1960, 1961. The genetic basis of natural variation. I, II and III. Genetics, **45,** 35–48 and 377–391; **46,** 25–38.

Naumenko, V. A., 1941. Fixation of certain mutations by artificial selection of corresponding modifications. *Compt. rend. acad. sci. URSS.* **32,** 1–15.

Reeve, E. C. R., 1960. Some genetic tests on asymmetry of sternopleural chaetae number in *Drosophila. Genet. Research, Cambridge* **1,** 151–172.

Reeve, E. C. R., and Robertson, F., 1954. Studies in quantitative inheritance. VI. Sternite chaeta number in *D. melanogaster. Z. induktive Abstammungs-u. Vererbungslehre* **86,** 269–288.

Rendel, J. M., 1959a. Natural and artificial selection. *Australian J. Sci.* **22,** 22–27.

Rendel, J. M., 1959b. Canalisation of the scute phenotype of *Drosophila. Evolution* **13,** 425–439.

Rendel, J. M., and Sheldon, B. L., 1960. Selection for canalisation of the scute phenotype in *Drosophila melanogaster. Australian J. Biol. Sci.* **13,** 36–47.

Robertson, F. W., 1959. Gene-environment interaction in relation to the nutrition and growth of *Drosophila. Biol. Contribs. Univ. Texas, Publ. No.* **5914.**

Robertson, F. W., 1960. The ecological genetics of growth in *Drosophila,* I and II. *Genet. Research, Cambridge* **1,** 288–318.

Sang, J. H., and McDonald, J. M., 1954. The Production of phenocopies in *Drosophila* using salts, particularly sodium metaborate. *J. Genet.* **52,** 392–412.

Schmalhausen, I. L., 1949. "Factors of Evolution," 327 pp. Blakiston (McGraw-Hill), New York.

Shatoury, H. H., 1956. Developmental interactions in the differentiation of the imaginal muscles of *Drosophila. J. Embryol. Exptl. Morphol.* **4,** 228–239.

Simpson, G. G., 1953. The Baldwin effect. *Evolution,* **7,** 110–117.

Stern, C., 1958. Selection for sub-threshold differences and the origin of pseudo-exogenous adaptations. *Am. Naturalist* **102,** 313–316.

Stern, C., 1959. Variation and hereditary transmission. *Proc. Am. Phil. Soc.* **103,** 183–189.

Tebb, G., and Thoday, J. M., 1954. Stability in development and relational balance of X chromosomes in *Drosophila melanogaster. Nature* **174,** 1109–1110.

Thoday, J. M., 1953. Components of fitness. *Symposia Soc. Exptl. Biol. No.* **7,** 96–113.

Thoday, J. M., 1956. Balance, heterozygosity and developmental stability. *Cold Spring Harbor Symposia Quant. Biol.* **21,** 318–326.

Thoday, J. M., 1958. Homeostasis in selection experiment. *Heredity* **12,** 401–415.

Waddington, C. H., 1940a. The genetic control of wing development in *Drosophila. J. Genet.* **39,** 75–139.

Waddington, C. H., 1940b. "Organisers and Genes," 160 pp. Cambridge Univ. Press, London and New York.

Waddington, C. H., 1941. Evolution of developmental systems. *Nature* **147,** 108–110.
Waddington, C. H., 1942. Canalisation of development and the inheritance of acquired characters. *Nature* **150,** 563–564.
Waddington, C. H., 1943. Polygenes and oligogenes. *Nature* **151,** 394.
Waddington, C. H., 1948. The concept of equilibrium in embryology. *Folia Biotheoret.* **B3,** 127–138.
Waddington, C. H., 1951. The evolution of developmental systems. *Rept. Australian and New Zealand Assoc. Advance Sci.* **28,** 155–159.
Waddington, C. H., 1952a. Canalisation of the development of quantitative characters. *In* "Quantitative Inheritance," pp. 43–47. H. M. Stationery Office, London.
Waddington, C. H., 1952b. Selection of the genetic basis for an acquired character. *Nature* **169,** 278.
Waddington, C. H., 1952c. Reply to Begg, 1952. *Nature* **169,** 625.
Waddington, C. H., 1953a. Genetic assimilation of an acquired character. *Evolution* **7,** 118–126.
Waddington, C. H., 1953b. The Baldwin effect, genetic assimilation and homeostasis. *Evolution* **7,** 386–387.
Waddington, C. H., 1953c. Epigenetics and evolution. *Symposia Soc. Exptl. Biol. No.* **7,** 186–199.
Waddington, C. H., 1953d. The evolution of adaptations. *Endeavour* **12,** 134–139.
Waddington, C. H., 1954. The integration of gene-controlled processes and its bearing on evolution. *Proc. 9th Intern. Congr. Genet., Caryologia, Suppl.* **6,** 232–245.
Waddington, C. H., 1955. On a case of quantitative variation on either side of the wild-type. *Z. induktive Abstammungs-u. Vererbungslehre* **87,** 208–228.
Waddington, C. H., 1956. Genetic assimilation of the bithorax phenotype. *Evolution* **10,** 1–13.
Waddington, C. H., 1957a. "The Strategy of the Genes," 262 pp. Allen and Unwin, London.
Waddington, C. H., 1957b. The genetic basis of the assimilated bithorax stock. *J. Genet.* **55,** 241–245.
Waddington, C. H., 1958a. Inheritance of acquired characters. *Proc. Linnean Soc. London* **169,** 54–61.
Waddington, C. H., 1958b. Theories of evolution. *In* "A Century of Darwin" (S. A. Barnett, ed.), pp. 1–18. Heinemann, London.
Waddington, C. H., 1959a. Evolutionary systems: animal and human. *Nature* **183,** 1634–1638.
Waddington, C. H., 1959b. Canalisation of development and genetic assimilation of acquired characters. *Nature* **183,** 1654–1655.
Waddington, C. H., 1959c. Evolutionary adaptation. From "Evolution after Darwin. I. The Evolution of Life" (Sol Tax, edt.), pp. 381–402. Univ. Chicago Press, Chicago, Illinois; see also *Perspectives in Biol. Med.* **2** and *Vestnik Československ. Zool. Spol.* **23,** 289–306.
Waddington, C. H., 1960. Experiments on canalising selection. *Genet. Research,* Cambridge **1,** 140–150.

GENETICS OF *Habrobracon*

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	Page
I. Introduction	295
II. Sex Types	297
A. Males	297
B. Females	299
III. Sex Determination	300
A. Method	301
B. Sex Linkage	301
C. Selection Experiments	302
D. Implications of the Theory	303
IV. Cell Size in Sex Types	304
V. Cytology	306
VI. Mutants	309
VII. Mosaics	310
A. Methods of Origin	310
B. A Selected Example	311
C. Behavior of Gynandromorphs	312
D. Autonomous and Nonautonomous Phenotypes	313
E. Eye Pigments	314
VIII. Linkage Maps	315
IX. Radiations	316
A. Effects on Adults	317
B. Effects on Mature Sperm	317
C. Effects on Unlaid Eggs	321
D. Modifying Agents	326
E. Genome Number and Sensitivity	328
F. Stages at Death of Lethal-Bearing Embryos	331
G. Cytoplasmic and Nuclear Injury	334
H. Ingested Radioactive Isotopes	337
I. Irradiated and Radioactive Hosts	339
X. Nitrogen Mustard	340
XI. Colchicine	342
XII. Concluding Remarks	343
Acknowledgments	343
References	343

I. Introduction

In 1917 P. W. Whiting working as a Harrison Research Fellow at the University of Pennsylvania found a small female wasp in a culture of the Mediterranean flour moth *Ephestia kühniella* Zeller. This female

initiated years of research by Dr. Whiting and his students for he saw in her and in her readily obtainable progeny the possibility of utilizing a hymenopterous insect conveniently for genetic study. The problem of sex determination presented a challenge and the advantages of male haploidy, 10-day generation, number of progeny per female (as many as 400), and ease of handling made the prospect of conducting research on this species, *Habrobracon juglandis* (Ashmead), a tempting one. The first sentence of the first publication (P. W. Whiting, 1918), "The problem of sex-determination is nowhere of greater interest than in the Hymenoptera," was prophetic and in the years that followed much experimental work and several preliminary theories preceded its solution, for *Habrobracon* if not for Hymenoptera in general.

In breeding tests with wild type stock, virgin females produced males only, mated females both males and females, and it was concluded that unfertilized eggs developed into males, fertilized into females. Preliminary cytological studies indicated that males were haploid with an abortive first meiotic division.

Work on the wasp was discontinued for an interval, but in 1919 a female was found on the window of a grocery store in Lancaster, Pennsylvania, and, since host caterpillars were available, the study was resumed. Technique of rearing host and parasite was described by P. W. Whiting (1921a) and this has been followed with only minor changes since that time. Very thorough experimental work has been done on the biological relationships of *Habrobracon* by Hase (1922) and by Genieys (1925). Genetic results were summarized in 1947 by Martin.

This parasitic wasp, now called *Bracon hebetor* Say by entomologists, is widely cited in the literature of economic entomology under several different names as an enemy of cereal-infesting moths. The genus was named *Bracon* in 1804 by Fabricius, the species, *hebetor*, by Say in 1836. Muesebeck *et al.* (1951), in their synoptic catalogue of Hymenoptera of America north of Mexico, place it in the superfamily Ichneumonoidea, the family Braconidae, the subfamily Braconinae. Before 1922, the names *Habrobracon brevicornis* (Wesmael) and *Habrobracon juglandis* (Ashmead) were used interchangeably so that in earlier publications one species may be discussed under the name of the other. Cushman in 1922 defined the two species, and restricted the former name to that occurring in Europe which parasitizes the corn borer (*Pyrausta nubilalis* Hübner), the latter name to that found also in America which parasitizes meal caterpillars. The name *Hadrobracon* in the first papers by P. W. Whiting was a misspelling with no recognized standing, having been copied from Chittenden (1897).

The European species, *Habrobracon brevicornis* (Wesmael), has also been used for genetic research. It successfully parasitizes larvae of *Ephestia* and can be reared by the same methods as those used for *H. juglandis*. According to Muesebeck (personal communication), its name should be *Bracon brevicornis* Wesmael. Inaba (1939, 1941, 1944) carried on experiments with a strain taken in Japan, called in her publications, *Habrobracon pectinophorae* Watanabe, bred in government agricultural stations for checking the rice plant pest, *Chilo simplex* (Butler). It also breeds successfully on *Ephestia* larvae. Comparison of specimens from Japan with *H. juglandis* indicated identity of structure and crosses between them produced progeny which were fully fertile. Mutant traits in *H. juglandis* introduced into *H. pectinophorae* segregated as expected, and linkage relationships were the same for both strains in the few tests made (R. L. Cornish quoted by P. W. Whiting, 1949). In this review, *Habrobracon* will be used as a common name to refer to *H. juglandis*.

II. Sex Types

With the discovery of the first mutant, recessive orange eye color, *o*, and the derivation of a homozygous orange stock, complications arose (P. W. Whiting, 1921b). Wild type, black-eyed females (+/+) mated to orange males (*o*) gave expected results, heterozygous black females (biparental) (+/*o*) and black males (supposedly impaternate) (+). When orange-eyed females (*o*/*o*) were crossed to black males (+), 1334 orange sons (*o*) and 999 black daughters (+/*o*) were produced and, unexpectedly, 57 black sons. Black must have been brought in by sperm, and if these eggs were fertilized, why did they develop into males? Five of the unexpected black males when mated to orange females produced a few black daughters, only 5.4 per male tested. Eighteen orange males mated to orange females sired orange daughters as expected, 29.6 per male tested. No male produced more than one kind of sperm. It was concluded that females are certainly diploid and arise from fertilized eggs, but it did not follow that all males come from unfertilized eggs and the explanation of these "anomalous" results was not found until the method of sex determination had been solved.

A. Males

From unfertilized eggs the typical or characteristic type is the haploid, impaternate male. These are all alike from homozygous mothers and approximate gametic ratios from heterozygous mothers. If females are mated, about one-third of the eggs are unfertilized and haploid-male producing. About two-thirds are fertilized. The sex of diploid offspring

depends upon the relationship of the parents. If the mother is "out-crossed" to an unrelated haploid male, all diploids are female but if she is "close-crossed" to a related haploid male, about half of the zygotes are female-producing and half potentially male-producing. These males are highly inviable. Many die as embryos and "bad" eggs from close crosses are numerous. First encountered as the black sons of orange females by black males, they were designated "patroclinous," for it was difficult to think of them as completely diploid with the preconceived idea of male haploidy, female diploidy (A. R. Whiting, 1925). The majority of the patroclinous males were found to be sterile, some were partially fertile, and bred as black. In none was fertility comparable with that of haploid males. Were these males androgenetic with sperm chromosomes only or biparental, haploid or diploid?

With the use of new recessive mutations, they could be tested genetically for diploidy, and later in 1925 (P. W. Whiting and A. R. Whiting, 1925) it was announced that they inherit from both parents factors modifying the same structure. Thus, two recessive wing mutations, one from each parent, reconstituted the wild type wing in black-eyed males as in their sisters while their orange-eyed brothers possessed the maternal wing character. As more mutations were found, evidence was consistent for diploidy in respect to every chromosome tested (A. R. Whiting, 1927, 1928; Torvik, 1931). Even when extremely heterozygous, these males always breed as dominants, producing one kind of sperm (all diploid), since in them, as in their haploid brothers, there is but one meiotic division in spermatogenesis, and that equational. The explanation of the occurrence of diploid males will be discussed in Section III on sex determination.

A. R. Whiting observed that no reduction in the number of gynogenetic offspring, haploid males, occurred when females were mated to the almost completely sterile diploid males, and that either these males produced very few active sperm or the sperm failed to enter the eggs (quoted in P. W. Whiting and Anderson, 1932). In special tests (MacBride, 1946), fifteen diploid males were mated in forty-two crosses and all were sterile. Nineteen females mated to diploid males were dissected and their seminal receptacles studied. Five were full of sperm, seven had very few, and seven had none. The living sperm resembled those of haploid males, but when eggs laid by females mated to diploid males were studied, only one showed a possible but questionable sperm nucleus. It was suggested that failure of penetration of sperm was the cause of sterility.

Diploid males were found in *H. brevicornis* (B. R. Speicher and K. G. Speicher, 1940) and in *H. "pectinophorae"* (Inaba, 1939). In the

former, their survival rate, diploid males/females, was 0.666, in the latter, 0.003 to 0.060. In *H. juglandis,* it ranged from 0.017 to 0.399 (Grosch, 1945).

Triploid males would be expected from crosses of diploid males to related females but none has been observed. In view of the high percentage of sterility in diploid males and the possibility of triploid males being missed during the early work, the fact that none has been identified is not conclusive evidence against the possibility of their occurrence.

A male was found by Clark which had paternal traits only (P. W. Whiting, 1943c). His mother was homozygous for the linked genes for honey and black body color and cantaloup eyes, $\underline{bl + + c\ ho +}/\underline{bl + + c\ ho +}$, his father had the alleles including genes for lemon body color, long antennae and legs and veinless wings, $\underline{+ le\ l + + vl}$. The exceptional male was obviously androgenetic. When virgin type females are X-rayed and then mated to recessive males, some androgenetic males appear (A. R. Whiting, 1946). Subsequent study of these males and their significance will be discussed in the section on radiation effects.

B. Females

Normally, females come from fertilized eggs and are biparental. In 1924 P. W. Whiting reported the occurrence of several exceptional females from virgin mothers. This was not observed again until 1930, and in 1934 a detailed report on them was made (K. G. Speicher, 1934). It was noted that when females from tapering antennae stock (*ta*) were outcrossed, some of their unmated daughters produced an occasional female. Such impaternate females were diploid, of normal morphology, viability, and fecundity. Summaries of seven loci studied showed that in forty-five cases the females were heterozygous while in thirty-nine they were homozygous for one or the other of the alleles carried by their heterozygous mothers, a 1:2:1 ratio. The constitution of the parental male as well as of the female affects the ratio of F_2 impaternate females. Their ratios increase with increasing age of mothers. Heredity obviously plays a part in their production since they occur only after crossing of certain strains.

The hypothesis was offered that these diploid impaternate females might be produced by the suppression of the second maturation division in the unfertilized egg or by the fusion of two reduced egg nuclei. The consistent occurrence of homozygotes and heterozygotes, indicating a 1:2:1 genotypic ratio for all loci tested (except those for sex and the sex-linked gene, fused), was at variance with the results to be expected according to these hypotheses. If the first division were reductional at the centromere, homozygosis of any locus would vary with its distance

from that point and a 1:2:1 ratio would not occur consistently for each pair of alleles.

It was realized from the beginning that other hypotheses could be advanced and one of these was the possibility that each set of homologous chromosomes of an impaternate female came from a separate group of tetrads, two primary oöcyte nuclei, both completing meiosis within a single egg. In a study of hundreds of individuals, K. G. Speicher and B. R. Speicher (1938) demonstrated cytologically that some unfertilized eggs in stocks which gave rise to these females were tetraploid before reduction and, therefore, diploid after reduction. No evidence of binuclearity or of a suppression of one meiotic division was found, but the first meiotic division in some eggs showed 20 dyads going to each pole. These cytological data and the fact that the impaternate females come in groups suggest that patches of tetraploid ovarian tissue are responsible for their occurrence. The behavior in meiosis appeared to be consistent with that of allotetraploids, synapsis of pairs and homologs. Possibly, at the time of synapsis, oöcytes were binucleate and nuclei fused in some later stage. The absence of impaternate diploid males which should, theoretically, occur in numbers equal to those of impaternate females, can be explained by their high inviability.

About 10% of diploid males are fertile. They produce few daughters, 1 to 6 each, and these are triploid. Tests were made of 34 triploid females (Bostian, 1936). Of their 2023 eggs, 32 hatched, 12 pupae were formed and 8 adults emerged, 2 males and 6 females. In controls from 17 diploid females, of 4790 eggs laid, 3884 hatched, 3789 pupae were formed, and 3491 adults emerged. Aneuploidy undoubtedly was responsible for the low fecundity of triploid females.

Inaba (1939) obtained 187 daughters of diploid males in *H. "pectinophorae."* All showed the dominant characters of their fathers and many were normal in appearance. Others were asymmetrical and irregular morphologically. Each triploid female stung caterpillars and laid many eggs. Of these, 14 hatched and 10 developed into adult males. In oögonial cells of triploid females, haploid, diploid, or triploid complexes were observed.

III. Sex Determination

Of the sex types in *Habrobracon*, the males from fertilized eggs were the most difficult to account for. When it was shown in 1925 (P. W. Whiting and A. R. Whiting) that the "patroclinous" males were really of biparental origin and diploid, search was made by genetic techniques for an odd sex chromosome or a differential sex-determining region in one chromosome pair. Cytological study (Torvik-Greb, 1935) indicated

no visible difference between the 10 pairs of chromosomes of diploid males and females. According to the principle of genic balance, under any conditions of euploidy, the haploid complex would be merely duplicated, and there should be one sex only. In view of these uncertainties, the central problem for several years was that of sex determination. Results are reviewed in detail by P. W. Whiting (1940, 1943a).

A. Method

In 1933 the hypothesis of complementary sex determination was suggested (P. W. Whiting) and, with the evidence for consistent sex linkage, proved to be the explanation. In brief: there are different kinds of haploid males, similar in appearance but containing different sex genes, an allelic series in which nine have been identified, *xa*, *xb*, *xc*, etc. Any combinations of two different alleles, *xa/xb*, *xc/xd*, etc. produces a female. Diploid males are homozygous for the sex alleles, *xa/xa*, *xb/xb*, etc. Since two-thirds of the eggs laid by mated females of this species are fertilized, one-third of the potential progeny of any type of cross consists of haploid males, half *xa*, half *xb*, for example, indistinguishable in appearance. From a "close-cross"—or better—two-allele cross, $xa/xb \times xb$, one-half of the eggs fertilized (heterosyngamy) develop into females, *xa/xb*, one-half (homeosyngamy) are potentially diploid male-producing, *xb/xb*. From an "out-cross"—or better—a three-allele cross, $xa/xb \times xc$, all fertilized eggs (heterosyngamy) develop into females, *xa/xc*, *xb/xc*. The nine alleles identified permit nine kinds of males, haploid or diploid, and thirty-six kinds of females, $(n^2 - n)/2$. Triploid females have two alleles identical and, presumably, the third different.

B. Sex Linkage

The sex-differentiating alleles were shown to be linked with the gene fused, *fu*, affecting antennae, legs, and wings, and the two loci are ten units apart. Crosses of females heterozygous for fused by fused males will illustrate the method whereby the theory is proved to be correct. The females are made homozygous for a recessive gene so that their haploid sons can be identified. Table 1 outlines the results to be expected. From two-allele crosses, progeny show sex linkage (A and B), from three-allele crosses, no sex linkage (C). Large numbers of tests have checked with these expectations.

Impaternate daughters of females heterozygous for fused, *fu*, were always heterozygous for fused (K. G. Speicher and B. R. Speicher, 1938), no homozygotes appearing. They would be expected in only 18% of females, 9% type and 9% fused.

Inaba (1944) found in *H. "pectinophorae"* a mutant gene, ebony body color, which was sex-linked and behaved in the same manner as fused. The crossing-over value between ebony and the sex-determining

TABLE 1
Kinds and Ratios of Progeny Expected from Two Two-Allele Crosses, A and B, and a Three-Allele Cross, C*

Eggs and Haploid ♂ ♂	Sperm A + *xa fu*	Sperm B + *xb fu*	Sperm C + *xc fu*
9 *vl* *xa* +	9 + diploid ♂ ♂	9 + ♀ ♀	9 + ♀ ♀
1 *vl* *xa fu*	1 *fu* diploid ♂ ♂	1 *fu* ♀ ♀	1 *fu* ♀ ♀
1 *vl* *xb* +	1 + ♀ ♀	1 + diploid ♂ ♂	1 + ♀ ♀
9 *vl* *xb fu*	9 *fu* ♀ ♀	9 *fu* diploid ♂ ♂	9 *fu* ♀ ♀

* The mothers are veinless and heterozygous for fused (*vl*/*vl* *xa* +/*xb fu*), the fathers, fused (+ *xa fu*, + *xb fu*, and + *xc fu*, respectively).

factor ranged from 13 to 33%. The sex linkage of ebony entirely disappeared in three-allele crosses but reappeared by inbreeding, even directly after outcrossing, thereby favoring the multiple allele rather than the multiple factor theory to be discussed below.

C. Selection Experiments

Three theories supplementary to that of complementary sex determination were suggested to explain the observed facts concerning close-crosses and outcrosses: (1) selective fertilization (P. W. Whiting, 1935a), (2) multiple factors (Snell, 1935), (3) multiple alleles (P. W. Whiting, 1940). In the first theory it was suggested that of the two kinds of reduced nuclei in the egg, only those unlike the sperm nucleus could effect syngamy if the sperm were from an unrelated male. B. R. Speicher (1936) soon demonstrated that of the four potential nuclei resulting from maturation in the egg, only one is actually functional. The other chromosome groups quickly degenerate without forming nuclear membranes.

According to Snell's theory of multiple factors, the *xa*/*xa* (or *xb*/*xb*) zygotes are female-producing if heterozygous for other sex-determining genes, *za*/*zb*. Thus sex differentiation in some inbred lines might be by *xa*/*xb*, but if by *za*/*zb*, fused would be nonsex-linked and females + *xb*/*fu* *xb* *za*/*zb*. Bostian (1939) proved this theory untenable and obtained the first proof of multiple alleles. He closely inbred for twenty generations his previously unanalyzed stock. It must have contained

three sex alleles. Females heterozygous for fused were always crossed to fused males (brothers in the last eleven generations), both sexes selected from fraternities with no diploid males, and with fused and nonfused females present in equal numbers (Tables 1C, 2). In spite of this selec-

TABLE 2

Plan of Selection Experiments in Which Nonfused Daughters and Fused Sons from Fraternities with no Diploid Males (82% Expected) Were Used for Breeding *

	Fused sons	
+ Daughters	1 *xa fu*	9 *xb fu*
9 *xa* + / *xc fu*	9	81
1 *xb* + / *xc fu*	1	9

* This was continued for twenty generations during which fraternities continued to appear showing diploid males and sex linkage of fused (18% expected). Original cross *xa* + / *xb fu* ♀♀ × *xc fu* ♂♂.

tion from fraternities in which segregation was of the nonsex-linked pattern, it was impossible to obtain a strain breeding pure for independence of fused and sex. A few fraternities were always of the backcross or two-allele type (Table 1A, 1B), showing sex linkage of fused, and these were the only ones containing diploid males. This not only disproved Snell's theory but strengthened the multiple allele theory showing that the fused-linked gene was still acting as the sex differentiator.

Horn (1943) conducted an experiment somewhat similar to that of Bostian but used two stocks with different sex alleles, four in all. Her results demonstrated that F_2 males segregating from crosses of *xa/xb* stocks with *xe/xf* stocks were still *xa*, *xb*, *xe*, or *xf* since they all sired diploid sons, one-half with *xa/xb* females and not with *xe/xf*, and one-half with *xe/xf* but not with *xa/xb*. Sex linkage of fused was shown in the progeny of each male in which diploid sons were sired but not in the progeny lacking diploid sons.

The detailed genetic map of the sex chromosomes is given in Section VIII.

D. Implications of the Theory

P. W. Whiting (1943a) regards the multiple sex alleles, *xa*, *xb*, etc., as differential chromosome segments which have been built up in the early evolution of the Hymenoptera and which are necessarily associated with haploid parthenogenesis. They consist of many factors determining the numerous sex differences, structural, functional, and behavioral,

characterizing the genus. These factors have, in the aggregate, duplicate effects such that all haploids or homozygous diploids are similar and male, but combinations of any two different alleles result in females, dominants, and heterozygotes for many factors. The terminology used, *xa, xb,* etc., is a convenient one, each symbol being a simple expression for what is undoubtedly a chromosome region with many factors, some dominant, some recessive, and all so close together as to be completely linked. Subsequent research on other superfamilies of Hymenoptera indicates that for them the theory will need some modification.

IV. Cell Size in Sex Types

Diploid males can be distinguished from haploid males even in the absence of genetic markers. The most easily identified difference is the "roughness" of the wings in the former due to their larger cells which are measured by counting the microchaetae within a given area on the upper surface of the wings, each microchaeta corresponding to a single cell (B. R. Speicher, 1935; Grosch, 1945). Grosch used six inbred stocks, three of which had recessive eye colors to serve as markers for the identification of the haploid male classes. Each recessive stock had the same sex alleles as one of the wild stocks. Crosses were *xa/xi* × *xa* or *xi* (*ai*); *xe/xf* × *xe* or *xf* (*ef*); *xg/xh* × *xg* or *xh* (*gh*). Length of animal, wing size, eye size, antennal flagellar and segment lengths, and eye facet size were measured, the last by Risman also (1942).

TABLE 3

The Correlation of Means of Wing Cell Size * and Ratio of Diploid Males to Females in the Three Kinds of Crosses Used †

Sex alleles	Mean number of microchaetae			Diploid ♂♂ / ♀♀
	Diploid ♂♂	Haploid ♂♂	♀♀	
ai	9.62 ± 0.07	16.33 ± 0.10	15.57 ± 0.10	0.3992 ± 0.0211
ef	9.35 ± 0.08	14.41 ± 0.11	14.09 ± 0.11	0.1116 ± 0.0082
gh	9.27 ± 0.10	13.14 ± 0.12	13.56 ± 0.13	0.0167 ± 0.0015

* Measured by michrochaetae per 0.01 sq mm.
† From Grosch, 1945.

The most striking difference, wing cell size, will be discussed here. For all three sex types, relative cell sizes were consistent within the three kinds of crosses (Fig. 1), smallest in *ai*, intermediate in *ef*, and largest in *gh*. There was negative correlation between cell size and survival frequency for diploid males—the smaller the cells, the higher

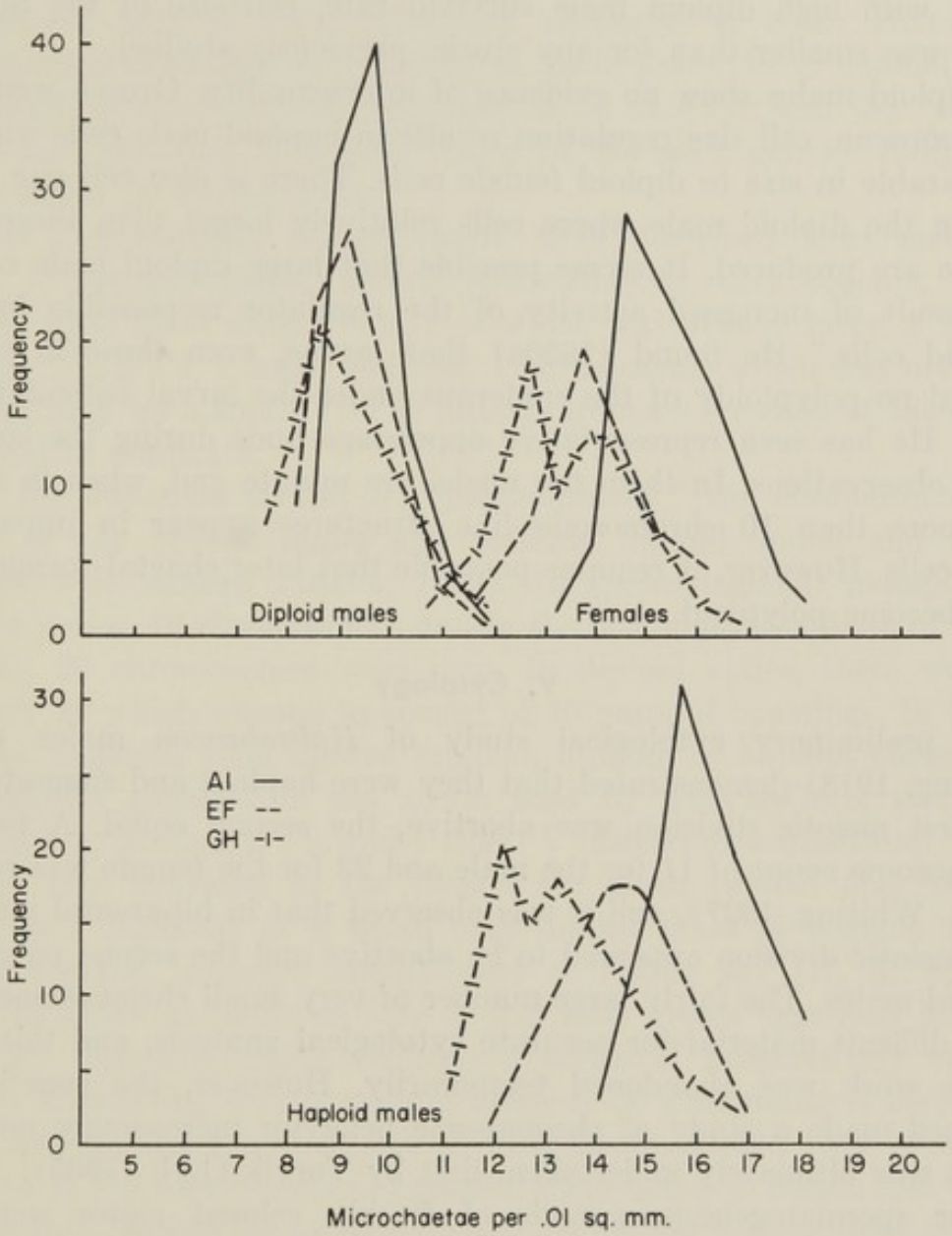


FIG. 1. Frequency arrays of microchaetae per 0.01 sq mm of radial region in right primary wing from the three sex types among offspring of two-allele crosses between pairs of inbred stocks with same sex alleles, *xa/xi* (*ai*), *xe/xf* (*ef*), and *xg/xh* (*gh*). The arrays include counts from 100 specimens of each of the nine types. Average area covered by an epidermal cell may be obtained in square micra by dividing 10,000 by the number of microchaetae corresponding to any given frequency. (From Grosch, 1945).

the survival rate (Table 3). When mean numbers of microchaetae per 0.01 sq mm were compared, it was clear that greater uniformity existed for diploid males from the three kinds of crosses than for the other classes (Table 3). Data indicate either lower production of extreme deviates in diploid males or more extreme selection against deviates in them than in the other sex types. Mitchell (1948) noted that within a

strain with high diploid male survival rate, cell size of the three sex types was smaller than for any stocks previously studied.

Diploid males show no evidence of intersexuality. Grosch writes, "In *Habrobracon,* cell size regulation results in haploid male cells which are comparable in size to diploid female cells. There is also cell size regulation in the diploid male where cells relatively larger than those of the female are produced. It seems possible that large diploid male cells are the result of increased activity of the regulator responsible for large haploid cells." He found (1950a) that larvae, even those in cocoons, showed no polyploidy of the epidermis under the larval cuticular structures. He has seen representative appendage buds during the course of other observations. In them the nuclei are minute and, when in mitosis, not more than 10 chromosome-like structures appear in impaternate male cells. However, it remains posssible that later chaetal-forming cells may become polyploid.

V. Cytology

A preliminary cytological study of *Habrobracon* males (P. W. Whiting, 1918) demonstrated that they were haploid and suggested that the first meiotic division was abortive, the second equal. A tentative chromosome count of 11 for the male and 22 for the female was reported (A. R. Whiting, 1927), and it was observed that in biparental males the first meiotic division appeared to be abortive and the second equal as in haploid males. The fairly large number of very small chromosomes made them difficult material for accurate cytological analysis, and this aspect of the work was abandoned temporarily. However, the genetic data obtained made a study of chromosome behavior increasingly necessary and it was ultimately undertaken, first by Torvik-Greb (1935).

For spermatogenesis, gonads of freshly eclosed males were used since they showed the highest percentage of cells in active division. Good mitotic figures of spermatogonia were rarely seen but enough were found to offer convincing proof of the fact that normally there are 10 chromosomes in each of these cells of the haploid male. In later stages of the first spermatocyte the cells were described as pear-shaped in preparation for the abortive first meiotic division. Ultimately, a small cytoplasmic bud appeared to be cut off at the narrow end of each pear-shaped cell. Best slides showed consistently 10 chromosomes in the "first" spermatocyte. In the "second" spermatocyte division the cytoplasm divided equally, and each chromosome divided equationally so that two functional and genetically identical sperm were formed from each primary spermatocyte.

Observation of spermatogenesis in diploid males showed that it followed the same course as in the haploid males. The cells were larger but

cell growth and division proceeded similarly in both. Homologous chromosomes exhibited no tendency to lie in pairs. As in haploid males, the "first" meiotic division appeared to be abortive and the "second," equational, resulting in diploid sperm of the same genetic composition as the males producing them.

The chromosomes, though small, were described from metaphase plates in spermatogonia, second spermatocytes, and oögonia. In the haploid male there were 4 V's, 1 of which was always large, 2 medium to large L's, 2 J's, 1 small rod, and 1 chromosome which was often rod-like but may be a small V. The diploid groups have each of these chromosomes in duplicate.

In the Japanese strain, *H. "pectinophorae,"* the chromosome complexes of the normal males and females, diploid males, and triploid females were studied (Inaba, 1939). In spermatogonial metaphase of haploid males, 10 chromosomes and in the oögonial metaphase of normal females, 20 chromosomes were seen. In diploid males, there were apparently 20 which seemed to consist of 10 pairs of homologs. In triploid females, oögonial cells showed triploid, diploid, or haploid chromosome numbers. In contrast to the strain used by Torvik-Greb, there were 5 V's, 2 L's, perhaps 2 J's, and 1 small V instead of a small rod.

The four ovarioles in the female are much twisted and recurved. Each ends cephalad in a small club-shaped mass of oögonia and, in gravid females, enlarges caudad into a uterine sac (B. R. Speicher, 1936) in which as many as four or five fully grown eggs may be stored (Fig. 2). The mid-region contains a series of progressively enlarged oöcytes alternating with masses of nurse cells. The youngest oöcytes are small and almost spherical but as they move caudad they increase in size and in the uterus each is approximately 450 μ in length and cucumber-shaped. During the growth period the oöcyte nucleus does not show the Feulgen reaction, and an analysis of the chromosome structure and behavior in these stages cannot be easily made with that technique.

The first maturation division of the egg is initiated before oviposition. Chromatin elements appear, at first elongate and granular, and always in close contact with the periphery of the nucleus. The elements then quickly shorten, still in contact with the nuclear membrane. Ten can be clearly counted. With disintegration of the nuclear membrane, chromosomes move to the center of the nuclear area and become arranged on a flat metaphase plate. A spindle forms at the same time. Speicher describes the most advanced eggs in the uterus as in early anaphase, but since the chromosomes have not completely separated, this author prefers to speak of this stage as late metaphase. Further progress in maturation is stopped until after oviposition.

The second maturation metaphase appears 20 to 30 minutes after

oviposition and, within an interval of 5 minutes after this, all chromatids separate from their partners, forming four chromatin groups. The innermost group becomes the functional egg nucleus. The polar nuclei have never been observed to form membranes, and no membranes develop between meiotic divisions.

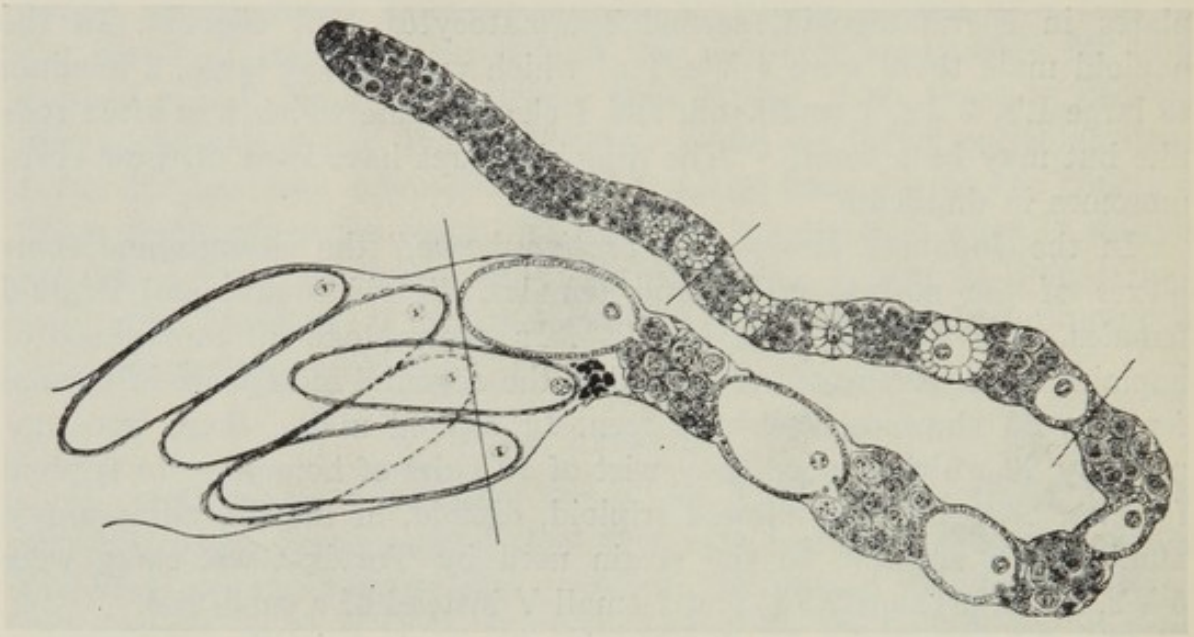


FIG. 2. A single ovariole. Uterine oöcytes are in first meiotic metaphase and the nurse cells associated with earlier stages have disappeared. Oögonia undergoing mitosis are in the anterior end of the ovariole.

Cleavage is approximately synchronous throughout the egg except for the dropping out of primary germ cells and occasional cleavage nuclei. At least until the eleventh cleavage there is no doubling in chromosome number in either fertilized or unfertilized eggs nor is there any evidence for chromosome elimination.

B. R. Speicher and K. G. Speicher (1940) found the chromosome numbers to be the same for *H. brevicornis* as for *H. juglandis.*

The formation of a cytoplasmic bud and its pinching off during the first spermatocyte abortive division in hymenopteran males have been taken for granted by most students of this group. The phenomenon has often been cited as an example of retention of the ancestral habit of undergoing two meiotic divisions. In some hymenopteran forms this has not been observed, and Walker (1949) undertook to study these "buds" in the sawfly *Pteronidea ribesii.* She believes that they can best be interpreted as the result of the "sticky" property of the interzonal body, that they are remnants of cytokinesis interfering with the completion of the last spermatogonial division, and that they have not the evolutionary significance commonly attributed to them. Earlier studies were made

from thin sections, while the use of thicker sections and of the newer smear techniques makes more accurate observation possible.

B. R. Speicher has checked the process in thick sections of *Habrobracon* testis, 20 μ. He observed that in these the spermatocytes appear attached to each other in clusters by heavy cytoplasmic strands, and he believes (personal communication) that these strands, and all fragments which appear when strands are cut off at various angles, give the impression of "buds" if the sections are cut 4–6 μ thick. What had appeared as centrioles in the bud are pretty certainly chondriosomal strands seen end on, for they show mainly following chondriosomal fixation and staining. Furthermore, as negative evidence, Speicher has never been able to arrange his fifty or more drawings of spermatogenesis into two separate prophases, let alone other stages, and he is fairly convinced that Walker is correct.

VI. Mutants

Recessive mutations, visible or lethal, are identified with special ease in *Habrobracon* because every chromosome acts as does the X in *Drosophila*. A visible mutation, dominant or recessive, appears at once in a haploid male if the mutant gene was present in the egg from which he developed, and in one-half of the haploid progeny of a female into whose composition has gone either an egg or a sperm nucleus with a mutant gene. Lethal mutations will be discussed in the section dealing with radiation effects.

By 1935 ninety visible mutations had been described (P. W. Whiting, 1932a, 1934b, 1935b), 44% X-ray induced, and about 90% recessive. During the succeeding years many have been added to these. They can be divided into those affecting size, pigment, morphology (most frequent), behavior, and sex. Some loci have multiple alleles, the orange (eye color) locus with five. Several affect eye or body color. Changes in antennae, in legs, and in wings have been fairly common. Behavior differences, slow motion (*sm*) and nervous (*ne*), were conspicuous because of the barely perceptible movements made by the former and the constant twitching of the antennae in the latter (A. R. Whiting, unpublished). These were completely recessive to type. Bunker and Speicher (1951) devised tests for checking accurately on the behavior differences in stock #33 of *H. juglandis* and the stock of *H. "pectinophorae."* Individuals of the former are sluggish and easily handled without etherization, of the latter, very active and unpredictable in their activities. Lots of ten were timed as they passed through a dark tube illuminated at one end, the number emerging at one-minute intervals tabulated. The average time for #33 females was 5.53 minutes; for

#33 males, 6.41 minutes; for *H. "pectinophorae"* females, 2.97 minutes; males, 3.44 minutes. This kind of difference can be observed between stocks #33 and #17-o^i, both *H. juglandis*. The latter fly erratically about, taking off unexpectedly after simulating death, whereas the former show no trace of this behavior. Sluggishness is recessive here too.

Intersexes have occurred by mutation. A single mutant male found in a bisexual fraternity illustrates weak male intersexuality (P. W. Whiting *et al.* 1934). His antennae and anterior abdominal tergites and sternites were like those of the female. Genitalia and instincts were of normal male character and he was fully fertile, his daughters being normal in appearance and fertile. One-half of their sons resembled him. Superficially, these males suggested gyandromorphs, but the bodies were symmetrical with all parts presumably of the same genetic constitution, and the type was perpetuated as a true breeding form and designated as gynoid (*gy*).

A single kind of intersex has appeared three times, 64 individuals in all (P. W. Whiting, 1943b; P. W. Whiting and R. Starrells, 1950; von Borstel and Smith, 1960). Each had large male ocelli, long male antennae, and female abdomen. Seminal receptacles were present and in some instances contained sperm. Although the instincts of the intersexes were male, normal brothers had mated with some of them. Ovaries were small and filled with undifferentiated cells resembling oögonia. Their wing cells resembled those of haploid males. Two of the mothers were virgin and the third presumably so, and each was heterozygous for an allele in the orange locus, $+/o$ or $+/o^i$. Their origin is interpreted by von Borstel and Smith as due to the mutation of a gene concerned with sex which occurred once spontaneously and once in the X-rayed +-bearing chromosome and once in an X-rayed o^i-bearing chromosome. In each case it proved to be linked with the orange locus with about 26% recombination. Whiting called them intersexual females, in spite of their apparent haploidy, because of the presence of ovaries, poison apparatus, and female genitalia, segmentation of antennae (male) occurring late in development after the turning point in sexual development.

VII. Mosaics

A. Methods of Origin

Mosaics appeared early in the experimental work on *Habrobracon*. In the paper reporting the first visible mutation, orange eyes (*o*), (P. W. Whiting, 1921b), 7 males with black eyes from crosses of orange females by black males bred as orange (47.4 daughters per male), and 1 orange male produced only black daughters (19). Sperm had entered these eggs

and, in the former cases had contributed to the formation of the head, in the latter, to the gonads. From our present information, it is clear that paternal parts may have been either haploid and androgenetic, or diploid and homozygous for the sex allele involved.

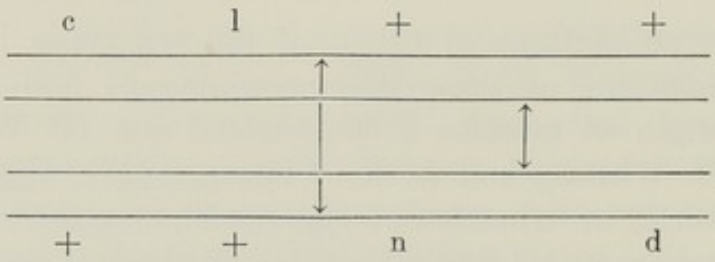
Since 1921 several hundred mosaics have been found. All can be classified into mosaic males, mosaic females, and gynandromorphs. The majority identified have been produced by unmated, heterozygous mothers during linkage tests so that these mosaics are male and haploid. In *Drosophila* most mosaics can be interpreted as due to chromosome elimination or deletion, a few to binuclearity of the egg, but chromosome elimination or gene deletion cannot be the basis of mosaic formation in haploid males. A few mosaics from fertilized eggs can be interpreted as in *Drosophila*. However, the phenomena now known to occur in *Habrobracon* reproduction permit an explanation other than chromosome or gene loss in every case.

When one considers the possibilities of origin of the sex types in themselves, kinds of combinations of these become numerous and a discussion of methods of origin of mosaics a complicated one (P. W. Whiting, 1931, 1932b; P. W. Whiting and A. R. Whiting, 1927). Eggs may be uninucleate, binucleate, or trinucleate; egg nuclei, haploid or diploid; no sperm, one, or two may be involved, although the last condition is rare since dispermy occurs in 1% of fertilized eggs only. Diploid egg nuclei or those resulting from fertilization may be heterozygous or homozygous for sex alleles, producing female or male parts respectively. Haploid regions may be either maternal or paternal in origin. When two or three haploid nuclei of maternal genotype are involved in mosaic formation, the question of their origin arises. A mutant stock derived from an X-ray experiment in which there occurred a relatively high incidence of mosaicism has been studied by von Borstel (1957). Females heterozygous for the semidominant lemon body color, a pupal lethal, produced occasional haploid males mosaic for the autonomous body colors. The lethal effect of the mutated gene was nonautonomous, for males would survive with only a small patch of wild type tissue. Cytological study of uterine oöcytes in mothers of mosaics showed in some a continuation of meiosis in the position of the normally blocked metaphase I nucleus. This suggested that removal of the block to meiosis enabled the products of meiosis to escape disintegration in the egg interior so that two or more could take part in development of the embryo.

B. A Selected Example

Several mosaics with two or three nuclei contributed by the egg have, because of the heterozygosity of the mothers for linked genes, demon-

strated that the chromosomes involved in the mosaicism can have come from one tetrad, and no exception to this has been found. No evidence against polar nuclei as the source of the additional nuclei has been obtained, therefore. An example found by Gilmore and reported by P. W. Whiting (1934a) was produced by a virgin female heterozygous for the linked genes, cantaloup eyes (*c*), long antennae (*l*), narrow wings (*n*), and defective venation (*d*). This mosaic male had type antenna and eye on the left side of the head, long and cantaloup on the right. Left wings were narrow, right defective. Gonads were genetically stratified for he bred as cantaloup, long, defective, then as type and, finally, as cantaloup, long, defective again. From the meiotic divisions of a single tetrad, three chromatids can be produced which will explain the mosaic. Genetic stratification of the gonads shown in the diagram is a rare occurrence.



c	l	n	d	not included
c	l	+	d	right side and gonad
+	+	+	+	gonad
+	+	n	*+	left side

C. Behavior of Gynandromorphs

In *Habrobracon*, clear-cut differences characterize the sexes. Males are primarily interested in mating and, on being introduced to females, run about excitedly flipping their wings. Stimulation is apparently by odor perceived largely by the antennae. Males that are actively stimulated by warmth and light and are in a vial with no females may attempt to mount each other. They are entirely indifferent to host caterpillars. Females may refuse to mate or may accept the males but they show no positive preliminary reaction to males or females. A female coming into the vicinity of a caterpillar assumes an aggressive attitude, thrusting her abdomen downward and forward, protruding her sting. If the caterpillar remains quiet, she will insert her sting in any part of the host's body, causing paralysis. She feeds subsequently at the puncture made with her sting, and when her eggs are mature, she deposits them under the paralyzed host.

Observations on fifty gynandromorphs (P. W. Whiting, 1932d) showed that, in general, reactions are determined by the head, regardless of the sex of the abdomen. Gynandromorphs with mixed heads may be either male or female in behavior. Instances of atypical behavior were classified as "nonstop," in which an individual will be stimulated by females but fail to mount, running back and forth repeatedly; "momentary reversal," in which response characteristic of one sex is shown by an individual with instincts in general of the opposite sex; "bisexual," in which the same individual gives female reactions toward caterpillars and male reactions toward females; "wires crossed," in which mating response is given toward a caterpillar or stinging reaction toward a female.

Because of the conflict between behavior and potential reproductive function, only four fertile gynandromorphs have been found (P. W. Whiting and Wenstrup, 1932). Two functioned as males and two as females. Female parts of the majority of gynandromorphs have been assumed to be biparental, male parts maternal. Breeding tests of the two functioning as females showed that their ovaries were heterozygous. The gonads of the two breeding as male were of maternal origin.

D. Autonomous and Nonautonomous Phenotypes

In the majority of mosaics in *Habrobracon,* each genetically different area is autonomous, separated from the adjoining one by a sharp line. In eyes mosaic for any two of the alleles in the orange series, however (wild type, dahlia, orange, and ivory), there is interaction, a gradual shading from the darker to the lighter part with no clear boundary to indicate where cells of one genotype stop and others begin. There is, then, in such haploid mosaics, some phenotypic "diploidy." Cantaloup (*c*), an eye color gene in another locus, is strictly autonomous in mosaics with its wild type allele (+), the line of division following facet boundaries (P. W. Whiting, 1932c).

A female heterozygous for ivory and for cantaloup eye colors ($+/o^i$ $+/c$) produced, in addition to sons of the four genotypes regularly expected, a male with ivory not-cantaloup (o^i+) and not-ivory cantaloup ($+\,c$) regions in the same eye (A. R. Whiting, 1934). No genotypic wild type tissue was present, but the genetically ivory part bordering the cantaloup was wild type black, a phenotypic complementary effect due to diffusion into the ivory part of some product dependent upon the presence of the dominant allele to ivory in the cantaloup region which remained autonomous with no diffusion in the opposite direction.

Similarly, feminization occurs in a high proportion of haploid mosaic males when the line of division passes through the genitalia. The physical change is always on one side of the line separating the genotypically

different regions and resembles the complementary behavior in the ivory-cantaloup eye mosaics described above where phenotypic wild type appears. Here we have phenotypic femaleness in genotypically male regions. The characteristic structure produced is a small protuberance suggesting a female gonapophysis. A sting may also be present (P. W. Whiting *et al.*, 1934) and such individuals have been designated as "gynandroid."

It was postulated (P. W. Whiting, 1933) that these mosaic males are composed of two types of tissue, each recessive for a different sex factor, the dominant alternatives to both of which are necessary for femaleness. One type of tissue may contain *A* and *b*, the other *a* and *B*. Some substance from one side diffuses to the other, producing phenotypic femaleness in genotypically male cells and regions. This furnished the clue to the sex-determining mechanism described in Section III.

E. Eye Pigments

The mutation from wild type to orange and to ivory, an allele of orange, resulted in the absence of some substance in the mutants which is present in wild type, and this is diffusible as demonstrated in mosaic eyes. The mutation from wild type to cinnabar in *Drosophila* in the same manner involved the loss of a diffusible substance. Reciprocal injections and feedings of wild type extracts between *Drosophila* and *Habrobracon* larvae or pupae gave a reasonable basis for postulating that the substance deficient in orange or ivory is the same as that deficient in cinnabar (Beadle *et al.*, 1938). Extracts of wild type *Drosophila* (+) modified orange (*o*) and ivory (o^i) eyes of *Habrobracon* in the direction of wild type whereas extracts of cinnabar (*cn*) had no effect. Extracts of wild type (+) *Habrobracon* modified cinnabar (*cn*) eyes of *Drosophila*, those of orange (*o*) had no effect.

Ephestia larvae have cinnabar-plus substance and the question was raised as to why orange and ivory larvae feeding on them develop unaltered orange or ivory eyes. It was suggested that cinnabar-plus substance may not be present in the blood of *Ephestia*. The authors suggested tentatively that the wild type alleles to cinnabar and orange are homologous and that their mutant alleles represent parallel mutations.

Anderson (1941) implanted *Habrobracon* eye discs or parts of them into *Habrobracon* larvae by the Ephrussi and Beadle technique. Of the combinations made, orange (*o*) and ivory (o^i) discs were the only ones affected, being shifted toward wild type in all hosts except orange and ivory. When autonomous whole carrot (wh^c) or the allelic white (*wh*) eye discs were implanted into ivory hosts, host eyes became red. When small fragments were implanted, host eyes became brown. The author concluded that brown was an intermediate step between ivory and red.

VIII. Linkage Maps

Linkage data have been collected at intervals during the years of work with *H. juglandis,* and many students have contributed to them, too many to be listed here. The genes showing linkage fall into eight

Linkage Group I

Sk—speckled eyes
12
r—reduced wings
13
gl—glass eyes
30
x—sex gene
10
fu—fused antennae
22
Sb—stubby antennae
42
bl—black body color
30
le—lemon body color
12
c—cantaloup eye color
14
l—long antennae
3
n—narrow wings
7
ho—honey body color
8
vl—veinless wings
15
ro—rough wing veins
12
bu—bulged eyes
37
cr—crescent eyes
41
**sl/co*—semi-long/coalescent antennae
33
ct—cut wings
32
rd—red eyes
37
gy—gynoid-feminized males
7
**ac/ey*—aciform antennae/eyeless

Linkage Group II

k—kidney eyes
28
dw—dwindling antennae
5
m—miniature body
11
o—orange eyes

Linkage Group III

bk—broken wings
25
**wh/pl*—white/pellucid eyes
9
st—stumpy feet

Linkage Group IV

sv—shot veins
23
td—truncated wings
27
ma—maroon eyes

Linkage Group V

wa—wavy wings
22
br—broad thorax

Linkage Group VI

ta—tapering antennae
40
un^2—undulating^2 wings

Linkage Group VII

**pk/ew*^{3}—pink eyes/extended^3 wings

Linkage Group VIII

wt—wet wings
18
bf—black feet

groups, as listed in the tabulation, which may or may not correspond to 8 of the 10 chromosomes. Some groups may be combined with further study, and some may be spurious because of translocations. Chromosome I, the sex chromosome, is 380 cross-over units in length, apparently the longest on record. Loci marked * are complex in that each of the two alleles contains a factor affecting a different trait or character of the wasp. Such alleles may be called complementary because the compound is wild type. No recombinations of these traits have been found. Localized chiasmata may account both for the loose linkage and for the relative frequency of the compound loci.

IX. Radiations

H. J. Muller, before the announcement of his discovery that both lethal and visible mutations could be induced in *Drosophila melanogaster* by X-rays (1927), suggested that similar tests be made with *Habrobracon*. A significant increase in both visible and lethal mutations was obtained, and detailed observations and predictions of the trends of subsequent experiments were presented (P. W. Whiting, 1928, 1929).

Through the years, accuracy of dose measurement has improved, and estimated exposures in earliest and latest experiments are not strictly comparable. In the following summaries, conditions of treatment are stated only when significant effects of differences in rates, etc. have been observed.

With the solution of the sex-determination problem, crosses could be controlled and the production of highly inviable diploid male embryos avoided. All later experiments deal with stocks known to contain different sex alleles, so that inviability can be attributed to irradiation effects. Actually, type stock #1 (*xg* and *xh*) and #33 (*xe* and *xf*) have been used in some experiments, #33 females and #17-o^i males in others. Sex alleles in #17-o^i stock have not been identified but diploid males have never developed from crosses of it with #33 and F_1 hatchability is about 97%. The presence of a recessive gene in the sperm has a special value as will be seen in the report on irradiation effects on eggs.

In most experiments, hatchability (conveniently observed because eggs are deposited on the surface of the paralyzed host) has been the criterion of effect. The majority of radiation-induced lethals act on embryos, and environmental factors such as crowding or shaking from the host do not affect results. At the suggestion of C. E. McClung made in 1935, freshly laid eggs were placed in mineral oil, Nujol, in which hatchability can be accurately observed, but no method has been devised for recovering the larvae with this technique. It is used by some investigators when hatchability is the only measure of injury. Others prefer to

retain the natural host so that larvae which emerge and survive can complete development. Female wasps are placed with active caterpillars which they soon sting and inactivate or with paralyzed ones previously stung by "nurse" wasps, females sterilized by X-rays.

A. Effects on Adults

The extreme resistance of adult wasps to irradiation was noted in early experiments (McCrady, 1930; Stancati, 1932; Maxwell, 1938; P. W. Whiting, 1938). The 100% lethal dose for haploid males is approximately 250,000 r (Heidenthal, 1945). Sullivan and Grosch (1953) observed that wasps of both sexes exposed to 144,200 and 180,250 r became sluggish but recovered within one half-hour, and they made careful studies of longevity without feeding. Haploid males lived longer than controls even after exposure to 180,250 r, mean longevity, 191.77 $\pm$ 4.30 hours, that of controls, 160.14 $\pm$ 3.00 hours; for females, length of life also continued to increase with dose, after 180,250 r, 276.86 $\pm$ 10.99 hours, controls, 231.00 $\pm$ 9.35 hours. Diploid males resembled haploid males in longevity. The reserve food supply in females undoubtedly contributes to their greater longevity, for they resorb their eggs when deprived of food (Flanders, 1942), the most mature ones first, and then progressively younger ones. Grosch and Sullivan (1952) reported that longevity of female wasps was not influenced significantly by internal beta radiation over the range investigated, 12.6 to 1445 μc/g. In fact, the experimentals showed a higher mean survival time than the corresponding controls in five out of nine experiments. Although egg production in some was halted 5 to 8 days after treatment, the sterilized females were fed and lived for weeks after laying their last eggs.

No explanation has been found for the great resistance of insects and for their longer lives after heavy X-ray doses. Perhaps the absence of dividing cells, except for those in gonads, will explain the former, and reduced activity after heavy irradiation, the latter. This resistance of adults has been of value in the study of irradiation effects on highly resistant stages of unlaid eggs. Flanders' observations have pointed out the importance of using only well-fed, egg-laying females for complete records on hatchability studies since partially starved control females deposit some eggs which shrivel and fail to hatch.

B. Effects on Mature Sperm

In all tests of irradiated sperm, males are left with females for about one hour after exposure and then removed. Thus, no sperm irradiated as spermatids are included.

Male sterility may be due to (1) absence of sperm, (2) inactivation

of sperm, or (3) sperm carrying dominant lethals. The first condition is highly improbable in any tests of haploid males made soon after exposure to a mutagen because of the large number of successive matings required to exhaust the sperm supply of a male (fourteen in less than 2 hours in one test). The eggs of females mated to males lacking sperm would show control hatchability, all progeny male. Complete inactivation of sperm would have the same effect as their absence, partial inactivation would increase the percentage of males above 33%, that of controls. With at least one dominant lethal in each sperm, hatchability would be about 33%, that of eggs not fertilized, and all survivors would be male. If some sperm contain dominant lethals, for example, 50%, hatchability would be 66%, and adult survivors 50% male and 50% female. Thus, in *Habrobracon* the first two phenomena can be distinguished from the third, and the second from the first, when matings are made soon after exposure to mutagens and one male has not been mated to a large number of females.

Sperm inactivation has been distinguished from induction of dominant lethals (Stancati, 1932; Bishop, 1937; P. W. Whiting, 1938; Maxwell, 1938; Heidenthal, 1945), and the results of the experiments agree in showing a constant reduction in the percentages of females produced with no increase in the percentage of males after doses up to 10,000 r, the dose at which practically every sperm carries at least one dominant lethal and no females are produced, males still developing from 33% of the eggs laid. Maxwell tested the effects of 41,000 and 142,000 r, and detected significant evidence for sperm inactivation. Total average males per day for the latter dose was 2.01, intermediate between mated controls (1.05) and unmated controls (3.12). Bishop used hatchability and Heidenthal both hatchability and adult survival as measures of change, and each dealt with stocks known to differ in sex alleles. Bishop found a decrease of 66% in female progeny for 5000 r and of 97% for 7500 r, with no compensating increase in males. Heidenthal noted that after very high doses, 153,320 and 168,000 r, numbers of males showed a significant increase. This she interpreted to mean that about 40% of sperm were inactivated.

Dominant and recessive lethal and visible mutation rates induced in mature sperm are estimated as follows: males from virgin mothers are X-rayed and mated at once to untreated virgin females. Hatchability records are kept of the eggs laid. F_1 females are bred unmated and egg hatchability is recorded for each. Surviving F_2 males are studied for visible mutations. If 50% of the eggs of an unmated F_1 female fail to hatch, she is heterozygous for one recessive lethal, if 25% fail to hatch, for two nonlinked lethals, etc. A female heterozygous for a visible muta-

tation will produce sons in a 50–50 ratio, wild type and mutant. By this method (devised by Heidenthal) recessive mutations in all chromosomes are detected because of haploid parthenogenesis. Their rates are estimated by the number of F_1 females with one or more recessive mutations over total number tested $\times$ 100.

Dominant lethal rate is estimated by the equation

$$D = 1 - \frac{m - (E \times 0.33)}{E \times 0.67} \times 100$$

in which m = hatchability of eggs from mated females, 0.33 the frequency of eggs not fertilized, 0.67 the frequency fertilized, and E the total number of eggs laid.

Heidenthal (1945) exposed haploid males (stock #1) to doses of X-rays ranging from 500 to 10,000 r, and mated them to untreated females (stock #33). The number of females per day decreased with

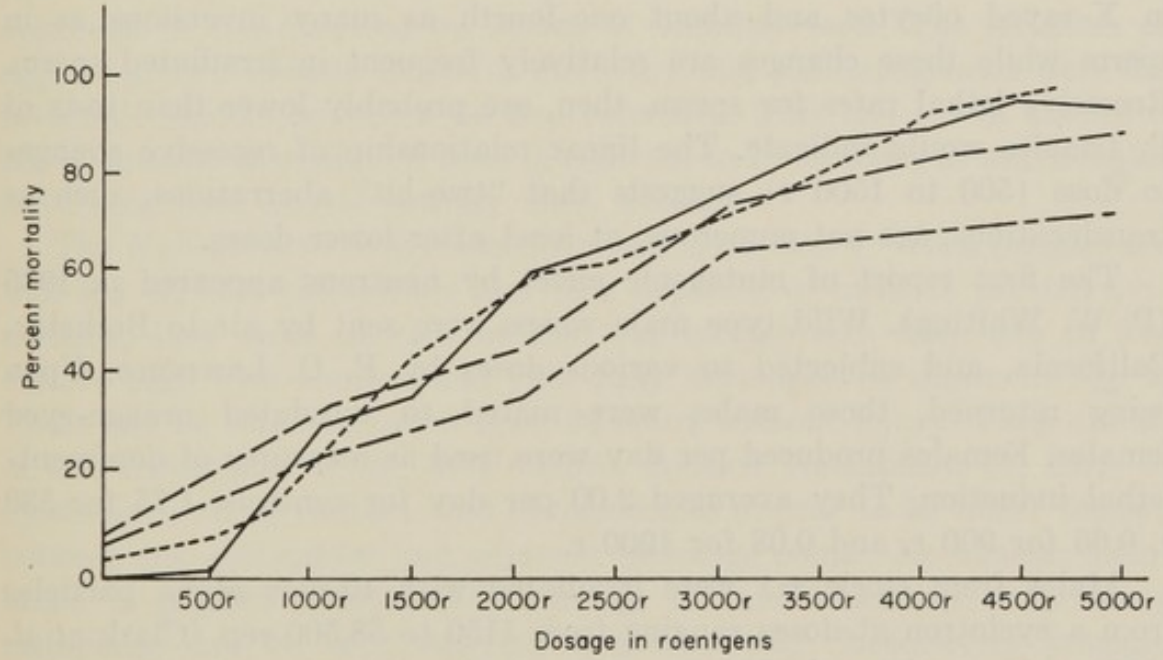


FIG. 3. The effects of exposure of mature sperm to increasing doses of X-rays on the incidence of adult wasps, the hatchability of *Drosophila* eggs, and the incidence of adult *Drosophila*. Key: adult wasps failing to emerge (———), Heidenthal; *Drosophila* eggs of Oregon R stock failing to hatch (—•—•), Demerec and Fano; (.....), Sonnenblick; adult *Drosophila* of Oregon R stock failing to emerge (- - - - -), Demerec and Fano. (From Heidenthal, 1945.)

increasing dose, and 10,000 r induced at least one dominant lethal in each sperm. The number of males per day remained relatively constant, indicating no inactivation. In Fig. 3 percentage of female mortality is plotted against dose. This gives a nonlinear relationship, with the 50% lethal point at about 1800 r and the 95% lethal point beyond 4500 r. Since the curve becomes nearly asymptotic at 5000 r, it has not been

extended to include doses of higher value. Figure 3 also shows the curves for dominant lethal rates in irradiated *Drosophila* sperm taken from the work of Sonnenblick (1940) and of Demerec and Fano (1944) redrawn for purposes of comparison with that for *Habrobracon*.

Recessive lethals induced in sperm increased approximately linearly with dose from 5–6% at 500 r, 10.7% for 1000 r, to 17% at 1500 r (Heidenthal, 1952, 1953). After 4200 r they were present in 60.8% of sperm tested (A. R. Whiting and Murphy, 1956).

Actually, the estimation of recessive lethal rates induced in sperm is not quite as simple as this technique would suggest. The possibility exists of the induction of changes not lethal *sensu stricto* which may be viable in F_1 females, but which result in unpredictable or irregular inviability in F_2 males after passing through meiosis in F_1. Translocations and large inversions are examples. In *Drosophila* Glass has found by salivary chromosome analysis (1955) the almost complete lack of translocations in X-rayed oöcytes and about one-fourth as many inversions as in sperm while these changes are relatively frequent in irradiated sperm. Recessive lethal rates for sperm, then, are probably lower than tests of F_1 females would indicate. The linear relationship of recessive changes to dose (500 to 1500 r) suggests that "two-hit" aberrations, such as translocations, are not numerous, at least after lower doses.

The first report of mutagenic effect by neutrons appeared in 1936 (P. W. Whiting). Wild type male wasps were sent by air to Berkeley, California, and subjected to various doses by E. O. Lawrence. Upon being returned, these males were mated to unrelated orange-eyed females. Females produced per day were used as measures of dominant-lethal induction. They averaged 2.00 per day for controls, 1.75 for 530 r, 0.66 for 900 r, and 0.08 for 1900 r.

Males from stock #1 were irradiated with 40-mev alpha particles from a cyclotron at doses ranging from 1150 to 58,500 rep (Clark *et al.* 1957). After a dose of 6700 rep, 98% of the sperm carried at least one dominant lethal, the same per cent as that induced by a 7000-r X-ray dose. Thus, for equal expenditures of energy, the same incidence of dominant lethality occurred for alpha particles and for X-rays. As a test for inactivation, males were irradiated with alpha particles, doses ranging from 65,000 to 320,000 rep. For sperm treated with 187,000 rep, there was no evidence of inactivation after 3 days, a small per cent of inactivation after 7 days, and complete inactivation after 10 days. Males treated with 238,000 rep and higher doses died before mating. Thus, for mature sperm of *Habrobracon*, the doses of alpha particles needed to cause immediate loss of fertilizing power are of the same magnitude as the doses that will kill the adult.

C. Effects on Unlaid Eggs

The earliest detailed report of the effects of X-rays on unlaid eggs of *Habrobracon* showed that treatment of unmated females followed by breeding without mating caused reduction in offspring per day. When females were mated to untreated males after irradiation, there was no appreciable change in ratio of females to males indicating that few, if any, recessive lethals were induced in the eggs since fertilization did not increase ratio of diploids. Treatment of mated females caused a decrease in female ratio, showing that more dominant lethals were induced in sperm than recessive lethals in eggs (P. W. Whiting, 1938).

The relation of mitotic stage to irradiation sensitivity was being widely tested at this time and each stage had been suggested as most sensitive by one investigator or another. Karl Sax called to the attention of this author the possibilities that *Habrobracon* offered for an approach to the question by means of viability tests. The seriation of eggs of known meiotic stages in the ovary and parthenogenesis were the favorable factors to which he had reference. This has led to extensive use of females in radiation tests in contrast to *Drosophila* in which males have been used almost entirely until recently.

The *Habrobracon* ovary was described in Section V. When females are well fed on host caterpillars and then deprived of hosts to prevent oviposition, they store in each of the four uterine sacs from two to five mature eggs with chorion and yolk fully developed as shown in Fig. 2. These can be retained for at least 36 hours without injury. They are all in late metaphase of the first meiotic division (metaphase I). Ends of dyads are still in contact, and chromosomes appear to be under tension between the centromere and adjacent exchange points. No nuclear membrane is present. Occasionally an egg in late diakinesis or in early metaphase can be seen entering a uterine sac. Successively younger prophase oöcytes (prophase I) occupy the ovarioles anteriorly. Synapsis occurs in very young oöcytes near the anterior tips of the ovarioles.

In a study of effects of mutagens on the oöcytes with hatchability of the subsequently laid eggs as the criterion, the possibility of selection within the ovarioles must always be considered. During the course of the early experiments (A. R. Whiting, 1940), as egg laying continued beyond the second day after exposure of the females to an X-ray dose of 2500 r, bits of debris were deposited along with mature eggs. When ovaries were removed and fixed 5 days after exposure, oögonial tips fell to pieces at a touch and instead of mature eggs, uterine sacs contained pieces of chorion and yolk, nurse cells, and young oöcytes. In a few instances, oöcytes in metaphase I with chorion and without nurse cells

were found in the part of the ovariole normally occupied by young oöcytes. Some ovarioles were completely empty. These observations led to the conclusion that in any study of eggs laid after the second day by females exposed to mutagens, internal selection would invalidate data as exact evidence for cytological stages at the time of exposure, and later experiments by this investigator have dealt only with eggs laid on the first 2 days after exposure.

LaChance (1955) has demonstrated that if oviposition begins immediately after X-irradiation (dose 3750 r), hatchability is lower than if egg laying is delayed by keeping females without host caterpillars as long as 72 hours after exposure. Tests were continued for 20 days. Three factors were suggested as possible causes of this effect: (1) preferential resorption of damaged eggs; (2) nonpreferential resorption and replacement by surviving cells among the resistant oögonia; (3) recovery from radiation damage, such as chromosome healing which perhaps could not occur readily while active egg laying is in progress. He concludes that delay in egg laying affects hatchability during the entire reproductive cycle. This author has not observed a significant change, after a delay of 24 hours, in hatchability of the eggs laid on the second and third days following exposure.

In practice, when a comparison of responses of metaphase I and prophase I oöcytes is desired, virgin females with stored eggs are exposed to experimental conditions, then set at once, unmated or mated to unexposed males, on host caterpillars where they are left for 6 hours. They are then removed, kept without hosts overnight, placed on hosts for 2 hours, transferred to fresh hosts for 4–6 hours, all at 30°C. Eggs laid during the first 6 hours were exposed in metaphase I, during the first 2 hours of the second day, in a mixture of stages, during the remainder of the second day in late prophase I. The second group is discarded. Hatchability records are taken 48 hours after placing the females with hosts (A. R. Whiting, 1945a).

Unfertilized eggs failing to hatch may contain dominant or recessive lethal changes or both. Fertilized eggs failing to hatch contain dominant lethals. (All lethals discussed are embryonic unless stated otherwise.) Unlike F_1 data from irradiated sperm, those from irradiated eggs can be used for the estimation of both dominant and recessive lethal rates. Tests of F_1 females can be made, as in experiments with irradiated sperm, for recessive lethal rates, and it has been found that the latter method is the more accurate (Atwood *et al.*, 1956).

The F_1 method of estimating lethal rates (the only method for dominants) is made by comparison of hatchability of eggs laid by unmated and mated females. From the former, the viable proportion of unfer-

tilized eggs (Vu) contains no lethals. From the latter, the viable proportion of fertilized (Vf) must be estimated since only two-thirds of the eggs laid are fertilized. If D equals dominant lethal rate; r, recessive lethal rate; m, the number of viable eggs from mated females; E, the total number of eggs laid, then

$$Vu = \frac{\#\ hatching}{E} = (1 - D)(1 - r)$$

$$Vf = \frac{m - (Vu \times 0.33E)}{0.67 \times E} \times 100 = 1 - D$$

$$D = 1 - Vf$$

$$\frac{Vu}{Vf} = \frac{(1 - D)(1 - r)}{1 - D} = 1 - r$$

$$r = 1 - Vu/Vf$$

In the first tests for relative sensitivity of metaphase I and prophase I oöcytes (A. R. Whiting, 1940), 2500 r was the dose used; females were unmated and eggs were collected as laid, numbered, and their hatchability noted. No eggs exposed in metaphase I hatched while those exposed in late prophase I were highly resistant. Responses of metaphase I were less extreme for lower doses, 212, 425, 850, and 1275 r. Lethal dose for this stage was between 1275 and 2500 r. No dose approached that high enough to be lethal to prophase I. Immature females with no eggs in metaphase I when irradiated gave hatchability ratios similar to those of the later eggs laid by mature females. There was found then unexpected sensitivity of metaphase I and resistance of prophase I. This work was continued with improved technique (A. R. Whiting, 1945a).

Unlaid eggs X-rayed in metaphase I and allowed to develop parthenogenetically showed linear decline in hatchability with a lethal dose of about 1500 r, later estimated to be 2000 r. They did not respond to time-intensity differences or to fractionation of dose; continued meiosis after doses several times lethal at rates not noticeably different from controls; showed chromosome fragments but no bridges in division I, bridges, fragments, or both in division II, both in early cleavage; gave a dose-action curve expressing dose percentage of eggs without chromosome fragments which at high doses converged toward the dose-hatchability curve.

Unlaid eggs X-rayed in prophase I showed a decline in hatchability from 1000 to 44,800 r with lethal dose estimated to be about 50,000 r; gave a hatchability curve which was linear at low doses but which in-

creased more rapidly after higher doses, first apparent after 15,000 r (Fig. 5); some reduction of injury by fractionation of dose (not confirmed in later experiments, unpublished); continued meiosis and early cleavage after lethal dose at rates not noticeably different from controls; showed chromosome fragments and bridges in division I, neither in division II, both in early cleavage. Unlaid eggs treated in either stage showed no significant change in hatchability when fertilized with untreated sperm (not confirmed in later experiments). It was suggested that high sensitivity of metaphase I might be due to permanence of chromosome breaks because of tension of chromosomes in this stage, resistance of prophase I to absence of tension.

A detailed study of effects on chromosomes was then undertaken, handicapped as always in *Habrobracon,* by their small size and large number (A. R. Whiting, 1945b). This confirmed the preliminary cytological observations outlined above. Bridges in division I after irradiation in prophase I were observed to be permanent since the nuclei did not move far enough apart to break them. It was suggested that this was one factor in the resistance of this stage, some broken chromatids being withheld from passing to terminal nuclei (one of which is the functioning oötid) in division II.

These early experiments showed dominant lethality to be much the most frequent consequence of X-irradiation of oöcytes as it is for sperm, and there was no significant evidence for recessive lethals. Later (1952), Heidenthal reported 5.1% of recessive lethals in metaphase I after 500 r, 14.2% after 1000 r; A. R. Whiting and Murphy (1956), 5.7% for 500 r, 15.2% for 1100 r, and 9.7% for prophase I oöcytes after 15,000 r, all obtained by the F_2 method. When recessive ratios estimated by the F_1 method were checked with the F_2 method, a significant and consistent discrepancy was noted for eggs irradiated in metaphase I. The apparent frequency of recessive embryo lethals was significantly greater by the F_1 than by the F_2 method. For eggs irradiated in prophase I no such discrepancy was observed (Atwood *et al.,* 1956).

For 500 r, in metaphase I eggs, recessive lethal rate by the F_1 method was 16.4%, by the F_2 method, 5.8%; for 875 r, 30.0 vs. 19.7%; for 1100 r, 49.4 vs. 15.2. For 5000 r, in prophase I eggs, recessive lethal rate by the F_1 method was 5.0%, by the F_2 method, 1.3%; for 15,000 r, 14.5% vs. 14.4%; for 25,000 r, 14.1% vs. 15.0%. The differences for metaphase I are statistically significant. Irradiation of this stage in nitrogen reduced the effectiveness of the X-rays but did not alter differences in ratios between F_1 and F_2 tests.

In three experiments on metaphase I and one on prophase I, the total recessive lethals (r') expressed at any time in development, in

larvae and pupae as well as in embryos, were scored by the use of adult survival data. For 875 r, r' in metaphase I by the F_1 method was 22.4%, by the F_2 method, 25.2%; for 1100 r, 26.2% vs. 23.9%; for prophase I, 15,000 r, 21.4% vs. 23.7%. The values of r' determined by the F_1 method do not differ substantially from those in F_2 even after metaphase irradiation. When mortality at different postembryonic developmental stages was observed it was found that larval death was significantly higher in mated than in unmated in metaphase I but not in prophase I, that pupal lethals were not significantly different for mated and unmated in either stage. Fertilization and resultant presence of normal alleles to dominant lethals postpones some deaths from embryonic in haploids to larval in diploids when these dominants have been induced in metaphase I. The F_2 method of determining lethal rates is, therefore, the more accurate. Dominant lethals for which death is postponed by heterozygosis have been called "conditionally delayed dominants."

Hatchability of unfertilized *Habrobracon* eggs exposed in first meiotic metaphase was used to compare the effectiveness of roentgen rays of 124 kv and 30 mev (Heidenthal *et al.*, 1955). Dose range extended from 100 to 1750 r for low voltage, and from 100 to 1500 r for high voltage studies. High voltage roentgen radiation was delivered by the 50-mev machine at the General Electric Research Laboratory in Schenectady, New York. Extreme care was taken to obtain accuracy of dose and the technique is described in detail. Stock #33 was used in which control hatchability was $92.6 \pm 0.6\%$. No statistically significant differences between the effects of the two types of radiation were found. Dose-action curves were approximately exponential, with no significant differences between slopes of the curves or between them and that found by A. R. Whiting.

Buretz (unpublished thesis, 1956) determined survival curves for metaphase I eggs irradiated with alpha particles and deuterons. Exponential curves based on hatchability were approximately the same in slope as those obtained by other investigators under different conditions of irradiation. There was no effect of LET on survival. The RBE was independent of LET for the range of energies used. Eggs irradiated in prophase I gave curves similar to those obtained by A. R. Whiting (1945a), and the degree of resistance of prophase I eggs over metaphase I eggs to alpha particles and deuterons was of about the same magnitude as that found for X-rays and fast neutrons.

Clark, Fluke, and Buretz (unpublished) have estimated for metaphase I eggs, that the 50% hatchability dose of X-rays is 400 r, of alpha particles and deuterons 400 rep, of fast neutrons 1.4×10^{10} N_F/cm^2, and of thermal neutrons 5.5×10^{12} N_{th}/cm^2.

D. Modifying Agents

The announcement by Thoday and Read (1947) of the protective effect of anoxia on X-ray-induced chromosome aberrations stimulated a concentrated effort on the part of many investigators to determine whether the protection came about by reduction of primary injury to the chromosomes in lowered oxygen tension or by increase in restitution. It was suggested by Lüning (1954) that a type of response associated entirely with breakage of doubled chromosomes, or those about to be doubled in which lateral union of sister chromosomes takes place, could furnish evidence for the breakage theory if such a type responded to a change in oxygen tension. Restitution would be impossible after such alterations in the chromosomes.

With the conviction that changes induced in metaphase I oöcytes were of this kind, it was decided to test their response to irradiation in atmospheres of lowered oxygen content. Prophase I oöcytes and mature sperm were also tested (A. R. Whiting, 1954; A. R. Whiting and Murphy, 1956). Giles (1952) had suggested that data obtained from cells differing in water content might furnish evidence on the nature of X-ray-induced injury, and mature eggs and sperm were examples of such cells.

Adults were exposed in small Lucite tubes through which air or nitrogen passed during the actual time of irradiation only. In one experiment helium, or a mixture of 20% oxygen and 80% helium, was used. Doses were 1100 r for metaphase I, 15,000 and 25,000 r for prophase I, and 4200 r for sperm. Tests were made for dominant lethal, recessive lethal, and visible mutation rates (the last two by the F_2 method). For metaphase I, conditionally delayed dominants occurred after exposure in nitrogen as after that in air. The mutation rates after 1100 r in nitrogen approximated those after 500 r in air. Differences $\pm \sigma$ difference were 39.3 ± 2.3 for haploids, 42.8 ± 2.1 for diploids. For prophase I after 25,000 r, difference for dominant lethals was 26.1 ± 5.2; after 15,000 r, for dominants 21.5 ± 3.2, for recessives 8.2 ± 2.3, for visibles 2.2 ± 1.8. For sperm, dominant lethals differed by 10.0 ± 0.82, recessives by 20.2 ± 6.5, and visibles by 8.1 ± 4.4. All differences for lethal mutations are significant, those for visibles are not, although consistently higher for air than for nitrogen.

When comparing response to irradiation in reduced oxygen by cells or chromosomes in different stages, consideration should be given to the nature of changes, whether one-hit or two-hit. For dominant lethals, response of metaphase I is highest (one-hit), % air/% nitrogen = 2.09; for recessive lethals, response of prophase I is highest (one-hit?),

% air/% nitrogen = 6.64. In respect to both dominant and recessive lethals, sperm responded least, % air/% nitrogen = 1.12 and 1.50.

For eggs irradiated in metaphase I, dominant lethal mutations, apparently associated with isochromatid breakage and lateral sister union and with tension, conditions which prevent restitution, decrease in the same ratio as do recessive lethal and visible mutations. This favors the breakage hypothesis. The greater response of oöcytes of both stages than of sperm suggests that some factor associated with larger amounts of water may be responsible for the oxygen effect.

The relation of oxygen concentration to the dose-action survival curves of unfertilized eggs was tested by Kenworthy (1956). He found that the effect of the absence of oxygen was of the same magnitude for both metaphase I and prophase I, 48.5% for the former, 52.0% for the latter at doses giving maximum difference. Doses used for metaphase I were 396–2450 r, for prophase I, 2100–44,100 r. The author suggested that similarity of response of both stages strongly supports the primary breakage hypothesis. Since restitution of damaged chromosomes is considered to be rare in metaphase I eggs and frequent in prophase I stages, data are of interest in deciding on induction of initial damage or restitution.

Two experiments were performed to test the effects of gases on radiosensitivity of immature stages (Clark and Herr, 1955). In experiment I, larvae in cocoons, prepupae and white pupae were irradiated in air and in nitrogen, doses up to 15,000 r for the first, to 26,000 r for the second, and to 150,000 r for the third. In experiment II, larvae in cocoons were irradiated in air, hydrogen, and carbon dioxide, doses to 12,000 r. In I the difference in sensitivity was significant and consistent. All three groups in which the resistance to X-rays increases about tenfold with increasing age, irradiated in nitrogen, were one-third as sensitive as in air. It held for both haploid males and diploid females. Thus, the modification by nitrogen is independent of the stage of development, the degree of sensitivity, sex, and ploidy. In II those groups irradiated in hydrogen, nitrogen, or carbon dioxide were significantly more resistant than those in air; those in carbon dioxide or nitrogen were equal, and both more resistant than those in hydrogen.

The use of ethylenediaminetetraacetic acid (EDTA), which forms complexes with metal ions and renders them biologically inactive, was considered of possible interest in the study of radiation-induced dominant lethals and temporary sterility (LaChance, 1959). Experiment A: I. Unmated starved females were divided into four groups and given a single meal 4 hours before irradiation; (1) fed on a solution of 0.09 *M* EDTA diluted from 0.1 *M* by the addition of sugar solution; (2) sugar

solution only + 2500 r; (3) EDTA + 2500 r; (4) sugar solution only. II. This was repeated with a concentration of EDTA reduced to 0.075 *M*. Experiment A was used for the study of temporary sterility, and hatchability was recorded. Experiment B: Females were treated as in A except that half of them from each group were outcrossed to #17-o^i males which were left with the females throughout the experiment.

Recovery from temporary sterility induced by X-irradiation was retarded in those females which had ingested the chelating agent prior to radiation treatment. The combination of chelation and irradiation resulted in much greater damage than either singly. The per cent of eggs of those laid on days 1–7 with at least one dominant lethal was 26.7 for 2500 r, 12.5 for EDTA-fed, 42.7 for EDTA-fed + 2500 r. Data for days 8–20, and perhaps for earlier periods, are for survivors of internal selection. They indicate that EDTA ingestion increases the number of radiation-induced lethals and that the two agents are synergistic in action. In the discussion, five possible modes of action of EDTA are suggested, but data do not distinguish any one as more probable than another.

Von Borstel and Wolff (1955) exposed newly laid eggs to ultraviolet radiation, wavelength 2537 A, in either the nuclear or the cytoplasmic region. Dose-hatchability curve was exponential for the former, sigmoidal for the latter. Subsequent exposure to ultraviolet radiation, wavelength 3600 A, resulted in photorecovery of the nuclear region, a dose reduction of approximately 2.2, and no effect on the cytoplasmic region. Hatchability before reactivation was 20.2 ± 3.24, after, 51.2 ± 5.59. They concluded, "Taken as a whole, these experiments indicate that, by the criterion of hatchability, photoreactivable injury is related to events within, limited to, or governed by the egg nucleus."

E. Genome Number and Sensitivity

Clark and Mitchell (1952) found that embryos X-rayed during cleavage which failed to hatch were arrested at interphase and that their nuclei became enlarged up to four times the diameter of those in controls. The larger number of nuclei in these inviable embryos than of those present at time of exposure indicated that cleavage had continued for a short period after irradiation. Relative sensitivity of the sex types for this stage must be determined from study of differences in survival rates of eggs laid by mated and unmated females. Embryos treated in late stages of development in which lethals have been induced may hatch but are arrested in larval or pupal stages, the sex of the latter group only being identifiable. Larvae in cocoons, prepupae, and white pupae, after all doses of irradiation, continue to develop to the pig-

mented pupal stage when inviables can be sexed. With this information, it has been possible to make detailed studies on relative sensitivity of haploids and diploids at different stages of development and to construct dose-action curves for each stage.

Of investigators working with different kinds of material, some have demonstrated a greater resistance, some no difference, and some an increase of effect with higher ploidy. Experiments in which dominant and recessive lethal rates have been determined for the same kind of cell in known mitotic stages have demonstrated that higher dominant than recessive lethal rates are induced at all exposures. If this is characteristic of all cells, diploids should be more sensitive than haploids, but any statement about cells of ploidy higher than diploid cannot be made. Some lethal effects, dominant in diploids, might be recessive in triploids where two normal allelic areas for the majority of changes are present. In haploid cells, mortality to be expected is $(D + r) - Dr$ where Dr is the rate of induction of a dominant and a recessive lethal in the same cell. In diploid cells, expected mortality is $(2D - D^2) + r^2$ where D^2 and r^2 equal the rate of induction of the same lethal, dominant or recessive, twice in one cell.

When consideration is given to the facts of the great differences in sensitivity between different mitotic stages of the same kind of cell and between different kinds of cells in the same stages and to the rapidly accumulating evidence against the old concept of uniform ploidy of all the cells in a multicellular organism, the interpretation of sensitivity differences in relation to supposed genome number becomes difficult.

It was found in early experiments that young *Habrobracon* larvae were more susceptible to irradiation than old, and male than female (A. R. Whiting and Bostian, 1931). With the possibility in mind that differential sensitivity of males and females might be interpreted on the basis of sex rather than on one of ploidy, Clark and Kelly (1950) used cultures of three kinds, one from virgin mothers, a second from three-allele crosses, and a third from two-allele crosses. Dose rates, 792 and 2860 r per minute produced the same effects for total doses. Clark and Mitchell (1951) used cultures of the third type only. Pupae were X-rayed at various ages. Eclosion rates were recorded and sex of inviables determined. Results were in agreement, and eclosion rates showed that X-rays had a greater effect on haploids than on diploids, male or female. Eclosion curves for both haploids and diploids are sigmoid, indicating multi-hit effects.

In an intensive study and summary of the sensitivity of haploid males and diploid females in various developmental stages carried out at the Brookhaven National Laboratory (Clark, 1957), consistent results

were obtained for each age group, and these are expressed graphically in Fig. 4. Increasing resistance to X-rays with increasing maturity is obvious.

During cleavage, the stage in which radiation-induced lethality rates for haploids and diploids are as expected on a cytological basis, mitotic divisions are synchronous and chromosome numbers constant (B. R. Speicher, 1936). Cell division continues at a high rate throughout embryonic development. Embryo inviability due to nuclear damage would

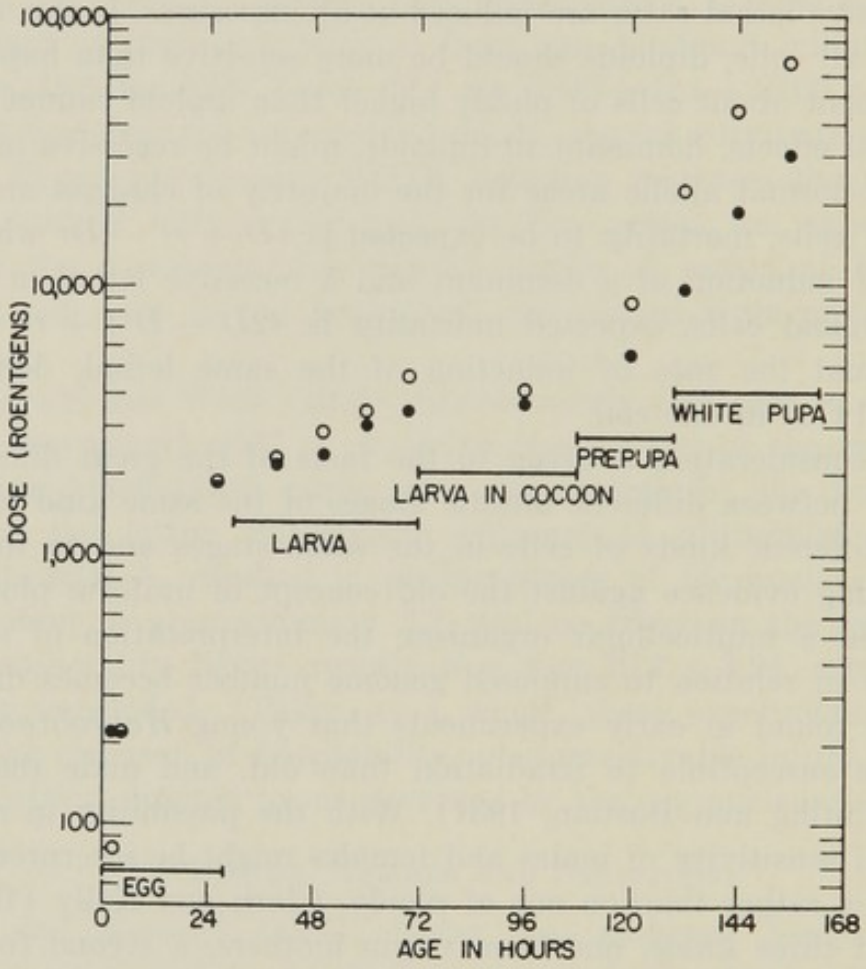


FIG. 4. The dose in roentgens administered at various stages of development required to give 50% eclosion of haploid males (●) and of diploid females (○). (Clark, 1957.)

seem to be unquestionable under these conditions. Higher nuclear sensitivity in later stages would be localized in mitotically active regions differing with age, in number, extent, and location and, possibly, in ploidy.

What actually are the states of ploidy in the larvae and pupae being X-rayed? What is the explanation of the beautifully consistent responses throughout the tests? These present a challenge and no convincing evi-

dence against nuclear injury as an important factor, although after high doses, some cytoplasmic injury would be expected on the basis of evidence from androgenetic male incidence to be discussed later. There seems to be no reason why cytoplasmic injury should differ with ploidy. The differences in response of haploids and diploids, even when the reverse of that expected on the basis of postulated chromosome numbers, furnishes strong evidence for chromosomal injury as an important factor.

Grosch (1950a, 1952) made a cytological study of cells in some organs of impaternate male larvae. He found evidence for polyploidy of cells in Malpighian tubules, fat body, midgut, and spinning gland. Epidermal cells, foregut and hindgut cells, and probably, nerve cells retain the haploid number. He cites literature which indicates that the somatic cells of an organism do not necessarily have chromosome constitutions which are equivalent quantitatively, and concludes with the statement: "Cytonuclear ratios should be approached with the reorientation of multinuclearity."

F. Stages at Death of Lethal-Bearing Embryos

The convenience of *Habrobracon* for obtaining and identifying X-ray-induced embryo lethals in oöcytes and spermatozoa and the smooth chorions of the eggs, which facilitate their study as Feulgen-treated whole mounts, inspired the undertaking of the observation of inviable embryos (A. R. Whiting *et al.*, 1958). All lethals act as do sex-linked lethals in *Drosophila* and for this reason no lethal-bearing haploid males survive and no homozygous lethal inviables are obtainable. The inviable classes available are dominant and recessive haploid embryo lethals and diploid embryos heterozygous for dominant lethals. X-ray doses used were those producing approximately comparable dominant lethal effects in heterozygotes, 1100 r for metaphase I oöcytes, dominant lethal rate 85.1%, 25,000 r for prophase I oöcytes, dominant lethal rate 85.3%, and 4500 r for sperm, dominant lethal rate 91.2%.

Inviable embryos were characterized at death (1) by the presence of a few scattered cleavage nuclei; (2) by a single-layered blastoderm; (3) by a general thickening of cell layers around anterior, posterior, and ventral sides with a slight anterior invagination of the stomodeum and polar cells still posterior to germ band; (4) by very definite stomodeum, proctodeum, and precerebral masses; visible evagination for appendage buds and polar cells within germ band; (5) by obvious segmentation and visible trachea (larvae which fail to hatch).

Observations demonstrated that dominants in haploids, dominants in heterozygotes, and recessives in haploids kill at increasingly later stages and that response of haploid embryos with more than one reces-

sive lethal in respect to mean stages at death depends on whether they were induced in oöcytes or sperm. In the former, there was no cumulative effect, no correlation between number of lethals and stage at death; in the latter, there was a correlation between what appear to be recessive lethals and stage at death which may be due to the presence in irradiated sperm of translocations and inversions.

For dominants in haploids and in heterozygotes the mode was class 1; for recessives induced in metaphase I it was class 4, in prophase I class 5, and in sperm about equally divided between 4 and 5.

A few F_1 females were obtained with egg hatchability as low as 3%. This would suggest that they were heterozygous for four or five recessive lethals. Mean stage at death for these lethals was 3.2, the lowest obtained for recessives; highest was 4.5 for eight females from irradiated prophase I oöcytes. Differences in mean stages at death between *D* and $+/D$ are about the same for metaphase I and prophase I, and there is a suggestion of delayed death in the latter. The striking delay in expression of lethality in inviables from X-rayed metaphase I oöcytes fertilized by control sperm mentioned above (Atwood *et al.*, 1956), would not show in this study since that postpones death to larval stage. Because of the high dose used for prophase I oöcytes, some cytoplasmic injury would be expected. However, no effect of an additional injurious factor can be identified in the data.

A detailed study of embryos inviable because of radiation-induced dominant lethal changes has been made by von Borstel (1955b) and von Borstel and Rekemeyer (1959). They divided them into three categories. Type I kills the embryos in early cleavage and is characterized by completion of meiosis at the normal rate, and a slowing up of the rate of mitosis with complete cessation after the second or third division. At about the eighth hour after oviposition, Feulgen-negative nuclei arise, and within 2–4 hours, 5–150 have appeared, after which they begin to enlarge. The Feulgen-positive nuclei can sometimes still be seen and they also enlarge. At 20 hours after oviposition, enlargement of the Feulgen-negative nuclei is nearly maximal, about two hundred times the normal size. Type II kills the embryo after blastula formation and before hatching if the embryo is haploid, after hatching, if the embryo is diploid and heterozygous (conditionally delayed dominants). Type III kills the embryo after blastula formation but before hatching whether the embryo is haploid or diploid. Control chromosomes postpone embryo death in this type but not to the extent of making it larval.

Type I follows irradiation of either oöcytes or sperm. About 80% of haploid inviable embryos and 60–70% of heterozygous inviables die in this stage. Type II is inducible in metaphase I oöcytes only. Type III

characterizes death of embryos from oöcytes irradiated in prophase I. The contrast between II and III cannot be made for sperm.

Irradiation of female *Drosophila* showed that inviable embryos consisted of Types I and III. Type I differed from that found in *Habrobracon,* however, for there were present no large Feulgen-negative nuclei.

In order to find out if Type I was due to chromatin loss in itself which is known to occur in irradiated metaphase I eggs, triploid females and translocation heterozygotes of *Habrobracon* were set and their inviable embryos fixed. They showed Types II or III dominant lethality.

It should be emphasized at this point that not only do chromatin bridges occur in each cleavage division after irradiation of *Habrobracon* oöcytes in metaphase I with lethal dose, but these bridges break in two places leaving a large fragment in the cytoplasm with each division. In aneuploids chromatin bridges are not formed.

Since contrived chromatin loss or deficiency is not associated with the Type I pattern and since the giant Feulgen-negative nuclei appear after the Feulgen-positive nuclei have stopped dividing, the possibility of their being retained or "rejuvenated" accessory or secondary nuclei comes to mind. Spotkov (1938) studied the maturing eggs of *Habrobracon* and described accessory or secondary nuclei which appeared in the cytoplasm during the formation of the first meiotic metaphase. He believed that they arose from granules extruded by the germinal vesicle in late prophase I. They divide amitotically, increase in size, and are Feulgen-negative. They normally disappear before maturation of the egg. Henschen (1928) also described them but thought that they arose from contents of the nurse cells which passed into the egg. Their retention or reappearance after the unlaid egg has been exposed to a lethal dose of mutagen seems plausible, but the relatively late entrance of an irradiated sperm makes their association with that more difficult to understand.

Von Borstel (1955b) suggested that Type I phenomena are associated with a defect in DNA synthesis induced by mutagens. If Type I pattern is not caused by altered chromosome behavior such as the breakage-fusion-bridge cycle, one intangible kind of lethal-inducing change must be epistatic to a second, known to be lethal-inducing. This unknown must be of such a nature that it is induced with relative ease in metaphase I, with difficulty in prophase I, and in addition, must respond with a different dose-action curve for each stage.

Von Borstel and Rekemeyer (1959) describe a mutation that "when homozygous in a female *Habrobracon,* mimics the Type I lethal syndrome in each egg laid."

G. Cytoplasmic and Nuclear Injury

It was noted in 1946 (A. R. Whiting) that, among several hundred mosaics found in *Habrobracon,* eleven only had regions which were unquestionably paternal in origin, and that among progeny from crosses of dominant females by recessive males (the condition in which androgenetic males can be identified), only six males had been reported showing traits exclusively paternal. In contrast, when unmated wild type females were heavily X-rayed, then mated to untreated males with recessive traits, there were frequently included among the expected wild type progeny, a few recessive males, normal in structure and behavior. These were fully fertile as were their numerous daughters. Their normal appearance and behavior, in spite of the heavily irradiated eggs from which they developed (29,300–42,000 r) suggested that this kind of experiment would furnish data of significance in respect to presence or absence of radiation-induced cytoplasmic injury.

It was stated in this preliminary report that androgenetic males developed from eggs irradiated in prophase I since they appeared in experiments in which egg hatchability had not been observed, and it was thought that a cell stage at which the lethal dose was 2000 r could not contribute normal cytoplasm to an embryo after 42,000 r. With the timing of eggs it became obvious at once that the androgenetic males developed from eggs X-rayed in metaphase I.

Further study (A. R. Whiting, 1948) showed that 1.57% of eggs X-rayed in metaphase I (14,420–28.840 r) developed into androgenetic males; that they would develop after any dose up to 54,000 r but not beyond it, although lethal dose for the egg nucleus is 2000 r.

Cytological study of 294 eggs irradiated in metaphase I and laid after exposure to 14,420–36,050 r from crosses of type females by recessive males showed that 6 or 2.04% were beginning development as androgenetic males, a percentage not different statistically from that of androgenetic males in control experiments, and it was concluded that androgenetic survivors represented, within this dose range, an approximate measure of their incidence. In these preparations chromatin bridges were seen in meiotic division II, which retarded movement of the egg pronucleus, occasionally to such a degree that the sperm pronucleus had divided and started development as a haploid androgenetic embryo. The almost complete absence of these bridges after exposure of prophase I will explain the failure of androgenetic males to develop in eggs X-rayed in this stage.

Method of origin and dose relationship of androgenetic males have been summarized (A. R. Whiting, 1955). It was stated in the conclusion

that "irradiated cytoplasm in metaphase I eggs may function normally after doses of X-rays many times greater than that lethal to the nucleus but that very high doses prevent its doing so, even in combination with an uninjured nucleus." The incidence of killing of nonirradiated nuclei by this kind of cytoplasmic injury first becomes apparent at 15,000 r. There is no evidence for the induction of genetic changes in untreated chromosomes by irradiated cytoplasm and, if mutations can be induced in chromosomes through an altered cytoplasmic environment, this type

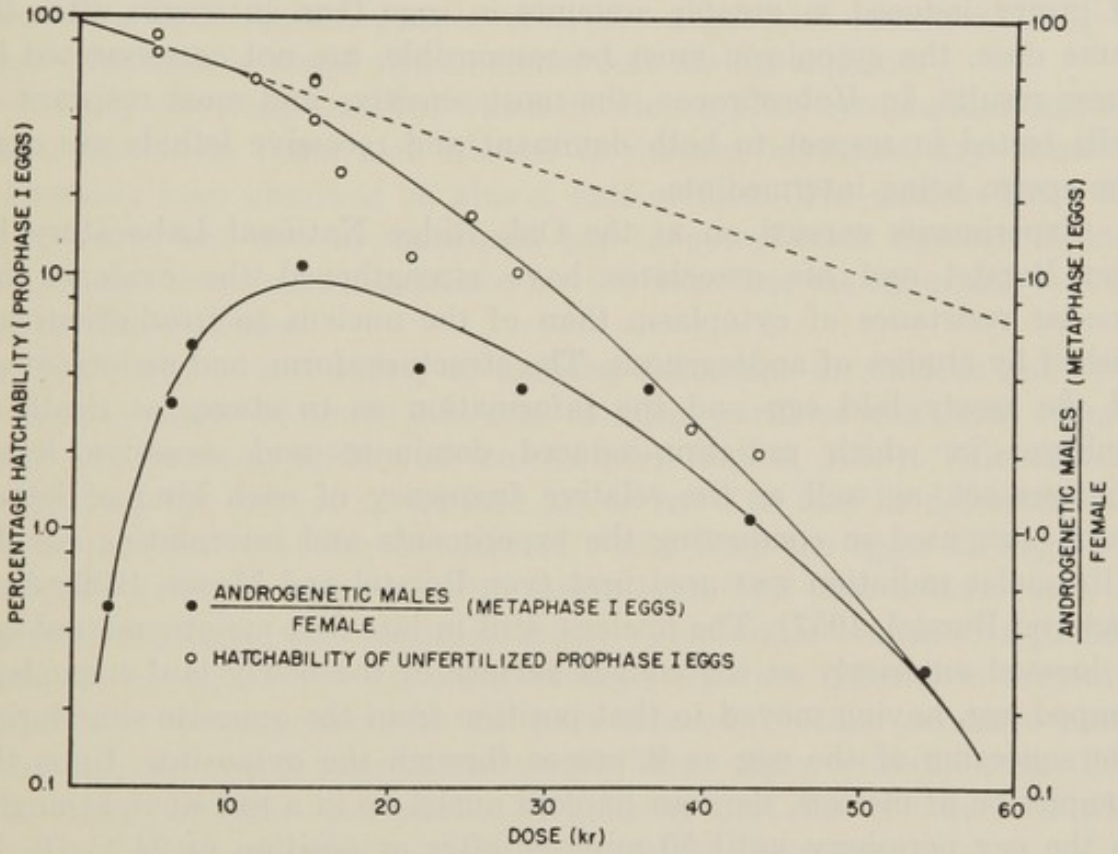


FIG. 5. Lethal dose of prophase I eggs is about 50,000 r, of metaphase I eggs, about 2000 r. Androgenetic males develop from the latter. Curves for prophase I hatchability and for androgenetic males per female exposed are similar in respect to points at which direction changes, and this is interpreted as the approximate dose above which appreciable cytoplasmic injury is induced in both stages.

of cytoplasmic change must be transitory for no visible mutations appeared in the 283 androgenetic males studied. Sperm enter the irradiated eggs from ½ to 6 hours after the exposure and there is some time for recovery if such occurs.

In Fig. 5, data on prophase I hatchability and on incidence of androgenetic males as correlated with dose are arranged graphically. Androgenesis rises with dose until about 15,000 r after which it falls, and no androgenetic males have been found following doses higher than 54,000 r

(64,000–144,000 r = 0/359). Hatchability percentages of eggs X-rayed in prophase I begins to fall below expectation on the basis of an exponential dose-action curve at about the same dose. Evidence indicates that injury is induced in both metaphase I and prophase I cytoplasm to the same degree per dose in spite of the great difference in lethal dose which in metaphase I (2000 r) must act on chromosomes, in prophase I (50,000 r), on both chromosomes and cytoplasm.

Early arguments that eggs should be more sensitive to X-rays than sperm because of their large amounts of cytoplasm or, that for any type of injury induced in greater amounts in eggs than in sperm after the same dose, the cytoplasm must be responsible, are not corroborated by these results. In *Habrobracon,* the most sensitive and most resistant of cells tested in respect to both dominant and recessive lethals are eggs, the sperm being intermediate.

Experiments carried on at the Oak Ridge National Laboratory by von Borstel and his associates have strengthened the evidence for greater resistance of cytoplasm than of the nucleus to irradiation furnished by studies of androgenesis. The structure, form, and meiotic stage of the newly laid egg and the information as to stages at death of embryos in which radiation-induced dominant and recessive lethal changes act, as well as the relative frequency of each kind of lethal, have been used in conducting the experiments and interpreting results. Ultraviolet radiation was used first (von Borstel and Moser, 1956; Amy and von Borstel, 1957). The nucleus, still in late first meiotic metaphase, is located anteriorly at the convex surface of the newly laid cucumber-shaped egg, having moved to that position from the opposite side during the squeezing of the egg as it passes through the ovipositor. Upon the completion of meiosis, the four haploid nuclei lie in a row at right angles to the egg periphery until 50 minutes after oviposition at 24°C (B. R. Speicher, 1936).

Nuclear and non-nuclear surfaces were separately inactivated by ultraviolet. The dose-hatchability curve for nuclear irradiation of the newly laid egg was exponential (incident radiation $LD_{50} = 60$ ergs/mm^2), that for the non-nuclear portion was steeply sigmoid (incident radiation $LD_{50} = 900$ ergs/mm^2). When the anterior end of the egg was shielded, the survival curve was also sigmoid, and this gave further evidence for the conclusion that this type of curve results from exposure of the cytoplasm.

Since wavelengths of ultraviolet radiation are absorbed selectively by different chemical groups, eggs were exposed to doses of monochromatic ultraviolet radiation at ten different wavelengths covering the range from 2220 to 3022 A. For non-nuclear surface irradiation, a broad

maximum of efficiency obtained, including the range from 2483 to 2753 A, with greatly reduced absorption efficiencies at the extremes. Comparison of known absorption spectra of biologically important compounds suggested to the authors that purines and/or pyrimidines (absorption maximum ca. 2600 A) might be the primary groups concerned with the deleterious effects, and that cyclic amino acids with an absorption maximum at 2800 A were probably involved as well. For eggs exposed on their nuclear surfaces, the action spectra were similar except that 2804 A was included in the area of maximum efficiency. Based on incident energy per unit, the ultraviolet radiation LD_{50} for the cytoplasm is about fifteen times that for the nucleus.

When surviving embryos which developed after cytoplasmic exposure were placed on hosts and allowed to mature, nonhereditary abnormal abdomens were observed on almost half of them. The authors state that these were not due to mutations but to cytoplasmic damage in local areas, and they furnish evidence for the idea that the sigmoidal curve is multi-hit in nature.

As a test of the effects of ionizing radiations on nucleus and cytoplasm, exposure of newly laid eggs to alpha particles was made (Rogers and von Borstel, 1957; von Borstel and Rogers, 1958). Polonium-210 plated on the end of a polished silver rod (10 mm in diameter) was used as a source at the distance of 1 mm from the eggs. All eggs were exposed within 5 minutes after being laid. The dose-action curve for nuclear response was exponential, for cytoplasmic response, sigmoidal, and, as for ultraviolet exposure, inviable embryos differed in respect to time of death, depending on the part exposed. Nuclear exposure caused early death, cytoplasmic, later death. With alpha radiation, the LD_{50} for the cytoplasm was about 3000 times that for the nucleus when incident alpha particles per unit area was the criterion.

Average nuclear diameter is about 2.8 μ immediately after oviposition, 5.7 μ in late anaphase after 5 minutes. The value 2.8 μ compares closely to the alpha particle target diameter of 2.41 ± 0.12. The suggestion is made that the critical target is the single chromosome set, presently to become incorporated in the pronucleus.

H. Ingested Radioactive Isotopes

Grosch (1950b) made a study of the effects of starvation on males and females of *Habrobracon*. The facts obtained were to prove of importance in later experiments with feeding of radioactive isotopes. Under conditions of complete starvation, females live longer than males, and larger wasps longer than smaller ones. Weight loss is chiefly from the abdomen which becomes flattened dorsoventrally. Histologically, the

greatest changes are observed in the number and size of the "fat" cells of the fat body of both males and females. During the last stages of starvation all developing eggs, as well as mature ones, are resorbed.

Most of the experiments concerned with the feeding of radioisotopes have dealt with females because preliminary experiments in feeding P^{32} to adult males proved to be unsatisfactory. Males cannot resist starving as well as females, some will not mate after being fed radioactive food, and often the few daughters produced when they do mate, accompanied by an increase of males, indicates a lowered sperm supply or sperm inactivation.

Twenty starved males of stock #17-o^i were fed honey adulterated with P^{32} at a concentration of 200 μc/g of mixture (Grosch, 1956). One day after feeding they were outcrossed to stock #33 females. The per cent of females in control fraternities was 61, in treated, 52. Incidence of males did not rise above that of controls, showing that the lowered female ratio was due to dominant lethals. Inviable pupae consisted of nearly twice as many females as those from controls, and in one specific check of 100 offspring, 87% of the inviables died as embryos. The level fed was biologically equivalent to about 2000 r of X-rays. If fed to females, the same mixture can permanently sterilize them and is equivalent in effect to 5000 r of X-rays.

Autoradiographs of males fed P^{32} show no radioactivity concentrated in the testis, an explanation perhaps of the relative ineffectiveness of radiophosphorus when fed to males. The mutagenic agents, beta particles, appear to be the emanations from the gut contents and soma. Synthesis does not occur in the testis of adult males in which spermatogenesis is largely completed by the time of eclosion.

Grosch and Sullivan (1952) fed honey containing P^{32} to starved virgin females at radioactive levels ranging from 10 to 1500 μc/g. The mean volume for distended crops from undwarfed adult females was determined as 0.49 ± 0.06 cu mm. Above 200 μc/g, egg laying ceased after a few days. Hatchability of eggs laid was correlated with the level of activity of material ingested. Below 200 μc/g of ingested mixture, egg laying continued throughout the life of treated females, with reduction in numbers after higher doses.

Virgin females were fed mixtures of honey and P^{32} at various levels of radioactivity lower than the sterilization dose of 200 μc/g (Grosch and Sullivan, 1953). Measurements of radioactivity were made daily on the following: (1) whole animals, anesthetized or dead; (2) separate parts, abdomen and anterior portion after cutting the petiole; (3) ovaries; (4) eggs; and (5) excreta. The biological half-life in egg-laying females is from 4 to 5 days. About 60% of the P^{32} lost is by way

of laid eggs. The radioactivity of eggs rises to a peak reached the second day after feeding, and after the third day drops to a low plateau. The shape of the hatchability curve is interpreted as indicating that lethality on the first 5 days is due to gamete-incorporated P^{32}, later to initial plus accrued total dose.

Starved virgin females were fed Sr^{89} citrate in a honey mixture at a level of 227 μc/g of mixture (Grosch and LaChance, 1956). Physical half-life, 55 days, compared with biological half-life, less than 1 day, indicated little retention. Radiostrontium showed up only in the oöcytes and not there after 2 days following its ingestion. Little more than 0.03% was lost by way of the eggs. Egestion in feces and excretion play the important role for this isotope, in contrast to P^{32} where most of it is eliminated in eggs.

Each of four groups of virgin females was fed a different beta-emitting isotope. Feedings were measured by precision weighing (Grosch *et al.*, 1956). As shown by lowered egg production and hatchability, the descending order of effectiveness was the same as the ascending order of physical half-life: P^{32}, Sr^{89}, S^{35}, and Ca^{45}. Results were in agreement with the concept that effectiveness of a given isotope is correlated with the number of beta particles received by the *Habrobracon* females during the first day after ingestion, and with the energy of these emissions.

Grosch (1959) summarizes results of the study of effects of mixtures of radiations on egg production and hatchability. Proportionate doses of X-rays and P^{32} and of gamma and X-rays did not prove additive in induction of permanent sterility or in lowering egg hatchability. They were not as effective as a 100% dose of either would have been alone. Dose rate was considered more important than kind of radiation. The high gamma component of treatment and low hatchability were positively correlated. One component of a mixture may have a predominating effect. This may be determined by the order of exposure.

I. Irradiated and Radioactive Hosts

Two series of experiments have been conducted in testing for effects of (a) irradiated host and (b) radioactive host on the female wasps feeding on them and on the progeny of these females. In the first (A. R. Whiting, 1951), host caterpillars were exposed to doses ranging from 40,000 to 160,000 r. Mated females deprived of food until only young oöcytes and oögonia remained in their ovarioles, were placed with the hosts immediately after exposure and transferred every third day to freshly irradiated caterpillars. In all, 10,934 gametes were tested for

dominant visible mutations and 16,472 for both dominant and recessive visible mutations. One mutant appeared, an F_2 male. Females appeared reluctant to sting and feed on the most heavily irradiated hosts although they did so.

Grosch and Sullivan (1954) injected radiophosphorus at forty-eight dilutions into *Ephestia* larvae paralyzed by sterilized female wasps, and transferred *Habrobracon* eggs laid on control caterpillars to these hosts or to controls. No difference in hatchability was noted between the two groups but a slight effect of the irradiated host was seen upon the percentage pupated, particularly above 600 μc/cc of injectant. There was also observed reduced spinning behavior. Considerable reduction in numbers completing metamorphosis occurred above 300 μc/cc of the injectant. Developmental delay and abnormalities occurred above 800 μc/cc and wing abnormalities were frequent. Females were more radioresistant than the haploid males in respect to all effects. Life span and egg production were not affected.

X. Nitrogen Mustard

With the publication of the discovery of the mutagenic effects of chemical substances (Auerbach and Robson, 1947), a study of the effects on adults, mature sperm, and unlaid eggs of nitrogen mustard, methylbis(β-chloroethyl)amine hydrochloride, was undertaken. The chemical was made up in a 10% solution in distilled water and was delivered to adults as an aerosol. Length of exposure was the variable and 90 minutes was the longest which adults could take and remain functional. Exposed males were kept for 22 hours before mating to control females, in order to avoid contamination of the unexposed eggs which occurred if matings took place at once. Egg hatchability was the criterion of effect.

At least one dominant lethal change was induced in every mature sperm by the shortest exposure given, 2½ minutes (A. R. Whiting and von Borstel, 1954). As length of exposure increased beyond 20 minutes, hatchability percentage of eggs laid by females mated to these males rose above 33% due to sperm inactivation, and adult survivors were haploid males. After 90 minutes of exposure, a hatchability percentage of 71.2 ± 2.27 was obtained, 53.5% sperm inactivation. Theoretically, an exposure of about 200 minutes should produce control hatchability, 98%, and all progeny would be haploid males.

Tests of hatchability over various periods of time after single matings were made, and in some cases continued for 21 days. In no female among the one hundred and three used was the sperm supply exhausted. Re-

peated matings gave no significant evidence for recovery of exposed sperm from either dominant lethal or inactivation effects.

Of metaphase I oöcytes, after a 5-minute exposure, 22.1 ± 4.48% hatched. After a 15-minute exposure, none hatched (von Borstel, 1955a). This suggested that the 100% lethal dose was approximately 10 minutes. Total lethality of prophase I oöcytes could not be obtained. Their lethal dose was as high as or higher than that of the females. Accurate information on hatchability of oöcytes exposed in prophase I was secured by means of a special technique. The schedule worked out for X-ray experiments did not apply here, some metaphase I oöcytes being retained until the second day of laying by the females exposed to the nitrogen mustard. Newly eclosed females with not more than one metaphase I egg were tested. After a 30-minute exposure period, hatchability was 85.3 ± 6.07%; after 60 minutes, 47.4 ± 5.07%. In order to test for recovery, one-half of the females with metaphase I eggs, exposed for 30 minutes, were set at once and one-half, 22 hours later. In neither group did eggs laid the first day develop while for those laid on the second day, a mixture of metaphase I and prophase I, hatchability was 56.0 ± 5.73% for the earlier setting and 52.9 ± 12.1% for the later in one experiment, 53.5 ± 4.96% and 59.6 ± 7.15% for a second. As pointed out above, hatchability of prophase I eggs after a 30-minute exposure was 85.3%.

It is of interest to contrast relative sensitivities of the three cell stages in respect to mutagenic agents. For X-rays, sensitivity increases in the order of prophase I, mature sperm, metaphase I; for nitrogen mustard, prophase I, metaphase I, mature sperm. It was suggested that the small size of sperm cells and their position in the body permitted greater accessibility of their chromosomes to nitrogen mustard.

Subsequent development of eggs which were exposed as metaphase I oöcytes to a lethal dose of nitrogen mustard was studied (von Borstel, 1955b). All were identical in their pattern of development, and presented the same appearance as that described for inviable embryos which were in Type I stage after exposure of eggs or sperm to X-rays, characterized by large Feulgen-negative neuclei.

Eggs undergoing early cleavage were placed in lens paper sacs and immersed in solutions of nitrogen mustard for half an hour (Clark and Beiser, 1953). The solutions were made up immediately before use and were kept at pH 7. After treatment the embryos were washed in a glycine-sodium bicarbonate solution and then in distilled water. Following exposures to a 0.001% solution, hatchability in cultures with haploid and diploid embryos from mated mothers was 22.2 ± 1.4%

(controls 94.4 ±1.4%); in those with haploid embryos only from unmated mothers, 58.1 ± 1.7% (controls 96.7 ± 1.2%). The percentage of females among total adult survivors after exposure of embryos to a 0.00075% solution was 23.1 ± 3.7 (controls 64.3 ± 2.0). Haploid males were more resistant than diploid females. No such differential toxic effect was noted between haploids and diploids treated in later embryonic stages.

Eggs treated during cleavage which hatched, developed into adults; death was consistently embryonic. Lethals induced in older embryos acted in larval or pupal stages. These results agree with those obtained by X-rays. Other chemicals were then tested (1955). Differential response occurred after different nitrogen mustards but when sodium azide or potassium cyanide was used, there were no differences in percentages of lethals between haploids and diploids; for the former, 46 ± 4.05 and 39 ± 3.05; for the latter, 66 ± 5.05 and 64 ± 3.05.

Examination of whole mounts of embryos treated during the cleavage stage with nitrogen mustard showed that they were arrested in development at the end of cleavage and had enlarged nuclei as after exposure to X-rays. The effects of the other compounds were not studied cytologically.

The authors suggested that the study of response of individuals differing in ploidy might furnish (1) a simple method of screening nuclear and non-nuclear poisons; (2) a method of distinguishing mutagens from nonmutagens; and (3) data on the mechanism of radiation damage by determining which chemicals are radiomimetic in this respect.

XI. Colchicine

Inaba (1941) treated eggs, larvae, pupae, and adults with 0.025, 0.05, and 0.1% aqueous solutions of colchicine for varying lengths of time, and found that 0.05 and 0.1% solutions were effective in producing polyploidy. After treatment of male larvae, polyploidy was observed in spermatogonial cells, but these degenerated so that no functional sperm were produced. Females exposed as adults gave rise to tetraploid eggs which, after reduction, developed into impaternate diploid females or males. Forty-four treated females produced 130 impaternate females and 6 impaternate males. Impaternate daughters of heterozygous mothers were distributed into 43 wild type and 13 recessives, 3:1, consistent with the results of K. G. Speicher and B. R. Speicher (1938) for spontaneously occurring impaternate females. Impaternate progeny were not produced before the ninth day after treatment and this led to the estimate that chromosome doubling took place in oögonia.

XII. Concluding Remarks

Several difficult decisions have had to be made during the writing of this review. Which of the more than four hundred publications on *Habrobracon* should be cited? Should fundamental genetics or radiation genetics be emphasized? Many excellent papers have been omitted. Radiation research has perhaps been overemphasized, and the inclusion of some experiments as genetics *sensu stricto* may be questioned.

Recently, this author has been told that research on *Habrobracon* "has run its course," "has little more to contribute," and she is reminded of a similar statement made to her about *Drosophila* just before the discovery of the significance of the salivary chromosomes. She is still sufficiently optimistic about *Habrobracon* to hope that work will be continued on it, and confident that, if not salivary chromosomes, some equally exciting discovery will be made to add to our knowledge of the mechanisms of genetics.

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References

Amy, R. L., and von Borstel, R. C., 1957. The effects of different wave lengths of ultraviolet on the *Habrobracon* egg. *Minerva med.* **2,** 419–422.

Anderson, R. L., 1941. Non-autonomous development of transplanted eyes in *Habrobracon, Proc. 7th Intern. Congr. Genet.* 47.

Atwood, K. C., von Borstel, R. C., and Whiting, A. R., 1956. An influence of ploidy on the time of expression of dominant lethal mutations in *Habrobracon*. *Genetics* **41,** 804–813.

Auerbach, C., and Robson, J. M., 1947. The production of mutations by chemical substances. *Proc. Roy. Soc. Edinburgh* **B62,** 271–283.

Beadle, G. W., Anderson, R. L., and Maxwell, J., 1938. A comparison of the diffusible substances concerned with eye color development in *Drosophila, Ephestia* and *Habrobracon*. *Proc. Natl. Acad. Sci. U.S.* **24,** 80–85.

Bishop, D. W., 1937. Induction of dominant lethal effects by X-radiation in *Habrobracon*. *Genetics* **22,** 452–456.

Bostian, C. H., 1936. Fecundity of triploid females in *Habrobracon juglandis*. *Am. Naturalist* **70,** 40–41.

Bostian, C. H., 1939. Multiple alleles and sex determination in *Habrobracon*. *Genetics* **24,** 770–776.

Bunker, H. P., and Speicher, B. R., 1951. Inheritance of physiological activity in *Habrobracon*. *Genetics* **36,** 545.

Buretz, K. M., 1956. The effects of various ionizing radiations on the survival of metaphase I and prophase I eggs of *Habrobracon juglandis* (Ashmead). Bachelor's Thesis, University of Delaware, Newark, Delaware.

Chittenden, F. H., 1897. Some little-known insects affecting stored vegetable products. *U.S. Dept. Agr., Div. Entomol. Bull.* **8,** 38–40.

Clark, A. M., 1957. The relation of genome number to radiosensitivity in *Habrobracon*. *Am. Naturalist* **41,** 111–119.

Clark, A. M., and Beiser, W. C., Jr., 1953. Effects of methyl *bis* (beta-chloroethyl) amine hydrochloride on haploid and diploid embryos of *Habrobracon*. *Nature* **171,** 178–180.

Clark, A. M., and Herr, E. B., Jr., 1955. The effect of certain gases on the radiosensitivity of *Habrobracon* during development. *Radiation Research* **2,** 538–543.

Clark, A. M., and Kelly, E. M., 1950. Differential radiosensitivity of haploid and diploid prepupae and pupae of *Habrobracon*. *Cancer Research* **10,** 348–352.

Clark, A. M., and Mitchell, C. J., 1951. Radiosensitivity of haploid and diploid *Habrobracon* during pupal development. *J. Exptl. Zool.* **117,** 489–498.

Clark, A. M., and Mitchell, C. J., 1952. Effects of X-rays upon haploid and diploid embryos of *Habrobracon*. *Biol. Bull.* **103,** 170–177.

Clark, A. M., Rubin, M. A., and Fluke, D., 1957. Alpha-particle-induced dominant lethals in the mature sperm of *Habrobracon*. *Radiation Research* **7,** 461–462.

Cushman, R. A., 1922. The identity of *Habrobracon brevicornis* (Wesmael) (*Hym.*, Braconidae). *Proc. Entomol. Soc. Wash.* **24,** 241–242.

Demerec, M., and Fano, U., 1944. Frequency of dominant lethals induced by radiation in sperms of *Drosophila melanogaster*. *Genetics* **26,** 348–360.

Flanders, E. E., 1942. Oosorption and ovulation in relation to oviposition in the parasitic Hymenoptera. *Ann. Entomol. Soc. Am.* **35,** 251–266.

Genieys, P., 1925. *Habrobracon brevicornis* Wesm. *Ann. Entomol. Soc. Am.* **18,** 143–202.

Giles, N. H., 1952. Recent evidences on the mechanism of chromosome aberration production by ionizing radiations. "Symposium on Radiobiology" (J. J. Nickson, ed.), pp. 267–284. Wiley, New York; Chapman & Hall, London.

Glass, B., 1955. A comparative study of induced mutation in the oocytes and spermatozoa of *Drosophila melanogaster*. I. Translocations and inversions. *Genetics* **40,** 252–267.

Grosch, D. S., 1945. The relation of cell size and organ size to mortality in *Habrobracon*. *Growth* **9,** 1–17.

Grosch, D. S., 1950a. Cytological aspects of growth in impaternate (male) larvae of *Habrobracon*. *J. Morphol.* **86,** 153–176.

Grosch, D. S., 1950b. Starvation studies with the parasitic wasp *Habrobracon*. *Biol. Bull.* **99,** 65–73.

Grosch, D. S., 1952. The spinning glands of impaternate (male) *Habrobracon* larvae: morphology and cytology. *J. Morphol.* **91,** 221–236.

Grosch, D. S., 1956. Lethality induced by feeding radiophosphorus to male *Habrobracon*. *Am. Naturalist* **90,** 200–202.

Grosch, D. S., 1959. Wasp egg production and hatchability after the mothers have been exposed to mixtures of radiation. *Atompraxis* **7/8,** 290–292.

Grosch, D. S., and LaChance, L. E., 1956. Fate of radiostrontium fed to *Habrobracon* females. *Science* **123,** 141–142.

Grosch, D. S., and Sullivan, R. L., 1952. The effect of ingested radiophosphorus on egg production and embryo survival in the wasp *Habrobracon*. *Biol. Bull.* **102,** 128–140.

Grosch, D. S., and Sullivan, R. L., 1953. The fate of radiophosphorus ingested by *Habrobracon* females. *Biol. Bull.* **105,** 296–307.

Grosch, D. S., and Sullivan, R. L., 1954. Sequelae of rearing *Habrobracon* on radioactive host larvae. *Growth* **18,** 191–205.

Grosch, D. S., Sullivan, R. L., and LaChance, L. E., 1956. The comparative effectiveness of four beta-emitting isotopes fed to *Habrobracon* females on production and hatchability of eggs. *Radiation Research* **5,** 281–289.

Hase, A., 1922. Biologie der Schlupfwespe *Habrobracon brevicornis* (Wesmael) Braconidae. *Arb. biol. Reichsanstalt. Land-u. Forstwirtsch. Berlin-Dahlem* **11,** 95–168.

Heidenthal, G., 1945. The occurrence of X-ray induced dominant lethal mutations in *Habrobracon*. *Genetics* **30,** 197–205.

Heidenthal, G., 1952. X-ray-induced recessive lethals in *Habrobracon*. *Genetics* **37,** 590.

Heidenthal, G., 1953. A comparison of X-ray-induced dominant and recessive lethals in first meiotic metaphase eggs and in sperm of *Habrobracon*. *Genetics* **38,** 668.

Heidenthal, G., Clark, L. B., and Gowen, J. W., 1955. Comparative effectiveness of roentgen rays of 124 kv and 50 mev on *Habrobracon* eggs treated in first meiotic metaphase. *Am. J. Roentgenol. Radium Therapy, Nuclear Med.* **74,** 677–685.

Henschen, W., 1928. Über die Entwicklung der Geschlechtsdrüsen von *Habrobracon juglandis* Ash. *Z. Morphol. u. Ökol. Tiere* **13,** 144–178.

Horn, A. B., 1943. Proof for multiple allelism of sex-differentiating factors in *Habrobracon*. *Am. Naturalist* **77,** 539–550.

Inaba, F., 1939. Diploid males and triploid females of the parasitic wasp, *Habrobracon pectinophorae* Watanabe. *Cytologia (Tokyo)* **9,** 517–523.

Inaba, F., 1941. Polyploidy in *Habrobracon* induced by colchicine treatment. *Cytologia (Tokyo)* **12,** 66–78.

Inaba, F., 1944. Genetical studies on *Habrobracon pectinophorae*. I. Sex-linked inheritance and sex-determination. *Japan. J. Genet.* **20,** 27–47.

Kenworthy, W., 1956. The effect of oxygen concentration on the dose-action survival curves obtained for *Habrobracon* eggs irradiated during meiotic prophase and metaphase. *Am. Naturalist* **90,** 119–126.

LaChance, L. E., 1955. Effects of delayed oviposition on X-ray induced sterility. *Nucleonics* **13,** 49–50.

LaChance, L. E., 1959. The effect of chelation and X-rays on fecundity and induced dominant lethals in *Habrobracon*. *Radiation Research* **11,** 218–228.

Lüning, K. G., 1954. Effects of oxygen on irradiated males and females of *Drosophila*. *Hereditas* **40,** 295–312.

MacBride, D. H., 1946. Failure of sperm of *Habrobracon* diploid males to penetrate the eggs. *Genetics* **31,** 224.

McCrady, E., 1930. A microscopic study of the effects of X-rays on the ovarioles. *Proc. Pennsylvania Acad. Sci.* **4,** 79–91.

Martin, A., Jr., 1947. "An introduction to the Genetics of *Habrobracon juglandis* (Ashmead)," 205 pp. The Hobson Book Press, New York.

Maxwell, J., 1938. Inactivation of sperm by X-radiation in *Habrobracon*. *Biol. Bull* **74,** 253–255.

Mitchell, C. J., 1948. Decrease in cell size associated with high diploid male viability in *Habrobracon*. *Genetics* **33,** 620.

Muesebeck, C. F. W., Krombein, K. V., and Townes, H. K., 1951. Hymenoptera of America north of Mexico: Synoptic Catalog, *U.S. Dept. Agr., Agr. Monograph* **2,** 1–1420.

Muller, H. J., 1927. Artificial transmutation of the gene. *Science* **66,** 84–87.

Risman, G. C., 1942. Cell size in *Habrobracon*. *Genetics* **27,** 166.

Rogers, R. W., and von Borstel, R. C., 1957. Alpha-particle bombardment of the *Habrobracon* egg. I. Sensitivity of the nucleus. *Radiation Research* **7,** 484–490.

Snell, G. D., 1935. The determination of sex in *Habrobracon*. *Proc. Natl. Acad. Sci. U.S.* **21,** 446–453.

Sonnenblick, B. P., 1940. Cytology and development of the embryos of X-rayed adult *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. U.S.* **26,** 373–381.

Speicher, B. R., 1935. Cell size and chromosomal types in *Habrobracon*. *Am. Naturalist* **69,** 79–80.

Speicher, B. R., 1936. Oogenesis, fertilization and early cleavage in *Habrobracon*. *J. Morphol.* **59,** 401–421.

Speicher, B. R., and Speicher, K. G., 1940. The occurrence of diploid males in *Habrobracon brevicornis*. *Am. Naturalist* **74,** 379–382.

Speicher, K. G., 1934. Impaternate females in *Habrobracon*. *Biol. Bull.* **67,** 277–293.

Speicher, K. G., and Speicher, B. R., 1938. Diploids from unfertilized eggs in *Habrobracon*. *Biol. Bull.* **74,** 247–252.

Spotkov, E. M., 1938. The centriole in the parthenogenetic and fertilized eggs of *Habrobracon juglandis*. *J. Morphol.* **62,** 49–89.

Stancati, M. F., 1932. Production of dominant lethal genetic effects by X-radiation of sperm in *Habrobracon*. *Science* **76,** 197–198.

Sullivan, R. L., and Grosch, D. S., 1953. The radiation tolerance of an adult wasp. *Nucleonics* **11,** 21–23.

Thoday, J. M., and Read, J., 1947. Effect of oxygen on the frequency of chromosome aberrations produced by X-rays. *Nature* **160,** 607–610.

Torvik, M. M., 1931. Genetic evidence for diploidism of biparental males in *Habrobracon*. *Biol. Bull.* **61,** 139–156.

Torvik-Greb, M. M., 1935. The chromosomes of *Habrobracon*. *Biol. Bull.* **68,** 25–34.

von Borstel, R. C., 1955a. Differential response of meiotic stages in *Habrobracon* eggs to nitrogen mustard. *Genetics* **40,** 107–116.

von Borstel, R. C., 1955b. Feulgen-negative nuclear division in *Habrobracon* eggs after lethal exposure to X-rays or nitrogen mustard. *Nature* **175,** 342–343.

von Borstel, R. C., 1957. Nucleocytoplasmic relations in early insect development. *In* "The Beginnings of Embryonic Development" (A. Tyler, R. C. von Borstel, and C. B. Metz, eds.), pp. 175–199. A.A.A.S., Washington, D.C.

von Borstel, R. C., and Moser, H., 1956. Differential ultra-violet irradiation of the *Habrobracon* egg nucleus and cytoplasm. "Progress in Radiobiology" (J. S. Mitchell, B. E. Holmes, and C. L. Smith, eds.), pp. 211–215. Oliver and Boyd, Edinburgh.

von Borstel, R. C., and Rekemeyer, M. L., 1959. Radiation-induced and genetically contrived dominant lethality in *Habrobracon* and *Drosophila*. *Genetics* **44,** 1053–1074.

von Borstel, R. C., and Rogers, R. W., 1958. Alpha-particle bombardment of the *Habrobracon* egg. II. Response of the cytoplasm. *Radiation Research* **8,** 248–253.

von Borstel, R. C., and Smith, P. A., 1960. Haploid intersexes in the wasp *Habrobracon*. *Heredity* **15,** 29–34.

von Borstel, R. C., and Wolff, S., 1955. Photoreactivation experiments on the nucleus and cytoplasm of the *Habrobracon* egg. *Proc. Natl. Acad. Sci. US.* **41,** 1004–1009.

Walker, M. C., 1949. Cytoplasmic bud formation in Hymenopteran spermatogenesis. *Nature* **163,** 645–649.

Whiting, A. R., 1925. The inheritance of sterility and of other defects induced by abnormal fertilization in the parasitic wasp, *Habrobracon juglandis* (Ashmead). *Genetics* **10,** 33–58.

Whiting, A. R., 1927. Genetic evidence for diploid males in *Habrobracon*. *Biol. Bull.* **53,** 438–449.

Whiting, A. R., 1928. Genetic evidence for diploid males in *Habrobracon*. *Am. Naturalist* **62,** 55–58.

Whiting, A. R., 1934. Eye colours in the parasitic wasp *Habrobracon* and their behaviour in multiple recessives and in mosaics. *J. Genet.* **29,** 99–107.

Whiting, A. R., 1940. Sensitivity to X-rays of different meiotic stages in unlaid eggs of *Habrobracon*. *J. Exptl. Zool.* **83,** 249–269.

Whiting, A. R., 1945a. Effects of X-rays in hatchability and on chromosomes of *Habrobracon* eggs treated in first meiotic prophase and metaphase. *Am. Naturalist* **79,** 193–227.

Whiting, A. R., 1945b. Dominant lethality and correlated chromosome effects in *Habrobracon* eggs X-rayed in diplotene and in late metaphase I. *Biol. Bull.* **89,** 61–71.

Whiting, A. R., 1946. Motherless males from irradiated eggs. *Science* **103,** 219–220.

Whiting, A. R., 1948. Incidence and origin of androgenetic males in X-rayed *Habrobracon* eggs. *Biol. Bull.* **95,** 354–360.

Whiting, A. R., 1951. Absence of mutagenic effect of heavily irradiated host on the parasitic wasp *Habrobracon*. *Anat. Record* **111,** 149.

Whiting, A. R., 1954. The effects of oxygen on the frequency of X-ray-induced mutations in *Habrobracon* eggs. *Genetics* **39,** 851–858.

Whiting, A. R., 1955. Androgenesis as evidence for the nature of X-ray-induced injury. *Radiation Research* **2,** 71–78.

Whiting, A. R., and Bostian, C. H., 1931. The effects of X-radiation of larvae in *Habrobracon*. *Genetics* **16,** 659–680.

Whiting, A. R., and Murphy, W. E., 1956. Differences in response of irradiated eggs and spermatozoa of *Habrobracon* to anoxia. *J. Genet.* **54,** 297–303.

Whiting, A. R., and von Borstel, R. C., 1954. Dominant lethal and inactivation effects of nitrogen mustard on *Habrobracon* sperm. *Genetics* **39,** 317–325.

Whiting, A. R., Caspari, S., Koukides, M., and Kao, P., 1958. Stages at death of X-ray-induced embryo lethals in haploids and in heterozygotes of *Habrobracon*. *Radiation Research* **8,** 195–202.

Whiting, P. W., 1918. Sex-determination and biology of a parasitic wasp. *Habrobracon brevicornis* (Wesmael). *Biol. Bull.* **34,** 250–256.

Whiting, P. W., 1921a. Rearing meal moths and parasitic wasps for experimental purposes. *J. Heredity* **12,** 255–261.

Whiting, P. W., 1921b. Studies on the parasitic wasp, *Hadrobracon brevicornis* (Wesmael). I. Genetics of an orange-eyed mutation and the production of mosaic males from fertilized eggs. *Biol. Bull.* **41,** 42–54.

Whiting, P. W., 1924. Some anomalies in *Habrobracon* and their bearing on maturation, fertilization and cleavage. *Anat. Record* **29,** 146.

Whiting, P. W., 1928. The production of mutations by X-rays in *Habrobracon*. *Science* **68,** 59–60.

Whiting, P. W., 1929. X-rays and parasitic wasps. *J. Heredity* **20,** 268–276.
Whiting, P. W., 1931. Diploid male parts in gynandromorphs of *Habrobracon. Biol. Bull.* **61,** 478–480.
Whiting, P. W., 1932a. Mutants in *Habrobracon. Genetics* **17,** 1–30.
Whiting, P. W., 1932b. Diploid mosaics in *Habrobracon. Am. Naturalist* **66,** 75–81.
Whiting, P. W., 1932c. Modification of traits in mosaics from binucleate eggs of *Habrobracon. Biol. Bull.* **63,** 296–309.
Whiting, P. W., 1932d. Reproductive reactions of sex mosaics of a parasitic wasp, *Habrobracon juglandis. J. Comp. Psychol.* **14,** 345–363.
Whiting, P. W., 1933. Selective fertilization and sex-determination in Hymenoptera. *Science* **78,** 537–538.
Whiting, P. W., 1934a. Egg-trinuclearity in *Habrobracon. Biol. Bull.* **66,** 145–151.
Whiting, P. W., 1934b. Mutants in *Habrobracon,* II. *Genetics* **19,** 268–291.
Whiting, P. W., 1935a. Sex determination in bees and wasps. *J. Heredity* **26,** 263–278.
Whiting, P. W., 1935b. Recent X-ray mutations in *Habrobracon. Proc. Pacific Acad. Sci.* **9,** 60–63.
Whiting, P. W., 1936. Dominant lethal genetic effects caused by neutrons. *Science* **84,** 68.
Whiting, P. W., 1938. The induction of dominant and recessive lethals by radiation in *Habrobracon. Genetics* **23,** 562–572.
Whiting, P. W., 1940. Multiple alleles in sex determination of *Habrobracon. J. Morphol.* **66,** 323–355.
Whiting, P. W., 1943a. Multiple alleles in complementary sex determination of *Habrobracon. Genetics* **28,** 365–382.
Whiting, P. W., 1943b. Intersexual females and intersexuality in *Habrobracon. Biol. Bull.* **85,** 238–243.
Whiting, P. W., 1943c. Androgenesis in the parasitic wasp *Habrobracon. J. Heredity* **34,** 355–366.
Whiting, P. W., 1949. The identity of *Habrobracon pectinophorae* Watanabe (Hymenoptera: Braconidae). *Entomol. News* **60,** 113–115.
Whiting, P. W., and Anderson, R. L., 1932. Temperature and other factors concerned in male biparentalism in *Habrobracon. Am. Naturalist* **66,** 420–432.
Whiting, P. W., and Starrells, R., 1950. Evidence for haploid intersexual females in *Habrobracon. Am. Naturalist* **84,** 467–475.
Whiting, P. W., and Wenstrup, E. J., 1932. Fertile gynandromorphs in *Habrobracon. J. Heredity* **23,** 31–38.
Whiting, P. W., and Whiting, A. R., 1925. Diploid males from fertilized eggs in Hymenoptera. *Science* **62,** 437–438.
Whiting, P. W., and Whiting, A. R., 1927. Gynandromorphs and other irregular types in *Habrobracon. Biol. Bull.* **52,** 89–121.
Whiting, P. W., Greb, R. J., and Speicher, B. R., 1934. A new type of sex-intergrade. *Biol. Bull.* **66,** 152–165.

GENETIC ASPECTS OF OMMOCHROME AND PTERIN PIGMENTS*

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Page

I. Introduction 349
II. The Pigmentary System and Its Chemical Bases 350
III. The Ommochromes 356
A. Kynurenine and 3-Hydroxykynurenine as Precursors of Ommochromes in Nonautonomous Mutants 356
B. Relations to Tryptophan Metabolism 359
C. Autonomous Mutants Affecting Tryptophan Metabolism 363
D. Homologous Mutants 364
E. Multiple Alleles and Modifiers Affecting Ommochrome Synthesis . . 366
IV. The Pterins 368
A. Effects of Genes on Pterin Pattern (Autonomous Mutants) . . . 368
B. Variations of Pterin Pattern Not Caused by Single Genes 371
C. Nonautonomous Mutants 372
D. Isoxanthopterin as a Secondary Sex Character 375
E. The Influence of Recessive Alleles 376
V. Pleiotropic Action of Genes Affecting Pigment 377
Ommochromes and Pterins 377
VI. Predetermination 389
VII. Modification by External Factors 391
VIII. Pterin Mutants in Vertebrates 392
IX. Taxonomic Questions and Concluding Remarks 393
References 395

I. Introduction

The action of genes results in the specific pattern of phenes, comprising the phenotype of the organism. Since its beginning, physiological genetics has been concerned with the problem of how the first step of gene action leads to phenotypic expression. In phages, bacteria, and fungi, biochemical genetics has yielded an intimate knowledge of the action of genes on metabolism. In contrast, very little is known about the complex processes of gene-dependent formation of the "phenotype" in higher organisms.

Gene-dependent formation of ommochromes (which are mainly restricted to arthropods) and pterin pigments presents models in which

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morphological phenes are relatively closely connected with gene-controlled chemical processes. The study of this topic has therefore resulted in one of the best illustrations we have of the problem of gene-dependent formation of the "phenotype" in higher organisms.

In addition, the ommochromes and pterins represent interesting groups of naturally occurring compounds, whose structure, biosynthesis, and physiological interrelationships might be elucidated by the study of mutants affecting them.

Both aspects of the genetic study of pterins and ommochromes, which are complementary to one another, will be discussed to sketch our present knowledge of these pigments in relation to genic action.

II. The Pigmentary System and Its Chemical Bases

Early investigations have shown that in the eyes of *Drosophila melanogaster* two different types of pigments can be distinguished: a brown and a red one. This was revealed by (1) a different behavior toward solvents: The red pigment dissolves in water, whereas the brown one does not (Mainx, 1938). The red pigment can be extracted from the heads by ethanol, acidified to pH 2.0, and the brown one, which remains, by methanol + HCl (1%) (Ephrussi and Herold, 1944); (2) a different time of formation during pupal development: In *D. melanogaster* the brown pigment appears at about 53–55 hours, the red one, about 71 hours after pupation (Danneel, 1941; Hadorn and Ziegler, 1958). Each component may also be affected by different mutations. For example, in mutant *brown* (*bw*) and mutant *purple* (*pr*) of *D. melanogaster* the formation of the red pigment is suppressed, the brown pigment is lost by action of genes *scarlet* (*st*), *vermilion* (*v*), or *cinnabar* (*cn*). Therefore, a combination of mutants such as *st* and *bw* in homozygous condition results in white eyes (Mainx, 1938; Crew and Lamy, 1932).

The brown pigments, called ommochromes (Becker, 1942) are found in the eyes of all arthropods as well as in many mollusca (Butenandt *et al.*, 1958; Butenandt, 1959). The chemical structures of these redox pigments were elucidated by Butenandt and his co-workers (Butenandt *et al.*, 1954a,b; Butenandt and Neubert, 1958). There are two different types of ommochromes, the ommatins and the ommins. The ommatins are alkali-sensitive pigments of the phenoxazone type. The first one, which was isolated in crystalline form was xanthommatin from the blowfly *Calliphora erythrocephala*. In nature it is mostly found in its stable yellow-brown oxidized form (Fig. 1), whereas the closely related rhodommatin is stable in its reduced form (Butenandt, 1959).

The same alkali-stable ommin, which has a higher molecular weight than the ommatins, has been found in all samples investigated. This ommin probably is a triphenoxazine thiazine.

The red, water-soluble component, which among insects is only found in Drosophilidae, is a pterin. Other pterins, which are yellow, faintly colored, or even colorless occur in the eyes, Malpighian tubules, testes, etc. of most insects. In addition, pterins are also found in the "pterinophores" in the skin of poikilothermic vertebrates and in their retinal pigment epithelium (cf. Ziegler, 1956b,c). As far as we know, all naturally

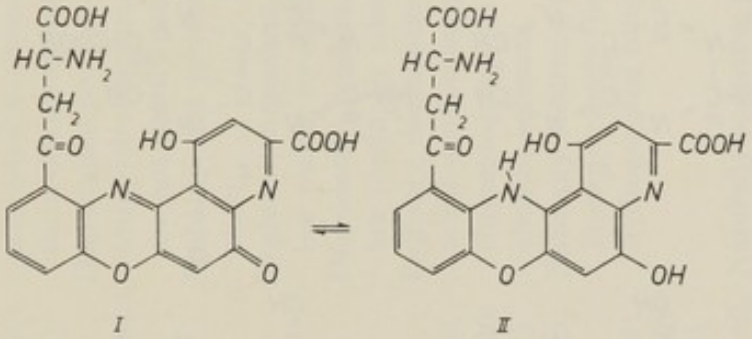


FIG. 1. Chemical structure of xanthommatin. Key: I = oxidized form (yellow-brown); II = reduced form (red).

occurring pterins are derived from 2-amino-4-hydroxypteridine (Fig. 2). In the last years, 2-amino-4-hydroxypteridine, 2-amino-4-hydroxypteridine-6-carboxylic acid (= pterincarboxylic acid), isoxanthopterin, xanthopterin, a xanthopterin-like compound, and biopterin (Fig. 2) were identified after chromatographic separation (Viscontini *et al.*, 1955; Forrest and Mitchell, 1955; Ziegler, 1956b). Most of these pterins found, except isoxanthopterin, seem to be degradation products of the naturally occurring pterins. Only in cases where xanthine dehydrogenase activity is blocked (see Section IV,C), 2-amino-4-hydroxypteridine may also occur in living tissue instead of the missing isoxanthopterin.

The natural products probably are the red pterin (consisting of three closely related pterins: drosopterin, isodrosopterin, and neodrosopterin; Viscontini *et al.*, 1955), the yellow pterin (= sepiapterin; Ziegler and Hadorn, 1958) and a nonfluorescing pterin, which immediately starts to fluoresce after irradiation at 365 mμ (Ziegler, 1956a). This latter compound is a derivative of tetrahydrobiopterin (possibly a *N*(8)-riboside; Ziegler, 1960a). It is known for certain that the yellow pterin also is a derivative of biopterin (Fig. 2) (Viscontini and Möhlmann, 1959; Ziegler, 1960a). It was isolated from *se*-flies of *Drosophila melanogaster* first by Forrest and Mitchell (1954a), and its tentative formulation was given as 2-amino-4-hydroxy-7,8-dihydro-8-lactylpteridine-6-carboxylic acid (Forrest and Mitchell, 1954b). However, evidence was given that the side chain at *C*(6) in the intact molecule does not show a free carboxylic group (Ziegler, 1956a). Forrest *et al.* (1959) suggest that it is 2-amino-4,6-hydroxy-6-(1-oxy-2-hydroxypropyl)pteridine. However, on the one hand, it reduces $K_3Fe(CN)_6$ and, on the other hand,

it is easily reduced to the tetrahydrobiopterin compound mentioned above (Ziegler, 1960a). Therefore it seems to be also a hydrogenated biopterin compound and might be the dihydro product.[1] The constitution of the red pteridines (drosopterins; Viscontini *et al.*, 1957) is still unknown. But the fact that red pteridines arise when 2-amino-4-hydroxy-

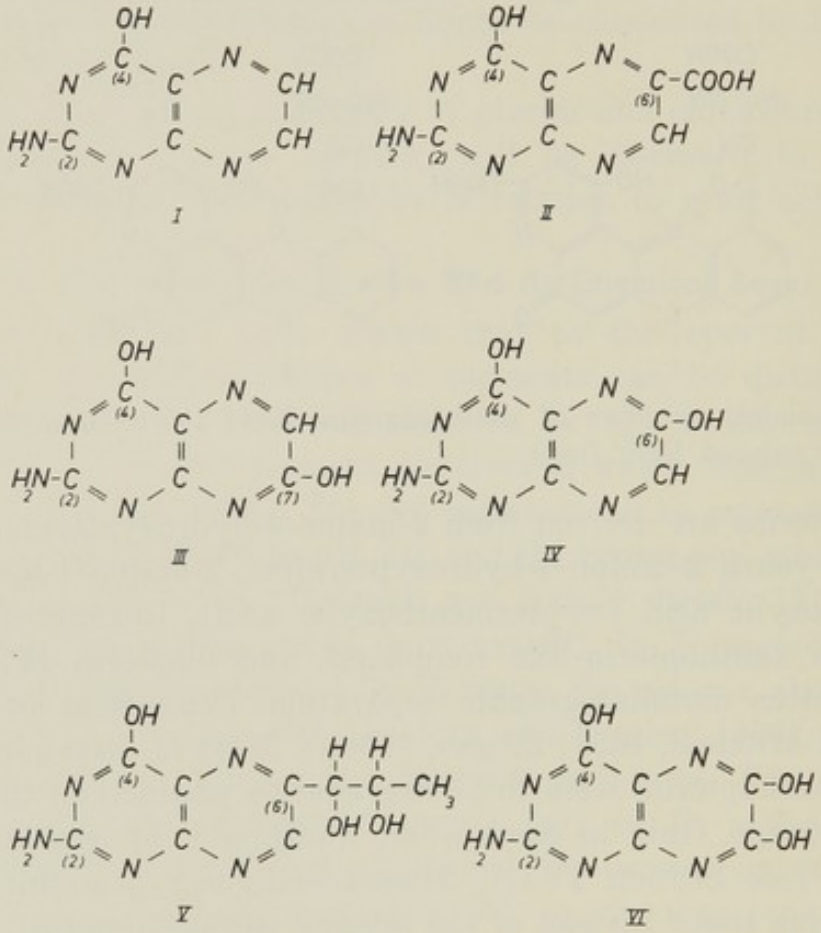


FIG. 2. Chemical structure of some pterins. Key: I = 2-amino-4-hydroxypteridine; II = 2-amino-4-hydroxypteridine(6)carbonic acid; III = isoxanthopterin; IV = xanthopterin; V = biopterin; VI = leucopterin.

5,6,7,8-tetrahydropteridine is reoxidized in the air (Viscontini and Weilenmann, 1959) supports the suggestion that the red pteridines may constitute final oxidation products. Formation of double bonds or condensation of two pteridines, which goes hand in hand with dehydrogenation, may cause the bathochromic shift.[2]

[1] In the meantime the dihydro structure has been confirmed: Taira, T. [*Nature* **189,** 231 (1961)] was able to perform enzymatic reduction with dihydrofolic acid reductase and TPNH to a tetrahydro compound. Vice versa, bacterial incubation experiments yielded an enzymatic oxidation of the tetrahydrobiopterin compound to the yellow pterin [Nathan, H. and Ziegler, I., *Z. Naturforsch.* **16b,** 262 (1961)].

[2] The basic structure of neodrosopterin as well as of drosopterin is intimately related to biopterin and therefore both are growth factors for *Crithidia fasciculata* [Ziegler, I. and Nathan, H., *Z. Naturforsch.* **16b,** 260 (1961)].

TABLE 1

The Pterins in the Eyes of *Drosophila melanogaster*

Compound	Synonym	Examples where it is accumulated	Remarks
2-Amino-4-hydroxypteridine	Fl4 (Hadorn and Mitchell, 1951) HB-1 (Viscontini *et al.*, 1955) 2-Amino-4-hydroxypterin	*se* (Ziegler and Hadorn, 1958) *sed* (Hadorn and Mitchell, 1951) *ry* (Hadorn and Schwinck, 1956) *cl* (Hadorn and Mitchell, 1951)	Probably in most cases a degradation product; when it occurs naturally it is converted to isoxanthopterin by xanthine dehydrogenase
2-Amino-4-hydroxypteridine(6)carbonic acid	Pterincarbonic acid	*se* (Ziegler and Hadorn, 1958) *ry* (Hadorn and Schwinck, 1956)	Degradation product of yellow pterin and tetrahydrobiopterin derivative, if they are not strictly protected from light
Isoxanthopterin	Fl3 (Hadorn and Mitchell, 1951)	*dor* (Counce, 1957)	Probably end product of pterin metabolism
Xanthopterin	X-pterin (Ziegler and Hadorn, 1958)	*se* (Ziegler and Hadorn, 1958)	Degradation product, probably from yellow pterin, by alkaline solvents
Xanthopterin-like pterin	*X*-pterin (Ziegler and Hadorn, 1958)	*se* (Ziegler and Hadorn, 1958)	Degradation product, probably from yellow pterin, by alkaline solvents
Biopterin	Fl4 (Hadorn and Mitchell, 1951) HB-2 (Viscontini and Möhlmann, 1959)	*se* (Ziegler and Hadorn, 1958) *sed* (Hadorn and Mitchell, 1951 *ry* (Hadorn and Schwinck, 1956) *cl* (Hadorn and Mitchell, 1951)	Probably degradation product from the tetrahydrobiopterin derivative when chromatographed in the dark in acid or alkaline solvents
Yellow pterin	Fl5 (Hadorn and Mitchell, 1951) Sepia pterin (Ziegler and Hadorn, 1958)	*se* (Forrest and Mitchell, 1954a; Ziegler and Hadorn, 1958) *sed* (Hadorn and Mitchell, 1951) *ry* (Hadorn and Schwinck, 1956) *cl* (Hadorn and Mitchell, 1951)	Naturally occurring pterin; structure unknown; closely related to biopterin; hydrogenated compound (dihydro ?)
Red pterin	Fl1 (Hadorn and Mitchell, 1951) Drosopterin (Viscontini *et al.*, 1957)	wild type (Hadorn and Mitchell, 1951) *b* (Hadorn and Mitchell, 1951) *l* (Hadorn and Mitchell, 1951)	Naturally occurring pterin; structure unknown; consists of three closely related pterins: drosopterin, isodrosopterin, and neodrosopterin
Tetrahydrobiopterin derivative		*se* (Ziegler, 1960a)	Naturally occurring pterin; not fluorescing; rapidly oxidized and decomposed by light

TABLE 2

The Pterins in the Eyes of *Ephestia kühniella*, *Plodia interpunctella*, and *Ptychopoda seriata*

Compound	Synonyms	Examples of mutants where it is accumulated	Remarks
2-Amino-4-hydroxy-pteridine	k_1 (Viscontini *et al.*, 1956) 2-Amino-4-hydroxypterin	*a Ephestia* (Hadorn and Kühn, 1953) *bch Ephestia* (Hadorn and Kühn, 1953) *"Stamm 6" Plodia* (Almeida, 1958b)	—
Isoxanthopterin	g (Viscontini *et al.*, 1956)	*bch Ephestia* (Hadorn and Kühn, 1953)	—
Xanthopterin	*h* (Viscontini *et al.*, 1956)	*a Ephestia* (Hadorn and Kühn, 1953) *ra Plodia* (Almeida, 1958b)	—
Xanthopterin-like pterin	$c_1 + c_2$ (Viscontini *et al.*, 1956)	*a Ephestia* (Kühn and Egelhaaf, 1959b) *dec Ptychopoda* before irradiation (Kühn and Egelhaaf, 1959b)	Converted to red pterin
Biopterin	k_2 (= HB-2 of *Drosophila mel.*; Viscontini *et al.*, 1956)	*bch Ephestia* (Hadorn and Kühn, 1953)	On k_2-spot also a nonfluorescent spot, which converts to pterin-carbonic acid on irradiation (Kühn and Egelhaaf, 1959b)
Red pterin	—	—	Similar to pterorhodin (Kühn and Egelhaaf, 1959)
2-Amino-4-hydroxy-pteridine(6)carbonic acid	i (Egelhaaf, 1956c) Pterincarbonic acid	*a Ephestia* (Hadorn and Kühn, 1953)	—
e	—	*a Ephestia* (Hadorn and Kühn, 1953) *ra Plodia* (Almeida, 1958b)	Unidentified
f	—	*a Ephestia* (Hadorn and Kühn, 1953)	Unidentified

TABLE 3
The Pterins in the Eyes of Other Insects

Compound	Synonyms	Occurrence	Examples for accumulation	Remarks
2-Amino-4-hydroxy-pteridine(6)carbonic acid	Pterincarbonic acid Blau 3 (Autrum and Langer, 1958)	*Calliphora erythr.*	—	Degradation product
2-Amino-4-hydroxy-pteridine	2-Amino-4-hydroxypterin	*Calliphora erythr.*	—	—
Yellow pterin	Xanthopterin B ? (Tsujita and Sakaguchi, 1955)	*Calliphora erythr.* *Bombyx mori*	*w Calliphora* (Ziegler, 1960b) *lem Bombyx* (Tsujita and Sakaguchi, 1955)	Found in epidermal tissue of *Bombyx*
Biopterin	—	*Calliphora erythr.*	—	—
Tetrahydrobiopterin derivative	—	*Calliphora erythr.*	—	—
Leucopterin	—	*Bombyx mori*	—	Found in epidermal tissue of *Bombyx*
Isoxanthopterin	—	—	—	Found in epidermal tissue of *Bombyx*

Tables 1, 2, and 3 summarize the chemical structures and synonyms of these compounds. In the following pages, 2-amino-4-hydroxypteridine itself and all its derivatives will be designated as pterins. The chemical structures are given in Fig. 2.

In the course of time many mutations were identified, which affect either the ommochromes or the pterins, or both of them. For economic reasons these mutants were studied intensively in *Ephestia, Bombyx,* and especially in *Drosophila,* where they occur as spontaneous as well as induced mutations. However, such mutations are widely distributed among insects as in *Plodia* (Almeida, 1958a,b), *Apis* (Green, 1955), *Calliphora* (Tate, 1947a), and many other insects, corresponding to the wide occurrence of both pigments in arthropods.

III. The Ommochromes

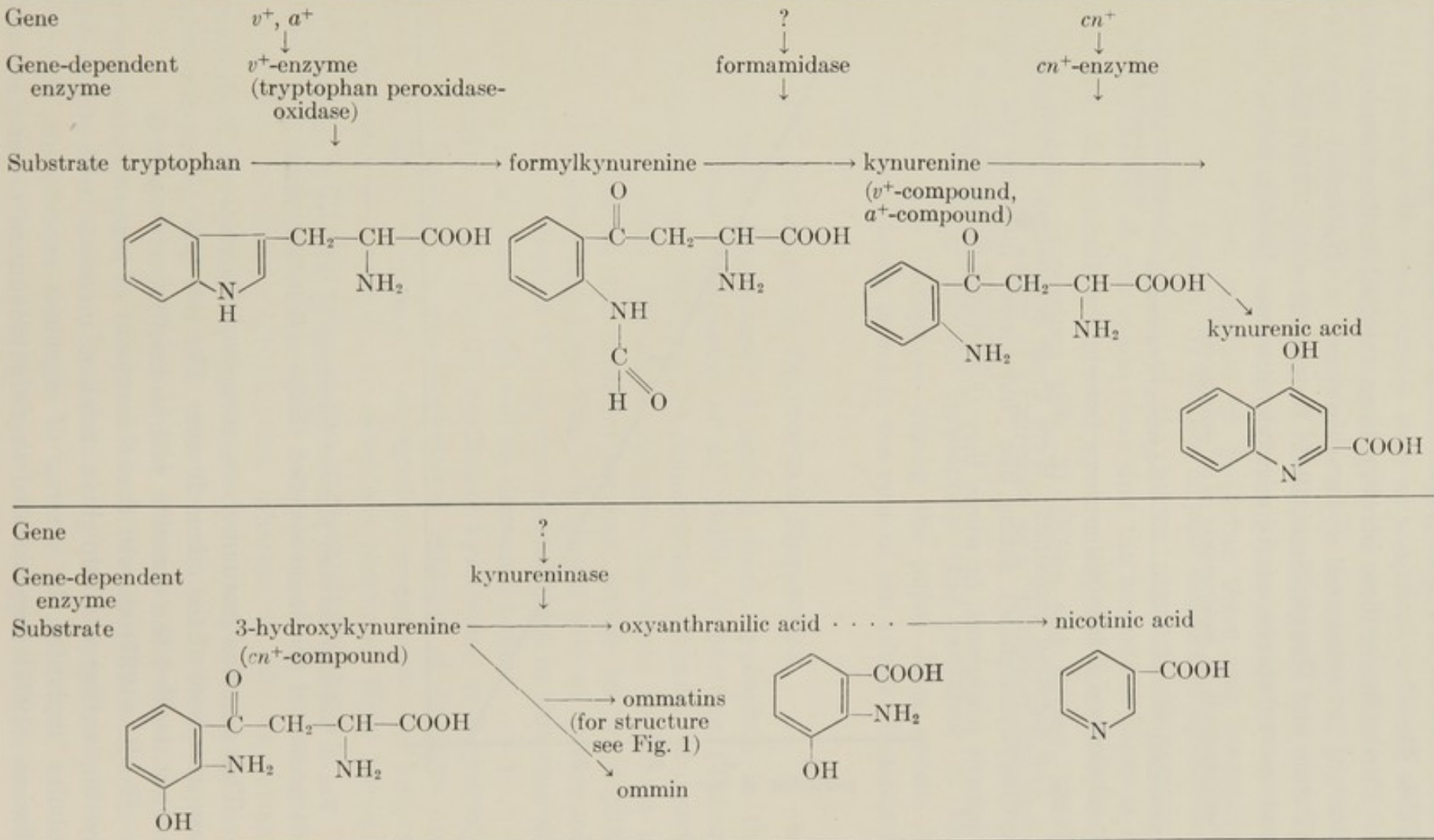
A. Kynurenine and 3-Hydroxykynurenine as Precursors of Ommochromes in Nonautonomous Mutants

The mutation $a^+ \rightarrow a$, which occurred spontaneously in *Ephestia kühniella* (Kühn and Henke, 1930), offered a fortunate opportunity, which resulted in one of the first great steps of biochemical genetics. This mutation causes red eyes instead of black ones. Furthermore, it is responsible for the lack of pigment in testes, brain, larval skin, and eyes. By transplantation experiments during the last larval period, Caspari (1933) first showed that the *aa*-eyes and testes show nonautonomous behavior: *aa*-testes, implanted into larvae of a^+a^+-genotype, are able to form wild type pigment. The reciprocal transplantation of a^+a^+-tissue into *aa*-larvae also causes pigment formation in the testes as well as in the eyes.

Also in *D. melanogaster,* two mutants, *cinnabar* (*cn*) and *vermilion* (*v*) were found which were unable to synthesize the brown pigment and showed a nonautonomous behavior in transplantation experiments (Beadle and Ephrussi, 1935). Again the conclusion was drawn that the wild type contains a substance which enables implants of the eye discs of these two mutants to form brown pigment. Moreover, the lymph of host-*cn*-mutants supplies a *v*-implant eye with the substance needed to perform pigment synthesis, whereas a *v*-host is not able to produce the lacking compound for a *cn*-implant (Beadle and Ephrussi, 1935).

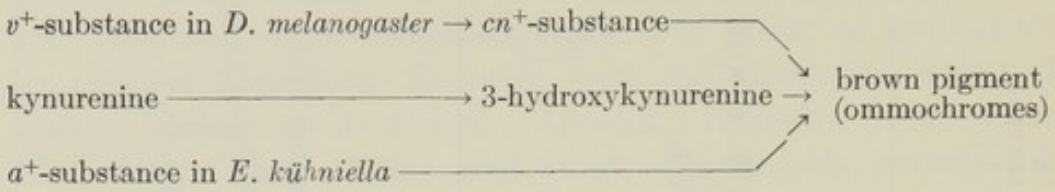
Several reviews tell the story of the "eye color hormones" up to 1942 (Ephrussi, 1942a,b; Plagge, 1939; Kühn, 1941a). The details of the steps, pursued by two groups of workers (Ephrussi, Beadle, and Chevais with *Drosophila;* Kühn, Caspari, Becker, and Plagge with *Ephestia*) will not be repeated here, except for noting that they resulted in the

TABLE 4
Pathway of Tryptophan Metabolism, Affected by Nonautonomous Eye-Color Genes in *Drosophila melanogaster* and *Ephestia kühniella*



elucidation of the first gene-controlled reaction chain (see below and Table 4).

Furthermore, these investigations resulted in the chemical identification of v^+-substance (= a^+-substance in *Ephestia*) as kynurenine (Tatum and Haagen-Smit, 1941; Butenandt *et al.*, 1940; Kikkawa, 1941) and cn^+-substance as 3-hydroxykynurenine (Butenandt *et al.*, 1949):



The question remained: Were these compounds (kynurenine and 3-hydroxykynurenine) acting like "hormones," inducing the formation of pigment precursors, or where they the precursors themselves?

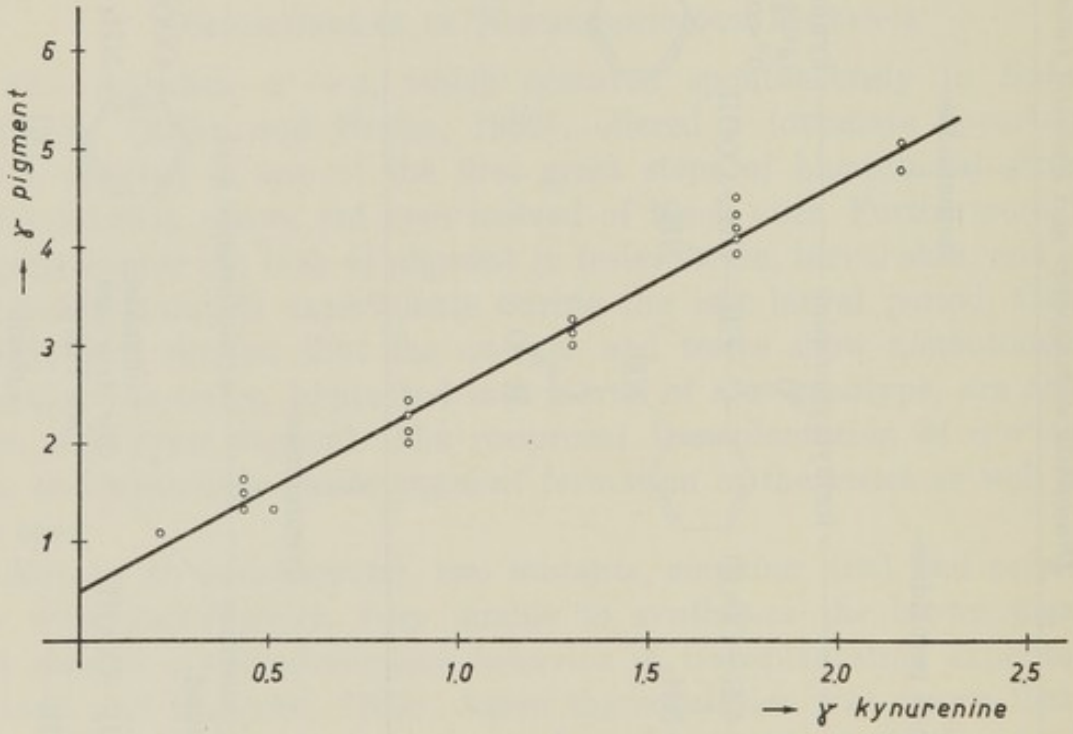


FIG. 3. Relation between pigment formation in the eyes of *a-Ephestia kühniella* and amount of kynurenine supplied (Kühn and Becker, 1942).

This question has now been unequivocally resolved. Both compounds are precursors of the ommochromes. The two types of experiments which mainly decided the question will be briefly reviewed here.

Beadle (1937) showed that the amount of pigment in v-eyes, induced by implanting v^+-Malpighian tubules, increases with the number of tubules implanted. In a series of ingenious experiments, Ephrussi and Chevais (1937) demonstrated that the amount of v^+- and cn^+-substance

(kynurenine and 3-hydroxykynurenine, respectively), provided by implanted eye discs of the *white*-alleles such as *w*-apricot, *w*-blood, *w*-331 (which form both compounds), increases with the amount not needed for their own pigment production and is reflected in the amount of pigment formation induced. The experiments of Khouvine, Ephrussi, and Chevais (cf. Plagge, 1939) showed that with increasing amounts of cn^+-extracts (= 3-hydroxykynurenine) supplied to *cn*-larvae there was increasing coloration of the eyes. Finally, by the use of pure samples of kynurenine and photometric determination of the brown pigment (skotommin) formed in *a-Ephestia,* it was made clear that the amount of pigment is proportional to the amount of injected kynurenine, and moreover, they are in stoichiometric proportions (Fig. 3) (Kühn and Becker, 1942). Both results exclude the action of kynurenine as a "hormone" and prove it to be a direct pigment precursor.

Finally, radioactively labeled tryptophan (the precursor of kynurenine) injected into *Calliphora* during the pupal stage results in labeled xanthommatin being found in the eyes of the adults (Butenandt and Neubert, 1955).

B. Relations to Tryptophan Metabolism

The v^+-substance, kynurenine, is derived from tryptophan in the metabolism of rabbits (Butenandt *et al.,* 1940), of bacteria (Tatum, 1939b; Butenandt *et al.,* 1940), and in ommochrome formation of insects (Butenandt *et al.,* 1940). Therefore, the gene-dependent variations in ommochrome metabolism are expected to show effects on tryptophan metabolism in the whole organism. This is a typical case of "pleiotropic effect of a gene" (see Section V); as the interrelations in this case are better known than in others, they will be discussed here.

If the organism is not able to synthesize kynurenine or 3-hydroxykynurenine, in analogy to the biochemical genetics of bacteria and fungi, the immediate precursor is expected to accumulate—as long as it is not degraded or converted by secondary reactions. What happens with tryptophan and kynurenine in the ommochrome mutants?

Green (1949b) stated that adult *v*-mutants of *D. melanogaster* accumulate free nonprotein tryptophan, whereas in the *cn*-mutant, which is not able to oxidize kynurenine to 3-hydroxykynurenine, more kynurenine is found than in wild type flies. In part this kynurenine seems to be degraded to kynurenic acid (Danneel and Zimmermann, 1954). A suppressor-gene of *v* (su^2-*s*) causes the appearance of brown pigment and a decrease in the amount of free tryptophan (Green, 1949b).

In *a-Ephestia,* Caspari (1946) showed that tryptophan in proteins

was slightly increased. These findings were confirmed by Butenandt and Albrecht (1952), who also showed increased protein tryptophan (in relation to total amount of nitrogen) in larvae and moths of several modifications of *aa*-animals (*aa*-dunkelrot; *aa*-orange; *aa*-hellgelb; and *aa*-klassisch-rot). With refined methods (chromatographic separation of tryptophan and fluorometric determination of tryptochrome after treatment with KIO_3 make possible the detection of <0.1 μg tryptophan on the paper sheet), Egelhaaf (1957) demonstrated a clear difference in free tryptophan between a^+- and *a-Ephestia* throughout the whole life cycle. All free tryptophan disappears in wild type *Ephestia* with oviposition; no tryptophan is found in the excreta (Egelhaaf, 1956a). The relatively large amounts of tryptophan, which are stored in the gut of *aa*-larvae are excreted to some extent with the meconia, but are partially retained in the coecal bladder. With the sensitive method mentioned above, Egelhaaf (1957) also demonstrated a concentration of tryptophan in the hemolymph of *aa*-larvae, which is about six times as high as in a^+a^+, where it is scarcely traceable. In *aa*-animals the concentration remains constant in adults. The presence of a larger volume of hemolymph during the larval period, and thus an increased amount of circulating free tryptophan in *aa*-animals, makes feasible the subsequent formation there of protein rich in tryptophan.

The proteins of *aa*-animals differ from normal a^+-protein: they are more resistant to autolysis (Caspari and Richards, 1948a) and show serological differences (Caspari, 1950). It is uncertain how far these differences are related to the altered tryptophan content.

Kynurenine, which in a^+-*Ephestia* appears during the second and third days of the pupal stage, is consumed in relatively large amounts mostly in ommochrome synthesis. Small amounts are excreted as fluorescent compounds, which are very similar to kynurenic acid. As expected, these a^+-excretory products are lacking in *a-Ephestia* (Egelhaaf, 1956a).

Two possible mechanisms by which the genes $v^+(=a^+)$ and cn^+ control the formation of both kynurenine and 3-hydroxykynurenine have been proposed by Caspari (1946) and Butenandt and Albrecht (1952): (1) the competent enzyme is not synthesized or is blocked in its activity, or (2) the primary step is a change in the protein constitution of the cell whereby its biochemical composition is changed (deficiency in tryptophan as substrate, caused by rapid incorporation into protein), and therefore the enzyme, even though it may be present in *aa*, cannot exert its activity.

Egelhaaf (1958), using isolated testes of larvae and ovaries of adults of *Ephestia* as a source of enzyme, first demonstrated that

a^+a^+-animals are able to convert added L-tryptophan into kynurenine, the amount of which was determined by the fluorometric method after chromatographic separation. Animals of mutant *aa* lack this ability.

Similarly in *D. melanogaster,* Baglioni (1959), using supernatants of homogenized flies, and estimating the amount of kynurenine by diazotization (Bratton-Marshall method), showed that, in contrast to the wild type, the *v*-mutant does not have the enzyme for formation of kynurenine from tryptophan. In *Ephestia* as well as in *Drosophila* the question remains: is the mutant unable to perform the enzyme formation or is the enzyme formed but blocked in its activity by an inhibitor?[3]

Knox and Mehler (1950), using liver preparations of many species, have shown that the first reaction in kynurenine formation from tryptophan is the peroxidation of tryptophan, which is followed by an oxidation, yielding formylkynurenine and H_2O_2 (Mehler and Knox, 1950). Kynurenine formamidase then hydrolyzes formylkynurenine, yielding kynurenine (Table 4).

Feeding experiments (Green, 1949b, 1952) have shown that the *v*-mutants of *Drosophila melanogaster* and *D. virilis* are able to convert formylkynurenine to brown pigment. In agreement with this finding Glassmann (1956) was not able to demonstrate any differences in the activity of formamidase between wild type and *v*. Seemingly, then, the enzyme involved with the first step, a tryptophan peroxidase-oxidase complex, is affected in the mutant *v* and by implication also in *a*.

It has been demonstrated in *Neurospora* that nicotinic acid is derived from tryptophan and that 3-hydroxykynurenine is situated in its biosynthetic pathway (Beadle and Mitchell, 1947). Tatum (1939a) showed, that *v-D. melanogaster* is dependent on an exogenous supply of nicotinic acid in a fully synthetic medium. It seems that other pathways of nicotinic acid synthesis are not used by *Drosophila*. Therefore, the nicotinic acid deficiency of *v-Drosophila* may be considered a further phene of the pleiotropic actions on tryptophan metabolism. After 3-hydroxykynurenine the pathway branches: oxyanthranilic acid, which is only on the pathway leading to nicotinic acid (Table 4) is not able to induce pigment formation in the *v*-mutant (Butenandt *et al.*, 1949). However, in *Bombyx mori,* mutant *w*-1, in which the conversion of kynurenine to 3-hydroxykyurenine is blocked, and *w*-2, in which the further metabolism of 3-hydroxykynurenine to brown pigment is blocked, seem to be able to synthesize nicotinic acid; Kikkawa (1941) therefore

[3] Egelhaaf, A. and Caspari, E. (1960) have recently shown in experiments with mixed homogenates that the inability of *aa* to oxidize tryptophan to kynurenine is not due to the presence of an inhibitor but to loss of activity of the enzyme catalyzing the reaction [*Z. Vererbungslehre* **91,** 373–379 (1960)].

suggested another pathway of vitamin synthesis, different from that found in mammals and in microorganisms.

The picture of gene action on ommochrome formation and its interrelations to tryptophan metabolism may be summarized in Table 4.

The quantitative studies of Egelhaaf (1957) mentioned above round out the experiments of Caspari (1933) and others who used transplantation experiments in *Ephestia* later on. It has been shown (cf. Plagge, 1939) that head and fat bodies of wild type, which are good donors of a^+-substance (kynurenine), also proved to be rich in kynurenine. Testes and ovaries, also very active donors of a^+-substance, do not store any demonstrable amounts of kynurenine, but they are a very active source of the a^+-enzyme (tryptophan peroxidase-oxidase) and therefore provide a permanent new supply of kynurenine by synthesis from tryptophan (Egelhaaf, 1958). Hemolymph, which is not an a^+-donor, is entirely devoid of kynurenine. Whether the different ability of various organs of *D. melanogaster* to induce pigment formation is a reflection of the absence or the presence of kynurenine or of the enzyme complex requires similar investigations.

The effect of the v^+- and cn^+-alleles on ommochrome synthesis is not restricted to the ommochromes of the eye. A mutant of *D. melanogaster,* which shows rusty red Malpighian tubules (mutant "red Malpighian tubules"), caused by ommochromes, has no pigment in the tubules after crossing *red/red* with *v/v* or *cn/cn* (Aslaksen and Hadorn, 1957).

Malpighian tubules of wild type larvae, which were exposed to 254 $m\mu$ irradiation, hypotonic solutions, or mechanical damage, form a red pigment after reimplantation, which seems to be an ommochrome. Malpighian tubules from *v*- or *cn*-mutants are not able to synthesize this pigment after UV-irradiation or mechanical damage except when those mutants are supplied with kynurenine or 3-hydroxykynurenine. These compounds also can be supplied by a "natural source," using a +-host for reimplantation (Ursprung *et al.,* 1958).

Tryptophan metabolism is also affected by mutant *g* in *Plodia interpunctella,* which causes yellow wings instead of red ones (Möhlmann, 1958). Even though the wing pigment (red and yellow) could not be identified with ommochromes (or any other known pigment), mutation *g* causes a lack of xanthurenic acid. This compound, contained in the scales, is ordinarily distributed over the whole wing. In mutant *g,* the further metabolism of 3-hydroxykynurenine seems to be blocked, and kynurenine, as well as 3-hydroxykynurenine, are excreted with the meconia. More intimate knowledge of the tryptophan metabolism in this organism depends on the identification of the numerous fluorescent compounds affected by the mutant *g.*

C. Autonomous Mutants Affecting Tryptophan Metabolism

Most genes affecting pigmentation are autonomous, and pigment formation cannot be induced by diffusible substances. The reason seems to be that the final steps of synthesis are accomplished on the "carrier granules" of the pigments. The precursors, which are transmitted by the hemolymph, are there metabolized into the final pigments. Because in most cases the pterins are also affected in these ommochrome mutants, we will treat the autonomous ommochrome mutants in detail in connection with the pterin mutants. Only effects on tryptophan metabolism will be discussed here.

We know, from transplantation experiments, that the autonomous mutants *scarlet* (*st*) and *cardinal* (*cd*) in *D. melanogaster,* which lack the ommochromes, are able to provide *v*- or *cn*-implants with kynurenine or 3-hydroxykynurenine, respectively (cf. Wagner and Mitchell, 1955), but their mode of gene action remains obscure. It is not known if *st* and *cd* accumulate 3-hydroxykynurenine as does the ommochrome-less *w*-2 mutant in *B. mori* (Kikkawa, 1941).

In *Calliphora erythrocephala,* (Tate, 1947a) has found a sex-linked and sex-limited white-eyed mutant. He explains the fact, that only females ($x^w x^w$) show white eye color, whereas males have brown eyes, by the presence of a normal dominant allele in the Y-chromosome ($x^w y^+$). Ommochrome formation in this autonomous mutant is not induced by simply supplying the *white* mutant larvae with 3-hydroxykynurenine (Hanser, 1959), but cold treatment (4°C) during a sensitive period (about 120 hours after pupa formation at 24°C) may induce it (Tate, 1947b). The influence of the mutation on pterins, which are present in the eyes, will be discussed later.

The red-eyed *ra*-mutant of *P. interpunctella,* which is very similar to *a-Ephestia* differs in the mode of genic action: Ommochrome formation cannot be induced in *ra* by parabiosis (during the pupal period the posterior ends of both partners are cut off and both animals are attached to each other with a ring of wax) with an ra^+-partner or a^+-*E. kühniella* partner; ra^+ as well as *ra-Plodia* are able to supply an *a*-partner of *Ephestia* with kynurenine (Almeida, 1958a). Therefore, mutant *ra* of *Plodia* is able to convert tryptophan into kynurenine; its genetic block is at some later step of ommochrome synthesis.

Another mutant, *br*, affecting ommochromes, was recently found in *Ephestia* (Kühn, 1957). Although its phenotype is similar to a^k (coffee-brown eyes), the new mutant was proved not to be an allele of *a* (Kühn and Egelhaaf, 1959a) as is a^k. Like *ra* in *Plodia* its ommochrome synthesis is affected at a later step: mutant *br* too is able to supply *a*-

Ephestia with kynurenine. Only the synthesis of ommin is completely blocked; xanthommatin is still present (Kühn and Egelhaaf, 1959a). The red ommochrome, present only in the mutant eye, might be the result of an intermediate step or of an alternate pathway in the biosynthesis of the missing ommin. In the testis, ommin and xanthommatin are absent. The faint red color, found in place of the brown-violet color present in the wild type, is due to the red "substitute pigment" just mentioned and a small amount of another redox pigment, which is also present in the wild type.

In *Phryne fenestralis* the mutant *alba* lacks v^+- as well as cn^+-substance and affects some synthetic process which takes place on the eye granules. This mutation causes a deficiency of all ommochromes present in wild type (eye, fat-body-like pigment tissue, testis sheath, Malpighian tubules) (Becker, 1942). *Pallida*, the other mutant of *Phryne fenestralis* which affects granule-bound syntheses, reduces the ommin content of the pigmented tissues drastically. The quantity of the ommatins in the eyes is only slightly reduced, but their behavior toward solvents indicates a qualitative change (Becker, 1942).

D. Homologous Mutants

Even before "v^+"- and "cn^+"-substances were identified with kynurenine and 3-hydroxykynurenine, respectively, quite a number of mutants were known within the genus *Drosophila* (in species *melanogaster, pseudoobscura, similans, virilis*) in which the ommochrome formation seemed to be affected at the same point (Sturtevant and Novitski, 1941). Consequently, transplantation experiments or injections of extracts between species (Howland *et al.*, 1937; Gottschewski and Tan, 1938) were able to provide the mutants with the kynurenine, viz., 3-hydroxykynurenine, lacking for pigment synthesis. These results are reviewed in detail by Plagge (1939) and the results in respect to homology of mutations are summarized here in Table 5.

In the last few years some other mutants were observed, which indicate a block in ommochome synthesis. These mutations cause light eyes as well as an accumulation of the precursors kynurenine and 3-hydroxykynurenine.

In the mutant *green* of *Musca domestica,* which has pale yellow-green instead of red-brown eyes, pigment formation is induced if the mutants are grown together with wild type flies. The wild type flies excrete some substance (probably kynurenine) into the medium, which favors bacterial conversion of tryptophan to kynurenine, which is eaten by the larvae (Ward and Hammen, 1957). Positive identification of kynurenine as the key compound would be achieved if the addition of kynurenine to the medium in which green-eyed mutants are being grown

TABLE 5
Mutants Affecting Steps in Ommochrome Synthesis*

Mutant	Organism	Pathway of synthesis	Product accumulated
		tryptophan	
vermilion	*Drosophila melanogaster*		tryptophan
vermilion	*Drosophila virilis* (analogous to v^{u} of *D. mel.* ?)		?
cardinal	*Drosophila virilis* (analogous to v^{s} of *D. mel.* ?)		?
green	*Musca domestica*		tryptophan
white eye	*Periplaneta americana*		tryptophan
snow	*Apis mellifica*		tryptophan
a	*Ephestia kühniella*		tryptophan
v	*Drosophila pseudoobscura*		?
		kynurenine	
cinnabar	*Drosophila melanogaster*		kynurenine, kynurenic acid
or	*Drosophila pseudoobscura*		?
scarlet	*Drosophila virilis*		kynurenine
candida	*Phryne fenestralis*		kynurenine (present, but not accumulated)
yellow	*Phormia regina*		kynurenine
ivory	*Apis mellifica*		kynurenine
white-1	*Bombyx mori*		kynurenine
o-series (*ivory, orange, dahlia*)	*Habrobracon juglandis*		?
white	*Calliphora erythrocephala*		kynurenine (present, amount unknown)
ra	*Plodia interpunctella*		kynurenine (present, amount unknown)
		3-hydroxykynurenine	
scarlet	*Drosophila melanogaster*		?
cardinal	*Drosophila melanogaster*		
cn	*Drosophila virilis*		?
white-2	*Bombyx mori*		3-hydroxykynurenine
g	*Plodia interpunctella*		kynurenine and 3-hydroxykynurenine accumulated
		?	unknown pigment in *Plodia interpunctella*
		pallida Phryne fenestralis ← *br Ephestia kühniella*	ommin
			ommatin

* kynurenine present, amount unknown

* For references see text.

mimics the effect caused by growing both the green-eyed mutant and wild type flies in one vessel.

A yellow mutant of *Phormia regina* is blocked between kynurenine (which is accumulated) and 3-hydroxykynurenine (which induces pigment formation) (Ward and Hammen, 1957). The same authors also report that a white-eyed mutant of *Periplaneta americana* accumulates tryptophan. Feeding tests are impossible here because this insect has a gradual type of metamorphosis.

The mutant *candida* (*cn*) of *Phryne fenestralis*—in contrast to *pallida* mentioned above—lacks all ommochromes. As extracts contain v^+-substance (kynurenine) but not cn^+-substance (3-hydroxykynurenine), the mutant seems to be homologous to the *cn*-mutant of *D. melanogaster* (Becker, 1942).

In experiments in which lyophilized mutants of *Apis mellifica* were fed to mutants of *D. melanogaster*, Green (1955) showed that the *snow*-(*s*)-mutant workers, which accumulate nonprotein tryptophan are homologous to *v*-Drosophila, whereas the kynurenine-accumulating *ivory*-(*i*)-workers are homologous to *cn*. The nonautonomous behavior is also seen in mosaics. Green (1955) suggested that the frequency of occurrence of these mutations indicates an identical mechanism based on a genic complex which was derived unaltered from a common ancestral form.

In Table 5 the mutations which block ommochrome synthesis are listed. A check of the activity of the tryptophan-metabolizing enzymes within these groups would be necessary to characterize those mutants which really are affected by the lack of the same enzyme and thereby are really homologous. Biochemical assays for the presence of enzymes involved in ommochrome synthesis from the gene groups in *D. melanogaster* and *D. pseudoobscura* established by Gottschewski and Tan (1938) seem to offer an especially promising opportunity to gain information about homologous genes and evolution of genic complexes in *Drosophila*.

Mutations which eliminate the ommochromes are also known in crustaceans, as for instance, in *Gammarus pulex*. The red-eyed mutant *so/so* lacks all ommochromes, whereas the carotenoids remain (A. Anders, 1956). In mutant *br/br* (red-brown eyes) the ring-shaped ommochrome granules, which comprise about 80% of the pigment granules, are absent, whereas the dotted type remains.

E. Multiple Alleles and Modifiers Affecting Ommochrome Synthesis

In both classical objects—*Ephestia* and *Drosophila*—multiple alleles of the *a*- and *v*-locus, respectively, are known. In *Ephestia*, a^k causes

a somewhat intermediate pigmentation of larval eyes, testes, imaginal eyes, and brain, whereas larval skin, which is light reddish in wild type, lacks pigmentation as in mutant *a* (cf. Plagge, 1939). The fact that *a*-animals, which contain implanted a^+-ovaries, are very similar to a^k, suggests that the intermediate phenotype of a^k is produced by an intermediate amount of kynurenine synthesized. For this amount of kynurenine produced, the threshold for pigmentation of larval skin is too high (cf. Plagge, 1939). Enzymatic proof of this suggestion would be desirable.

Recently another red-eyed mutant of *Ephestia* was described (Caspari and Gottlieb, 1959). The mutation is located at the *a-locus*. The mutant animals have darker eyes than those of the *aa*-strain, and the percentage of males having pigmented testes is higher. Caspari and Gottlieb (1959) showed that the differences are not caused by a different allele but rather by a pair of major modifiers (*M-a* and *m-a*).

By an intensive study of *vermilion* pseudoalleles in *D. melanogaster*, Green (1952, 1954) was able to divide them into two groups: One group (*v*-1, *v*-2) is suppressed by *su*-2-*s* or *su-g-v*, yielding wild type flies, whereas the other group (*v*-36*f*, *v*-48*a*, *v*-51*b*, *v*-51*c*) cannot be suppressed. Moreover, partial starvation during the larval period causes brown pigmentation of the eyes of the first group; the second group does not react. In combination with *brown* (*bw*), the first group (named *v-s*) shows some ommochrome, whereas the second group (*v-u*) has entirely white eyes. Even though there are major differences between both groups, they both ordinarily accumulate nonprotein tryptophan and in both cases ommochrome synthesis can be induced by larval feeding of kynurenine as well as of formylkynurenine. Green (1954) speculates that the enzyme responsible for the oxidation of tryptophan is present in the *v-s* group, but is inactivated by products of the mutant gene, whereas in *v-u* mutants it is blocked irreversibly. Indeed, enzymatic assay (Baglioni, 1959) has shown one-tenth of kynurenine production in *v*-1 (a member of the *v-s* group) as well as in *v*-36*f* (member of the *v-u* group) but the method of assay did not allow a decision as to whether blockage or inactivation of the enzyme had occurred. As to the origin of the alleles, spontaneous mutation caused *v-s* as well as *v-u* mutants, whereas induced mutations were only *v-u* (Green, 1954).

Another well-known series of pseudoalleles, the *lozenge*-mutants, are very complicated to analyze. Clayton (1957) checked the distribution of brown pigment (in *lz/lz; bw/bw*-animals) in Carnoy-fixed slides and classified the members of the *lozenge*-series in a decreasing series of pigment content. Since the "quantity," judged in this way, is also affected by the distribution of the pigment, spectroscopic evaluation of the extracted material would be interesting. However, the amount of brown as well as of red pigment (Green, 1949a) seems not to be the

primary result of gene action in *lozenge,* but only the result of structural abnormalities of the eye. Therefore, increasing distortion of the ommatidia within the pseudoallelic series in general goes along with a decreasing amount of brown pigment. Some exceptions exist, which may be caused by some independent action of the gene on pigment formation (Clayton, 1959). This typical pleiotropic action of the *lozenge*-locus and the complexity of the resulting phenotype cause difficulties in relating phenotypic groups to the three groups of closely neighboring loci within *lozenge,* found by Green and Green (1949).

IV. The Pterins

A. Effects of Genes on Pterin Pattern (Autonomous Mutants)

All studies on the effect of genes on pterin pattern were made after separation of the pterins by paper chromatography. Extracts or squashes of heads or other organs were chromatographed mostly in alkaline solutions (e.g., propanol: 1% ammonia = 70:30) and their quantitative determination made by fluorometric measurement on the paper sheet.

The occurrence of hydrogenated biopterin derivatives, which are very unstable (see Section II and Table 1) makes it probable that the quantitative changes reported for some of the pterins under genic influence might only indicate, in part, a change in degradation products derived from different unstable naturally occurring pterins. Experiments with the mutant *sepia* of *D. melanogaster,* described below, underline the differences in products obtained chromatographically, when degradation of hydrogenated pterins is prevented. For the true outline of compounds contained in the different mutants, it is essential that special care is taken that artifacts are not created during extraction and chromatography.

1. *Drosophila melanogaster*

Pterins are found in the eyes, the Malpighian tubules, and in the testis; the pterins of the eye are listed in Table 1. A survey in which 23 mutants were compared to wild type with respect to the red pterin of the eye as well as isoxanthopterin and a yellow pterin (this compound was measured together with HB-1 which is 2-amino-4-hydroxypterin and HB-2 which is biopterin) of the whole fly was given by Hadorn and Mitchell (1951). Some mutations, e.g., *purple* and *prune,* cause a drastic reduction of red pterin, which together with the ommochromes predominantly constitutes the "eye color." These mutations hardly affect the yellow pterin, or 2-amino-4-hydroxypterin and biop-

terin, or isoxanthopterin (="Fl 3") of the whole animal. In contrast, the *brown* mutant almost completely lacks pterin; only ommochromes remain.

The *sepia* mutant, which was studied more intensively (Ziegler and Hadorn, 1958) also does not synthesize any red eye pigment, but it accumulates large amounts of xanthopterin, a xanthopterin-like pterin, yellow pterin (="*sepia* pterin"), 2-amino-4-hydroxypterin, and biopterin. By very mild treatment, Ziegler (1960a) recently showed that large amounts of a tetrahydrobiopterin derivative are present in *sepia,* but almost no xanthopterin, xanthopterin-like pterin, and 2-amino-4-hydroxypterin, and only small amounts of biopterin were found. The increase in these three pterins found in earlier investigations (Ziegler and Hadorn, 1958) is in reality only a reflection of an increase of the hydrogenated pterins (tetrahydrobiopterin derivative and yellow pterin) in the living tissue.

The *se*-mutant, crossed with mutants which contain genes blocking ommochrome synthesis (*se/se*; *v/v*), results in flies which are yellow when hatched (Danneel, 1955). These progeny grow darker until, after some days, they are brown, even though the only color-producing compound present is the yellow pterin. This darkening might be caused by complex formation with tryptophan (or similar compounds) found in the granules on which the pigment is bound (see Section V,A,1). Both pterins and riboflavin can form complexes with tryptophan which cause a bathochromic shift (Fujimori, 1959).

2. *Ephestia kühniella, Plodia interpunctella, and Ptychopoda seriata*

Hadorn and Kühn (1953) studied fluorescent compounds in wild type *Ephestia,* in the *a*-mutant, and in the *biochemica* (*bch*) mutant.

Most of the fluorescent compounds were identified as pterins later on (Viscontini *et al.,* 1956; see Table 2). Recently, Kühn and Egelhaaf (1959b) have shown that both the wild type and the *a*-mutant contain a red pterin similar to pterorhodin, which is a condensation product of xanthopterin and 7-methylxanthopterin. The *a*-mutant contains decreased amounts of this red pterin (Egelhaaf, personal communication), increased amounts of xanthopterin, a xanthopterin-like compound, 2-amino-4-hydroxypterin, pterincarbonic acid, and biopterin. *Biochemica,* which is phenotypically very similar to the wild type, lacks all pterins except isoxanthopterin, 2-amino-4-hydroxypterin, and biopterin, which are accumulated at about fourfold the concentration found in the wild type (Hadorn and Kühn, 1953; Kühn and Berg, 1955). As mentioned previously, the presence of 2-amino-4-hydroxypterin, xanthopterin,

xanthopterin-like compound, and biopterin may be only extraction artifacts, as has been shown in the *se*-mutant of *D. melanogaster*.[4]

The main gene-dependent differences, accumulation of isoxanthopterin in *bch* and of xanthopterin and xanthopterin-like compound in *a*, is already evident in the egg (Egelhaaf, 1956c) and may be followed throughout development (Reisener-Glasewald, 1956).

An important observation for understanding the action of genes on naturally occurring pterins and their interrelationships was recently made by Kühn and Egelhaaf (1959b) who noted that in *a-Ephestia*, two blue-green fluorescent compounds (spot "c-1a" and "c-1b") are accumulated, which in earlier investigations (Viscontini *et al.*, 1956) were characterized as closely related to xanthopterin. Storage of the chromatograms in the dark converts these spots into red pterin.

In the *decolorata* (*dec*) mutant of *Ptychopoda*, the red pterin is not found in the eyes before the animals are irradiated by light of short wavelength (365 mμ is the most effective) (Hanser, 1948). Chromatograms of eyes, not previously irradiated, show the two c-spots, but no red pterin (Kühn and Egelhaaf, 1959b). Apparently the c-spots, which disappeared in chromatograms of eyes irradiated before, are precursors (or at least degradation products of a naturally occurring precursor in the eye) of the red pterin. Since the *Ephestia* mutant *bch* has no c-spots, its pathway to red pterin is apparently blocked before this step is reached.

The pattern of pterins in wild type *Plodia interpunctella* is similar to the pattern in wild type *E. kühniella* and the pattern in red eyed *Ephestia* mutant *a* is similar to red eyed *ra* in *Plodia* (Almeida, 1958b; see Table 2). These organisms differ only in their blocks in ommochrome synthesis (see Section III,C). Another mutant, the black-eyed "Stamm 6" of *Plodia*, which shows larger amounts of all pterins, resembles somewhat the *bch* mutant of *Ephestia*.

3. *Other Insects*

The eye pterins of *Calliphora erythrocephala* were described by Autrum and Langer (1958) (see Table 3). In the *white* mutant *wa* which is blocked in ommochrome synthesis (Hanser, 1959; see also Section III,C), pterins are not absent as they are in all other *white* mutants described; only the relationship between the yellow pterin and the tetrahydrobiopterin derivative is changed in this *white* mutant (Ziegler, 1960b). Chromatograms show much more tetrahydrobiopterin compound

[4] The isolation from *Ephestia* of a pterin which begins to fluoresce after irradiation (Egelhaaf, 1956c) makes us suspect that the living eye of *Ephestia* contains very sensitive hydrogenated pterins, similar to those in *Drosophila*.

in wild type females than in *wa*-females and a threefold increase in yellow pterin in the mutant over the amount found in wild type. Since previous investigation has shown that the yellow pterin and the originally nonfluorescent hydrogenated compound are identical in Calliphorinae, *Drosophila,* and *Rana* (Ziegler, 1960a), we might assume that gene *wa* of *Calliphora* causes a shift in the equilibrium:

yellow pterin $\rightleftarrows$ photosensitive, nonfluorescent pterin
(tetrahydrobiopterin derivative)

This shift seems to be closely connected with the loss of ommochromes.

The "classical" pterins in the wings of butterflies may also be affected by gene mutations. In the white-winged mutant of *Colias erate poliographus,* for example, xanthopterin and "xanthopterin B" are absent although leucopterin and "leucopterin B" are found in both the mutant and wild genotype. Electron microscope studies have revealed that variation in the chemical nature of the pterins is accompanied by an altered organization of the pigment granules in the scales (Yagi and Saitoh, 1955).

B. Variations of Pterin Pattern Not Caused by Single Genes

Pterin metabolism is not only affected by the action of special genes themselves, as was shown in the examples just cited, but it is also influenced by the "genetic background." In adult flies of the *ey*-2 mutant of *D. melanogaster* the ratio Fl 5:Fl 4 (= yellow pterin:2-amino-4-hydroxypterin + biopterin) in the eyes is 0.64; in wild type adults the ratio is 1.74 (Goldschmidt, 1954). When the *ey*-2 gene is transferred into the cytoplasm and genome from wild type stock (by mating *ey*-2 ♂♂ with wild type ♀♀; and mating again *ey*-2 ♂♂ obtained in F_2 to virgins of wild type stock), the pattern is no longer distinguishable from that of wild type; after seven backcross generations the ratio Fl 5:Fl 4 was 1.38. Further analysis showed that the "altered ratio" of the original *ey* stock depends on its genetic background, chiefly on the second and fourth chromosome in this stock (Goldschmidt, 1958). After having exchanged these chromosomes with those of the *Berlin* stock, the pattern of *ey*-2 animals became normalized.

The amounts of both 2-amino-4-hydroxypterin and biopterin found possibly indicate the quantity of the tetrahydrobiopterin compound originally present (inasmuch as both of the compounds are degradation products of the tetrahydro derivative). One might speculate that in this case, as in *Calliphora,* a shift between the yellow and the tetrahydro compound occurs. This shift would be intimately related to the hydrogen-

carrying processes of the animals, which are influenced by the new genetic background.

In *D. melanogaster* (wild type stocks Samarkand and Oregon), inbreeding causes elimination or concealment of some fluorescent compounds (xanthopterin and isoxanthopterin, among others) (Hoenigsberg and Castiglioni, 1958). Because all workers have measured, in addition to the natural compounds, unnatural decomposition products, and omitted measurement of unstable compounds, and because of a general lack of understanding of the metabolic relationships among the pterins, it is difficult to explain most of the effects reported.

To add to the complications, related pigments such as riboflavin (see Section V,A,5,a,ii) also seem to be influenced by the genetic background: in *E. kühniella,* strain B II, riboflavin accumulates in the testes first during the prepupa as in wild type and then, in addition, a second peak of riboflavin accumulation occurs in the testis sheath during the late pupal stage (Caspari, 1958).

C. Nonautonomous Mutants

Most pterin mutants of *D. melanogaster* show autonomous behavior in transplantation experiments. Among the offspring of a cross between wild type and a *cn;bw*-stock was a mutant which showed red-brown eyes when ommochromes were present, and light orange eyes when the ommochromes were eliminated by *cn/cn* (Hadorn and Schwinck, 1956). By outcrossing experiments, these investigators showed that the new mutant (called *rosy*-2) was an allele of *rosy* (*ry*). It is characterized by a reduced amount of red pterin in the eyes and complete absence of isoxanthopterin in the eyes and testes, an increase of xanthopterin, a xanthopterin-like pterin, the yellow pterin, 2-amino-4-hydroxypterin, and biopterin (Hadorn and Schwinck, 1956; Hadorn and Graf, 1958). In contrast to other mutants, implantation of *ry* eye imaginal discs into wild type hosts causes an increase in drosopterin synthesis, accompanied by a marked decrease of yellow pterin, 2-amino-4-hydroxypterin, and biopterin, and synthesis of isoxanthopterin. The reciprocal experiment, implantation of wild type eye imaginal discs into *rosy* host larvae, causes a *rosy*-like pterin pattern in the implanted +-eye.

Reciprocal transplantations showed that the genotype of the host exclusively determines the appearance of isoxanthopterin (Hadorn *et al.*, 1958), which is present in the testis of the wild type and absent in *rosy*.

Formation of red drosopterins and of isoxanthopterin in a *ry* host can be induced by implantation of either wild type eye discs, Malpighian tubules, or larval fat body during the last larval instar (Hadorn and Schwinck, 1956). Apparently, formation of these pterins is caused by a compound ("*rosy*$^+$-Stoff"; Hadorn and Graf, 1958), which also can be

supplied by genotypes such as *white, brown, white-apricot, cardinal, ma, p, mah, rs*^{2}. In all these autonomous mutants synthesis is blocked in steps subsequent to the one controlled by *rosy*. A quantitative effect of "*rosy*$^+$-Stoff" was shown by injection of one or two pairs of +-Malpighian tubules (Hadorn and Graf, 1958).

Isoxanthopterin is enzymatically formed from 2-amino-4-hydroxypterin by the action of xanthine dehydrogenase (Wieland and Liebig, 1944; Krebs and Norris, 1949). Enzymatic assay showed that *ry* and another nonautonomous mutant, *maroon-like* (*ma-l*), which has an effect on pterins very similar to that of *ry*, are unable to convert 2-amino-4-hydroxypterin to isoxanthopterin, whereas more than forty other mutants in addition to wild type showed xanthine dehydrogenase activity (Forrest *et al.*, 1956). Mutants such as *w-apricot*, which lack nearly all pterins after hatching, have been reported to convert 2-amino-4-hydroxypterin fed to the larvae into isoxanthopterin (Forrest *et al.*, 1956).

The question arises: what causes the lack of xanthine dehydrogenase activity in *rosy* and *maroon-like* mutants? We have already noted that Malpighian tubules injected into *ry*-mutant larvae cause enzyme activity and that tubules treated with ultraviolet light, for the most part, lose this ability (Graf *et al.*, 1959). It thus seems most unlikely that the "*rosy*$^+$-Stoff," carried by the Malpighian tubules, is the xanthine dehydrogenase itself. The irradiation experiment also strongly indicates that the transplanted tissue produces some substance in the host rather than that it acts as a carrier of the enzyme itself. The presence of a simple inhibitor in the mutant, which is destroyed by the implanted +-tubules, is also very unlikely, because no fractions of *ry*- or *ma-l*-extracts showed xanthine dehydrogenase activity (Glassmann and Mitchell, 1959a).

Reaction with antibodies against partially purified wild type xanthine dehydrogenase showed that *ma-l* contains a much larger amount of a cross-reacting compound than *rosy*. A maternal effect, which, contrary to *ry*, is shown by *ma-l*, does not depend upon the simultaneous presence of *ry*$^+$ in the mother: Attached-X females, containing *st*; *ry* mated to *m ma-l;st* males yield male progeny of genotype *m ma-l st ry/st ry*$^+$ and show *ma-l*$^+$ phenotype. This indicates that synthesis of some "compound x," which is necessary for xanthine dehydrogenase activity, is blocked in *ma-l*, but only its utilization is prevented in *ry* (Glassmann and Mitchell, 1959b).

Following this interpretation, injection of Malpighian tubules from wild type into *ry* seems to supply the xanthine dehydrogenase, already present in *ry*, with a cofactor necessary for its action.

Recently, Ursprung reported (1959) another mutant in *D. melanogaster, bronzy*, which also shows nonautonomous behavior with regard

to red drosopterin and isoxanthopterin formation in transplantation experiments. It is suggested that *bronzy* is an allele of *maroon-like.*

Very little is known about the simultaneous action of "*rosy*$^+$-Stoff" toward isoxanthopterin formation and the synthesis of red pterin (Hadorn and Graf, 1958). Although it seems well established that it is xanthine dehydrogenase itself which causes the formation of the isoxanthopterin by conversion of 2-amino-4-hydroxypterin, it is possible that the addition of a necessary cofactor or activator to xanthine dehydrogenase is necessary before it can act in drosopterin formation.

In the mutant *sepiaoid* (*sed*), the amount of drosopterins in the eyes is reduced, whereas the yellow pterin and biopterin show an enormous increase. This pterin pattern of the eyes shows an autonomous behavior upon transplantation into wild type hosts. A wild type eye implanted into a *sed* host is able to synthesize its normal amount of drosopterin, although isoxanthopterin is produced neither in the *sed*-host, nor in the implanted wild type eye, showing a nonautonomous behavior with respect to this compound (Hadorn and Goldschmidt, unpublished; cf. Hadorn, 1958).

Experiments of Nawa *et al.* (1957) lead to the conclusion that the xanthine dehydrogenase of the mutant *sed* has small amounts of an electron acceptor *in vivo:* enzyme preparations from wild type or *bw* catalyze the oxidation of 2-amino-4-hydroxypterin to some extent even if methylene blue is absent; an extract of *sed* does not work under these conditions.

Conversion of 2-amino-4-hydroxypterin to isoxanthopterin by xanthine dehydrogenase takes place in many parts of the body of the insect, but formation of the red pterin, which also involves in some way xanthine dehydrogenase, must take place on granules as we find them mostly in the eyes. The special contribution of these granules (see Section V,A,1) is still not clear.

These findings, as well as the fact that in contrast to other pterins isoxanthopterin seems not to be bound to the pigment granules (see Section V,A,2), indicate that isoxanthopterin formation is not a step in the biosynthesis of the eye pterins, but is part of an alternate pathway. The failure of injection of isoxanthopterin to stimulate drosopterin synthesis (Graf *et al.*, 1959) supports this view. The final step in this alternate pathway seems to be the formation of isoxanthopterin from 2-amino-4-hydroxypterin. The latter compound therefore accumulates in *ry*.

Accumulation of the yellow pterin, 2-amino-4-hydroxypterin and biopterin by *ry* is consistent with the possibility that in the eye of wild type the tetrahydrobiopterin derivative may be converted finally into the red pterin. Chromatographic separation shows positive correlation between the degradation products (2-amino-4-hydroxypterin and biop-

terin) in the eyes of *ry* (Hadorn and Graf, 1958), which may be a reflection of the increased amount of the tetrahydro compound. In the testes and in the Malpighian tubules of *ry* no accumulation of both compounds (by reason of missing drosopterin synthesis) takes place (Handschin, unpubl.; cf. Hadorn, 1958). Only the 2-amino-4-hydroxypterin accumulates in *ry* because it is not derived from the tetrahydrobiopterin compound here. It may be the end product of pterin metabolism in *ry*, whereas in wild type it is further converted to isoxanthopterin. Quantitative determinations of the 2-amino-4-hydroxy equivalents for the missing isoxanthopterin are only a few of the numerous data necessary to elucidate the action of *rosy* on the normal pterin pattern.

Further investigation is required to explain why *ry* eyes, implanted into a wild type host, show an increase in red pterin accompanied by a decrease in yellow pterin, 2-amino-4-hydroxypterin, and biopterin, but injection of +-Malpighian tubules into *ry* causes an increase in drosopterin and a decrease in 2-amino-4-hydroxypterin + biopterin, while the yellow pterin remains constant (Hadorn and Graf, 1958). The relation between the pterin to be converted and the kind of enzyme supply by the surrounding tissue seems to have an influence on the final pterin pattern.

Other mutants, which may show differences in xanthine dehydrogenase activity are *lemon* and *lethal lemon*, which constitute a multiple allelic series in *Bombyx mori* (Tsujita, 1955). Pterins (leucopterin and isoxanthopterin) are found in the epidermal tissue of the silkworm *Erisilkworm* as well as the Chinese tussar silkworm (*Anthereae pernyi*) (Sakaguchi, 1955; see also Table 3). In the mutant forms, which contain a large amount of yellowish pigment in the larval epidermis, increased amounts of "xanthopterin B" and decreased amounts of isoxanthopterin are found (Tsujita and Sakaguchi, 1955). Because irradiation transforms "xanthopterin B" into a blue fluorescent compound (Tsujita and Sakaguchi, 1955) one might suggest that it is either closely related to or identical with the yellow pterin in *Drosophila*, which upon irradiation yields blue fluorescent pterincarbonic acid and 2-amino-4-hydroxypterin. The necessary experiments to prove this identity, however, remain to be done. Because in *lem* "xanthopterin B" is found instead of isoxanthopterin, check of xanthine dehydrogenase activity seems to be a promising method for further investigation of *lem* gene action.

D. Isoxanthopterin as a Secondary Sex Character

Hadorn and Mitchell (1951) have shown that the testes of *D. melanogaster*, in contrast to the ovaries, contain large amounts of isoxanthopterin. Transplantation experiments (Hadorn *et al.*, 1958)

show that this accumulation of isoxanthopterin is dependent on the surrounding tissue: Testes implanted into females during the third larval instar contain only about one-third the amount of isoxanthopterin of those implanted into males.

The finding of Hadorn and Ziegler (1958), that the eyes of males (measured during late pupal period) contain twice as much isoxanthopterin as those of females, also proved not to be controlled by the chromosomal constitution within the eye: Implantation of ♂ eye imaginal discs into female hosts showed low isoxanthopterin levels characteristic of females and vice versa (Hadorn and Ziegler, 1958).

This nonautonomous sexual difference parallels somewhat the action of *rosy* gene. The female genotype would correspond to the *rosy* mutant. Accordingly, *rosy* testes implanted into wild type females contain only very small amounts of isoxanthopterin, but implantation into wild type males causes high levels of this compound to appear (Hadorn *et al.*, 1958).

The unsolved question remains: Is the limited amount of isoxanthopterin, produced in females, caused by (1) reduced activity of xanthine dehydrogenase, or by (2) the presence of another substrate with increased affinity for this polyvalent enzyme; or (3) do the smaller eyes of males (Ziegler, 1960b) result in an excess of unused pterin precursor which is converted into isoxanthopterin as an end product of pterin metabolism? In any case, the higher isoxanthopterin level of male eyes and testes originates outside the tissue where this pterin is finally found. Its formation seems to be a secondary sex character.

E. The Influence of Recessive Alleles

Many mutations affecting eye color or other characters are conventionally classified as recessive because the phenotypes of heterozygotes and of the wild type are the same. Thus, the alleles *sepia* and *white* in *D. melanogaster* are classified as recessive. The effect of homozygous *white* consists in the disappearance of all pterins after hatching; the effect of homozygous *se* has been described (see Section IV,A,1).

The "recessive" alleles show a marked influence on the quantity of pterins present in heterozygotes (se^+/se and w^+/w); the se^+/se heterozygotes contain increased amounts of xanthopterin, xanthopterin-like pterin, 2-amino-4-hydroxypterin, and biopterin (indicating an increase in the tetrahydrobiopterin compound in the living tissue, according to our present knowledge), so that an intermediate level between wild type and *se*-mutant is reached (Ziegler and Hadorn, 1958). Because the

"recessive" *se*-allele has no influence on drosopterin formation, the phenotype of se^+/se and se^+/se^+ is the same in this respect.

In heterozygous condition, *sed* causes an increase of yellow pterin in the eye but has no influence on the testis (Graf and Hadorn, 1959).

In heterozygous females (w^+/w), *white* causes a small decrease in drosopterin, xanthopterin, and xanthopterin-like pterin, and a marked increase in both the yellow pterin and HB-pterins (2-amino-4-hydroxypterin and biopterin). Heterozygous *white* combined with the *se*-allele in homozygous condition (w^+/w; *se/se*) causes a clearly different action: in a genotype where no red pterin is formed, *white* causes a reduction of *all* pterins (Ziegler and Hadorn, 1958). The phenotypes of w^+/w; *se/se* and w^+/w^+; *se/se* animals are the same (brown eyes, because no red pterin is formed).

In all these experiments influences of genic background and of heterosis have been excluded, confirming that the influence of the "recessive" allele is a locus-specific one.

Although more intimate knowledge is necessary to interpret the action of both these recessive genes, we may now suggest that generally *w* causes a break in the path of drosopterin synthesis. The unbroken chain might be tetrahydrobiopterin derivative → yellow pterin → red pterin. In the heterozygote w^+/w, therefore, the first two compounds are accumulated. In w^+/w; *se/se*, where the reaction chain is restricted by another gene (*se*), *w* acts as an additional block and causes a decrease in all pterins.

V. Pleiotropic Action of Genes Affecting Pigment

Ommochromes and Pterins

1. *Pigment-Carrying Granules*

The ommochromes as well as the pterins in the eyes of arthropods are normally bound to granules which can be stained with iron-hematoxylin. These granules were shown in wild type *Ephestia* by Hanser (1948), who suggested that they are protein granules. Caspari and Richards (1948b), by ribonuclease digestion combined with pyronine staining, proved that ribonucleoprotein is present. The ommochrome-carrying granules in the testis sheaths also contain ribonucleic acid (Caspari, 1955). Large amounts of pyronine staining material are first found around the nuclei but the substance diminishes 3 days after pupation. There is evidence that part of this material is secreted into the

lobuli and another part is consumed in pigment formation, possibly in the formation of the precursor granules (Caspari, 1955).

In *D. melanogaster* the composition of these "core-granules" was established by chemical analysis (Table 6; Ziegler and Jaenicke, 1959).

The pleiotropic action of gene *wa* in *E. kühniella* is caused by the absence of the core-granules (Hanser, 1948) which in turn explains the absence of ommochromes as well as of pterins (Hadorn and Kühn, 1953). In the eyes of *wa* imagoes the small amount of pterin (probably

TABLE 6
Chemical Composition of the Core-Granules of Single Heads of *Drosophila melanogaster**

	wild type (μg)	*white*-mutant (μg)
Protein, precipitable with trichloracetic acid	1.40	1.85
Ribonucleic acid		
by estimation of ribose	0.17	0.23
by estimation of phosphate	0.22	0.22
Lipid phosphate	2.2×10^{-3}	2.4×10^{-3}
Ratio, protein:ribonucleic acid	8:1	8:1

* From Ziegler and Jaenicke, 1959.

isoxanthopterin), which alone is still present, gradually drops (Reisener-Glasewald, 1956). This agrees with the special character of this pterin which is not bound to granules. Earlier findings (Kühn and Schwartz, 1942) indicating that *wa* is able to provide *a*-mutant with kynurenine are in good agreement: synthesis of this diffusible compound is not blocked; only further metabolism on the eye granules cannot take place. Accordingly, Egelhaaf (1958) has found that testes and ovaries of *wa*-animals are able to convert tryptophan to kynurenine as well as does wa^+.

We have seen that the final steps in the synthesis of ommochromes and pterins both take place on these core-granules. These processes are closely bound to respiratory metabolism: addition of KCN (in dilution of 1:10,000,000) blocks the formation of ommochromes from exogenously supplied kynurenine in isolated pupal eyes of *D. melanogaster* (Danneel, 1941). In addition, the radiation-induced formation of red pterin in the *dec* mutant of *Ptychopoda* (see Section IV,A,2) is O_2-dependent (Kühn and Egelhaaf, 1959b). This agrees with the *in vitro* synthesis of xanthommatin from 3-hydroxykynurenine by oxidative condensation (Butenandt *et al.*, 1954a). It has been suggested that tyrosinase participates in this reaction *in vivo* (Butenandt, *et al.*, 1956). This enzyme controls the dopa-dopaquinone redox system which

mediates the oxidative condensation of 3-hydroxykynurenine (Butenandt, 1959).

A number of enzymes of the tricarboxylic acid cycle, as well as protyrosinase (activated by the "activator" of the hemolymph), have been demonstrated in the core-granules of *D. melanogaster* (Ziegler and Jaenicke, 1959). In the *white* mutant—in contrast to *wa* in *E. kühniella*—the core-granules are still present, but show a characteristic change in the binding of two enzymes: Examination of enzyme activity in solutions of different osmotic pressures showed that in the wild type, succinic dehydrogenase is very tightly bound and protyrosinase is very loosely bound to the granules; binding of the same enzymes to the granules of the *white* mutant shows the opposite behavior (Ziegler and Jaenicke, 1959).

The *white* mutant is able to synthesize the pterin nucleus. Isoxanthopterin appears in the eyes at the same time and in about the same amounts as in wild type (20 hours after pupation, the earliest time at which heads can be dissected), and small amounts of yellow pterin, 2-amino-4-hydroxypterin, and biopterin occur about 40 hours after pupation. But at the time when rapid increase of all eye pterins starts in the wild type or in the *se* mutant (about 62 hours after pupation), these small amounts of pterin begin to disappear in *white*, leaving the eye entirely free of pterins a few days after hatching (Hadorn and Ziegler, 1958). Apparently the core-granules in *white Drosophila* can neither convert the precursors of eye pterins, delivered by the hemolymph, into the eye pterins nor convert the precursors into ommochromes. The fate of the precursors will be discussed later.

Earlier findings of Ephrussi and Chevais (1937) are in agreement. They showed that a *D. melanogaster w-apricot*-host supplies implants of *vermilion* genotype with v^+-substance (kynurenine) more efficiently than a wild type host. This may be explained by the fact that in a *w-apricot*-host kynurenine is exclusively used by the implant, because the eye granules of the *w-apricot*-host are not able to synthesize considerable amounts of ommochromes, whereas in a wild type host the kynurenine must be shared with the host eye.

Morita and Tokuyama (1959) have shown by the "double extraction method" that the decrease of red pigment within the pseudoallelic series w^+, *w-sat*, *w-co*, *w-e*, *w* is paralleled by a decrease in brown pigment. Quantitative estimation of the pterins after chromatographic separation (Ziegler, unpublished data) shows that with increased eye color (amount of red pterin) the amount of total pterin rises, but in some genotypes, which are devoid of any red pterin (e.g., *w-bf*), 2-amino-4-hydroxypterin, biopterin, and xanthopterin are present.

It may be suggested that this inability of the mutant *white* to synthesize the eye pterins is somehow connected with the changed structure of the enzyme proteins. There are indications that in *white* the tightly bound protyrosinase is involved in the respiratory chain: all conditions which specially block protyrosinase (e.g., EDTA or temperature $> 28°C$) also block respiratory activity of the eye granules in the *white* mutant but have no effect on the wild type. CO-blocking of respiration in the *white* mutant cannot be reversed by irradiation at about 400 mμ whereas it is reversed in wild type (the tyrosinase system is a copper-containing system; the Cu/CO-complex is not split by light as is the Fe/CO-complex) (Ziegler, unpublished data).

In spite of these clues we are still far from completely understanding the details of the connection between gene-dependent changes in granule structure and failure to synthesize pigments.

Two white-eyed mutants in *D. melanogaster* which exhibit temperature-dependent pigment synthesis will be discussed later.

It may prove fruitful to check other *white* mutants which seem to be widely distributed among insects [e.g., *Phormia regina* (Dickler, 1943); *Lucilia cuprina* (Mackerras, 1933); *Culex molestus* (Gilchrist and Haldane, 1947)] to see if pleiotropic gene action (deficiency of ommochromes as well as of pterins) is due to the absence of "core-granules" or to a change in their (enzymatic?) composition.

As we have seen, in "*white*" *Calliphora* the block of ommochrome synthesis on the eye granules does not cause absence of pterins (see Section IV,A,3), but a shift in the equilibrium of yellow pterin ⇆ tetrahydrobiopterin derivative. Whether this pleiotropic action is caused by changed structure of the core-granules or by changed light conditions (absence of ommochromes might cause free light admittance and thereby dehydrogenation of the very light-sensitive hydrogenated compound), i.e., a case of "physiologically conditioned" pleiotropic action, remains to be elucidated. In any case, *white Calliphora* should not be listed among "*white*" mutants, because this particular mutant contains pterins which are absent in other mutants called "*white*." Because the red pigment in *Drosophila* is a pterin, its disappearance in the *white* mutant is of no more importance to "comparative genetics" than the disappearance of phenotypically invisible hydrogenated pterins in other insects. Thus a revision of earlier appraisals (Sturtevant, 1947) of *white* mutations is indicated.

An attempt has been made to explain the pleiotropic effect of the *white* gene in *Drosophila* in connection with the submicroscopic structure of the core-granules (Ziegler, 1960c). It was shown that the pigment granules (diameter 0.7–1.4 μ) are not solid masses, but are similar to a

bag containing a number of "subgranules" (maximal diameter 0.4 μ), which show osmiophilic grains in linear arrangement. The osmiophilic grains seem to reflect the natural site of the ommochromes and pterins. In the *white* mutant, the "subgranules" as well as the grains are absent; only a ball of osmiophilic material (diameter 0.5–0.6 μ) is found within the core-granules. Only the granules of the *white* mutant have a very high osmotic pressure; they burst when treated with 0.33 M sucrose solution. Possibly, the granules of the *white* mutant seem to retain precursors of pterins and ommochromes in a soluble, osmotically active form. The existence of a characteristic submicroscopic arrangement of these core-granules opens a wide field of study concerning gene-dependent variations of pigments and their relation to electron microscopic structure, much as it has done for chlorophyll pigments and chloroplast structure (Wettstein, 1957). The question whether all "subgranules" of a pigment granule contain either pterin or ommochrome or whether each one contains both pigments could be solved by using suitable mutants (e.g., *brown* or *scarlet* in *D. melanogaster*).

Autonomous mutations affecting the eye granules need not eliminate ommochromes and pterins at all. But those mutations which block pterin synthesis in a more or less drastic way (e.g., *brown* or *claret* in *D. melanogaster*) usually affect ommochrome synthesis to some degree [reduction of brown pigment to 80 and 30%, respectively, in the mutants mentioned above (Nolte, 1952, 1955)] and vice versa. Possibly this pleiotropic action is due to a disturbance of (oxidative?) final steps, which take place on the eye granules and are common to both pigments.

Pleiotropic effects of eye color genes in many cases affect size, aggregation, number, and volume of pigment granules in addition to their actual pigment content [e.g., mutant *lightoid* in *D. melanogaster* (Nolte, 1954); *scarlet, brown, white-mottled* 4 in *D. melanogaster* (Nolte, 1950)]. We are far from the elucidation of the hierarchy of gene action in these cases and of the first gene-controlled change in metabolism which finally changes the phenotypic "eye color."

An explanation of gene action on the eye pigmentary system in the *lozenge-clawless* mutant of *D. melanogaster* may be derived from the arrangement of pseudoalleles according to pigment content. Different results are found depending on whether the red (Green, 1949a; Clayton, 1958) or the brown (Clayton, 1957) pigment or the "phenotypic eye color" is measured. This leads to the conclusion that abnormal cell differentiation is the primary cause of abnormal pigmentation (Clayton, 1959; see Section III,E). One of the characteristics of *lz*-eyes is the scattered pigment deposits, which form clusters and clumps of ommochrome pigment (G. Anders, 1955). Similar clumps, independent of granules, are

produced in eye imaginal discs as a result of tissue damage caused by mechanical damage or UV-irradiation (Anders and Ursprung, 1959) as well as after analogous treatment of the Malpighian tubules (see Section III,B). A working hypothesis for the interpretation of how artificial or natural disturbances of the tissue result in the formation of ommochrome clusters must be proved by experiments: Tissue damage causes the conversion of protyrosinase into tyrosinase by the "activator," a reaction which either does not take place, or does so only sparingly, in undisturbed tissue (Ziegler and Jaenicke, 1959). The further action of tyrosinase in the conversion of 3-hydroxykynurenine to ommochromes (Butenandt *et al.*, 1956) is highly probable.

An intimate connection between the size of the eye and the amount of pterin present has been shown by Taira and Nawa (1959) who checked *BB*, *bar*-3, *L*-2, and *Dp* in *D. melanogaster*. Reduction in size of the eye was found to be directly related to the reduction of the amount of eye pterins, whereas the pterins present in the testis remained unaffected. Therefore, these mutations seem not to affect pterin synthesis, but only the size of the eye. The reduction in the amount of pterins is only a secondary phene.

The pleiotropic action of ommochrome synthesis controlling genes on the color of the eye granules in the primary and secondary pigment cells and in the basal cells is more easily interpreted: The pathway of synthesis branches into formation of very dark ommins and somewhat lighter ommatins, which may be present in either reduced (wine-red) or oxidized (yellowish-brown) forms. A genetic block before the branching point of the biosynthetic pathway (see Table 5) can therefore cause the disappearance of several different pigment granules.

Pictures of eye granules of *Ephestia* mutants are given by Kühn and Berg (1955) and Hanser (1948), and for *Drosophila* mutants by Zeutzschel (1958).

Because no red pterin is present in the ocelli of *Drosophila*, the mutant genes *v*, and *cn* cause red eyes, but white ocelli (Danneel, 1955).

Mutant *rt* in *E. kühniella* offers an example of the influence of genic action on the structure of cytoplasmic particles (Caspari, 1955). The gene *rt* causes the delay of the onset of pigmentation on the precursor granules in the testis sheath: It occurs in prepupae rather than during the last larval molt with unpigmented granules still present even in 4-day-old pupae. Pigmentation in this mutant results in red instead of brown color (reduced form of ommochromes instead of oxidized one?) with granules of reduced size and altered shape. Subsequent metabolic processes seem to intensify a change of the metabolic level of these cytoplasmic particles.

2. *Interaction of Ommochrome- and Pterin-Affecting Genes*

The mutation *a* in *E. kühniella,* which blocks the conversion of tryptophan into kynurenine and therefore causes the total absence of any ommochrome pigment, also affects the pattern of pterins (Hadorn and Kühn, 1953; Kühn and Egelhaaf, 1955). Xanthopterin as well as the xanthopterin-like compound and an unidentified pterin "e" are markedly increased; the red pterin, which causes the red eye color, appears to be slightly decreased (Egelhaaf, personal communication). Parabiosis with an a^+-partner as well as injection of kynurenine—both of which induce ommochrome formation—also normalize the pterin pattern (Kühn and Egelhaaf, 1955). Kühn (1956) suggests that this highly striking pleiotropic action of gene *a* is not caused by competition for a common precursor but for a site of final synthesis at the granules. Competition for a common precursor seems to be unlikely, considering the strikingly different nature of the pterins and ommochromes.

Considering the redox character of both pigments, the possibility exists that the final synthesis of pterin is connected with a reduction of the accompanying ommochromes. More intimate knowledge about the structure of all pterin compounds occurring in the *living* eye during development is required before explanations of this phenomenon in *Ephestia* can be proved.

In regard to the action of this pleiotropic mutant *a* on the histological picture of the primary pigment cells (Kühn and Berg, 1955) one might conclude that the appearance of faint yellow granules in *a* instead of big ones in a^+ coincides with the changed pterin pattern. The red pterin of mutant *a* is found in the secondary pigment cells. In mutant *biochemica,* where xanthopterin and the xanthopterin-like compound as well as the red pterin are absent, only colorless core-granules remain in the primary pigment cells; dark ommochrome granules are found in the other pigment cells.

Combination of *a* mutant with *bch* (*bch/bch*; *a/a*) (Kühn and Berg, 1956) results in red eye color [caused by the red pterin, because mutant *a* is unable to synthesize ommochromes (Kühn and Egelhaaf, 1959b)], whereas the pattern of the other pterins completely corresponds to that of *bch* and not to *a*: only isoxanthopterin is present in large amounts plus some 2-amino-4-hydroxypterin and biopterin.

The most interesting combination of *wa/wa*; *bch/bch* results in the total absence of all pterins except isoxanthopterin, which is present in considerable amounts (Kühn and Berg, 1956). Homozygous *wa* prevents the formation of core-granules (Hanser, 1948) and this result therefore is a further indication that biosynthesis of isoxanthopterin is the result

of a branch pathway in pterin metabolism, which does not involve granules as does the biosynthesis of eye pterins. The accumulation of isoxanthopterin in *bch/bch* agrees with the marked reduction in the ratio xanthopterin: isoxanthopterin found in all tissues of *biochemica* (compared with wild type and mutant *a*) (Egelhaaf, 1956a). Examination of xanthine dehydrogenase relationships might prove interesting here.

3. *Effect of Eye-Color Genes on Pigmentation of Other Organs*

Already in 1933 Caspari had shown that mutant *a* of *E. kühniella* has a pleiotropic action: besides the change in eye color of the moth, the eyes and the cuticle of the caterpillar, and the testis and brain of the imago are changed in color. Addition of kynurenine restores all these phenes. The knowledge that all these pigments are ommochromes (Becker, 1942) provided an obvious explanation for this pleiotropic effect.

Different organs respond to kynurenine according to their individual thresholds for initiation of the effect. Larval eyes of *a-Ephestia* proved to be more sensitive than larval skin (Kühn and Plagge, 1937). The white color of the excreta in mutant *a*, compared to the orange-yellow color in wild type (Wolfram, 1948), also might be due to the lack of ommochromes.

The distribution of pterins in the different organs of the abdomen in wild type, mutants *a* and *bch* of *Ephestia* was thoroughly studied by Egelhaaf (1956b). The wild type eyes contain about 20–30 times more fluorescing compounds than does the abdomen. Within the abdomen the Malpighian tubules, testes, and ovaries contain the highest levels. The action of $a^+ \rightarrow a$ on the metabolites of ommochromes has been discussed in Section III,B. The *a*-mutation affects not only pterins in the eyes (Section IV,A,2) but causes also quantitative differences in the abdomen. The wild type contains both isoxanthopterin and xanthopterin; xanthopterin is extremely reduced in mutant *a* (Hadorn and Egelhaaf, 1956). The question remains whether the increased amount of eye pterins (especially of xanthopterin) in *a* compared to wild type is partially due to a "shift" of pterins from the abdomen to the head, possibly caused by the increased space on the ommochrome-less eye granules. In any case, this depletion of pterins in the abdominal organs of mutant *a* is not due to an intensified excretion; hardly any fluorescent compounds are found in the excreta (Hadorn and Egelhaaf, 1956).

The *bch* mutation of *E. kühniella* causes no drastic change in the pterin pattern of the abdominal organs. However, the ratio xanthopterin: isoxanthopterin in the abdomen is shifted in favor of isoxanthop-

terin as it is in the eyes (Egelhaaf, 1956a). The relationship between eye-color mutations and the ommochromes and pterins present in other organs are known in only very few cases. Some of these relations, studied by Hubby and Throckmorton (1960) in different species of the genus *Drosophila* will be discussed later.

The mutation $w^+ \rightarrow w$ in *D. melanogaster* causes complete depletion of ommochromes and pterins in the eyes and also in the testes and Malpighian tubules. All pterins which are still present in the abdominal organs of the *white* mutant at hatching time (Hadorn, 1954a) are excreted with the meconia as "simple" pterins such as isoxanthopterin, 2-amino-4-hydroxypterin, and others (Hadorn and Kürsteiner, 1955). Another part of the precursors of the eye pterins and ommochromes, however, seems to remain in the core-granules in osmotically active form. Other mutants, like *se*, show neither excretion of pterins (Hadorn and Kürsteiner, 1955) nor differences in the amount of pterins in the testis. The color of the Malpighian tubules of *se* is listed as "bright yellow" (Brehme and Demerec, 1942) and has increased amounts of yellow pterin, 2-amino-4-hydroxypterin, and biopterin (Ziegler, unpublished). However, most of the pterin precursors of the missing red pterin accumulate within the eye as yellow pterin and tetrahydrobiopterin compound (see Section IV,A,1).

In *sepiaoid,* in contrast to *sepia,* yellow pterin, 2-amino-4-hydroxypterin, and biopterin are accumulated in the testis in large amounts (Graf and Hadorn, 1959), whereas these three pterins are clearly reduced in the testis of *claret, w-apricot,* and *pink-peach.*

The *rosy*-mutation causes aggregation of yellow and orange colored balls of material to be excreted in the Malpighian tubules (Hadorn and Schwinck, 1956). Their chemical composition is unknown.

From the few cases studied we have some knowledge of the pterin metabolism of different organs under the influence of genes. However, a comparison of the list of eye pterins which are present in different mutants of *Drosophila* (Hadorn and Mitchell, 1951) with that of Malpighian tubule color (Brehme and Demerec, 1942) emphasizes how far removed we are from completely understanding or explaining this relationship.

4. *Effects on Physiological Processes*

Mutants which are affected in pterin or ommochrome synthesis, in many cases also show differences in viability, temperature sensitivity, or other parameters. For example, the changes of pterin pattern and decrease of viability are two phenes in the pleiotropic action spectrum of the *rosy* mutant in *D. melanogaster. Rosy* is semilethal at 25°C in

the late pupal or early imaginal stage (Hadorn and Schwinck, 1956), which might not be due directly to changes in pterin metabolism but to a block in xanthine dehydrogenase activity. Decrease of temperature to 18°C increases viability as well as the amounts of all pterins, except isoxanthopterin, which cannot be synthesized even at the lower temperature. The increase of red pterin and of all other pterins (Hadorn and Graf, 1958) shows that the generalized pterin synthesis, in this case, improves along with better viability.

Mutant *a* of *E. kühniella* shows reduced viability and delayed development (Caspari, 1933)—both perhaps caused by the change in tryptophan metabolism and protein composition. Combination of *bch* with *wa* in heterozygous condition (*bch/bch*$^+$; *wa/wa*$^+$) somewhat lowers viability; combination in homozygous condition (*wa/wa*; *bch/bch*) causes a marked decrease in viability (Kühn and Berg, 1956).

Change of the pterin pattern as well as female sterility are phenes, which are caused by pleiotropic action of the gene *deep-orange* (*dor*) in *D. melanogaster* (Counce, 1957). Here also the relationships in the hierarchy of gene action are difficult to understand at the present state of our knowledge. In one respect this mutant seems to be unique: it is the only mutation thus far found which increases the amount of isoxanthopterin in mutant females compared with wild type females.

The highly pleiotropic action of *lozenge-clawless* in *D. melanogaster*, reviewed extensively by Hadorn (1954b), is still not explained although some clues about eye phenes are now available.

A connection between tryptophan metabolism and the action of a tumor-suppressing gene (*su-tu*, third chromosome) in a tumorous strain (*tu*, second chromosome) of *D. melanogaster* was found by Glass and Plaine (cf. Glass, 1957). Excess of tryptophan supplied with the food inhibits the action of the tumor-suppressing gene. However, accumulation of nonprotein tryptophan in *vermilion*-flies, which was found by Green (1949b), is not able to parallel the tryptophan-induced inhibition of *su-tu* by food—also *su-tu* has no effect on the expression of *vermilion* gene. Combining the tumor-causing gene *tu* in *D. melanogaster* with *bw*, *v*, or *cn* highly increases penetrance of *tu* (Kanehisa, 1956). He also suggests that the link between the two phenes is a disturbance in tryptophan metabolism caused by the ommochrome-affecting genes.

The mutant *lemon* of *B. mori* is characterized by yellow larval skin, due to changes in the pterin pattern (Section IV,C), and by lack of black melanin. In addition, the cuticular layer of the hypodermis, especially in the mandibles, does not develop properly, causing inability to chew food in *lemon* or to hatch from the egg in *lemon-lethal*. Quantitative determinations (Tsujita and Sakaguchi, 1957) showed that the amount

of chitin as well as of tyrosinase and dopa-oxidase activity are reduced in mutant *lem-l* compared with wild type. Dopa is accumulated in considerable amounts in the mutant. It is highly probable that the change in pterin pattern has no direct relationship to the other phenes noted, but that they all are due to a still unknown block in metabolism.

An interesting question for geneticists is how pigment deficiencies in the eyes caused by genic action finally affect the behavior of insects toward the light. Use of suitable mutants may offer opportunities for physiologists to elucidate the action of pterins and ommochromes in light perception.

Lack of isolation between the ommatidia is suggested as being responsible for the fact that white-eyed mutants of *D. melanogaster* and *D. pseudoobscura* (Kalmus, 1943) as well as of *Culex molestus* (Gilchrist and Haldane, 1947) show no differences in perception of light intensities compared with the wild types, but fail to react to a moving contour between a bright and dark area of the visual field. The mutants *w-a* and *bw v* in *D. melanogaster* and *prune* in *D. subobscura* show very weak optomotor reactions (Kalmus, 1943), corresponding to their reduced pigment content.

Retinograms of wild type and *white Calliphora* are different (Autrum, 1955). The higher sensitivity and higher "on-effects" shown by the *w*-mutant in response to variously colored light of equal quanta and also the double-peaked efficiency curve of sensitivity in the wild type may be reasonably explained by the complete absence of ommochromes in the mutant form. Because the ommochromes are absent in primary as well as in secondary pigment cells of the *w*-mutant, the ommatidia cannot be protected; light may invade from neighboring rhabdomeres. The same intensities of light excite more ommatidia in the mutant than in wild type. The second peak in the wild type efficiency curve (at about 630 mμ) is due to the red color of the ommochromes which permit red light to pass through. The possible role of pterins in general, and the shift between the yellow and tetrahydrobiopterin compound (see Section IV,A,3), in particular, in the phenomena described above remain to be explained.

Fujito (1956) stated that the mutant *tan*-3 of *Drosophila* is less strongly phototactic than wild type. If formation of brown pigment (ommochromes) was increased by feeding kynurenine or 3-hydroxykynurenine, positive phototaxis also increased.

Wild type and mutants of *D. melanogaster* (*white, brown, sepia, vermilion, white-apricot*) to which colored light (most efficient at 366 mμ) was offered as an alternative to "dark," showed greater response to light, if the flies used had dark eyes (Fingerman, 1952). This type of

experiment, altered in such a way that light of equal intensity but of different wavelengths is used, may answer some questions mentioned above, if the test organisms used are mutants whose ommochromes and pterins are exactly known in their naturally occurring state.

5. *Relations to Other Heterocyclic Compounds*

a. *Pigments.* (i) *Melanin.* Changes in pterin formation, which are accompanied by missing melanin production, already have been shown to occur in mutant *lem-l* of *B. mori* (Section V,A,4). In vertebrates close relationships between melanophores and pterins are known (see Section VIII), but in insects no mutants suitable for the study of gene-dependent interactions between pterin metabolism and melanin synthesis seem to be known.

The mutant *eruca nigra* of *Ptychopoda seriata* shows a pleiotropic action on melanin metabolism as well as on ommochrome formation: increased melanin pigmentation of the caterpillar stage is accompanied by a decrease in red and yellow pigment (Kühn, 1941b).

(ii) *Riboflavin.* Close relationships seem to exist between pterins and riboflavin (cf. Ziegler, 1956b). Both have the pyrimidine-pyrazine nucleus and share possibly some common steps in biosynthesis (Weygand and Waldschmidt, 1955).

By paper chromatographic methods, separation of yellow pterin and riboflavin has not been possible. Therefore quantitative determination in series is complicated (see Hadorn and Ziegler, 1958) and it was measured together with the yellow pterin. Taira and Nawa (1958), using the bacteriological method of assay, stated that mutant *se* of *D. melanogaster* contains much more flavin than *v* or *bw*. Some relationship between "xanthopterin B" (which seems to correspond to the yellow pterin) and flavin is suggested.

Using bacteriological assay, Caspari and Blomstrand (1958) pointed out that the pigment deposited in rodlets in the outer layer of the testis sheath of *Ephestia* is at least in part riboflavin. In the wild type it appears gradually during the last larval instar, reaches a plateau in the early pupa, and disappears in the late pupa. It might then be transferred to the Malpighian tubules where riboflavin is, indeed, found after disappearance from the testis sheaths. Gene *a* causes the appearance of considerably higher amounts of riboflavin in the testis sheaths, but in *a* it seems to disappear somewhat earlier than in wild type. The pleiotropic action of gene *a* therefore includes not only tryptophan metabolism of proteins, but also metabolic processes which cannot be explained in detail at the moment as the action on pterins and riboflavin.

In mutant *wa* of *Ephestia* the appearance of riboflavin in the testis

sheaths is inhibited at all times. In principle, the *wa*-mutant is able to synthesize riboflavin, even though the total amount in *wa*-larvae is always lower than in wa^+. It was suggested that the failure of riboflavin to accumulate in the testis sheaths may be caused by its destruction (Caspari, 1958) since failure to accumulate riboflavin in the testis sheaths alone without destruction ought to cause equal amounts in the *whole* animal.

b. *Purines.* There is concrete information in one case for the influence of a gene on pterin—as well as on purine—metabolism: Blockage of xanthine dehydrogenase [which is able to convert hypoxanthine to xanthine and hence to uric acid, as well as 2-amino-4-hydroxypterin to isoxanthopterin (Krebs and Norris, 1949; Wieland and Liebig, 1944)] in the mutants *rosy* or *maroon-like* of *D. melanogaster* affects both pterins and purines. In these mutants hypoxanthine instead of uric acid is accumulated and excreted (Mitchell *et al.*, 1959). In other mutations, which cause marked differences in the amount of ommochromes or pterins (*vermilion, brown, sepia, sepiaoid, white*), no difference is found in the content of uric acid during the pupal stage (Taira and Nawa, 1958). According to Danneel and Eschrich-Zimmermann (1957), flies which do not contain any pterins in the eyes (*brown* or *white*) seem to lose all uric acid, whereas flies which have either red or yellow pterin (wild type, *cinnabar, vermilion, sepia*) retain uric acid at least to some extent.

Members of the *oily* pseudoallelic series in *Bombyx* lack epidermal uric acid (Jucci, 1932). In addition, some members of this series lack riboflavin in the Malpighian tubules and contain reduced amounts of brown pigment in the eyes and eggs. These findings, whose causal relationships are not well understood at the present point of our knowledge, are reviewed by Kikkawa (1953).

VI. Predetermination

Backcrossing of a^+a heterozygote females with *aa* males in *E. kühniella* revealed that a compound formed either under the influence of the nuclei in the oöcyte or originating from the surrounding tissue is present in the egg. This compound may cause pigmentation of larval eyes and larval skin during the first larval instars. Gradually the pigment-causing agent drops in its efficiency until the eye of the imago shows the genotypic red color (Caspari, 1936; Kühn and Plagge, 1937). The fact that implantation of a^+-donor into an *aa*-mother may cause predetermination proves that the active compound is not synthesized in the eggs under the influence of a^+ in the oöcyte but is transferred from the surrounding tissue to the egg (Kühn and Plagge, 1937).

The more "active compound" is supplied to the eggs (for instance, by implantation of a^+-testis into an *aa*-mother) the longer lasting is an influence observed in the development of the larva. In addition, a larger percentage of eggs deposited by this mother shows predetermination (cf. Plagge, 1939).

3-Hydroxykynurenine seems responsible for this predetermination: Egelhaaf (1958) demonstrated the presence of 3-hydroxykynurenine rather than of kynurenine in the ovaries.

Also in *D. melanogaster,* Graf (1957) showed, by quantitative determination of kynurenine in the eggs, that the presence of this metabolite in ommochrome synthesis is dependent on the genotype of the mother (Table 7). This kynurenine is metabolized during embryonic development and gradually disappears.

TABLE 7
Amount of Kynurenine in 200 Eggs of *Drosophila melanogaster**

Parental genotypes	Fluorescence
♀ ++ × + ♂	120 ± 3.0
♀ +*v* × *v* ♂	110 ± 5.0
♀ *vv* × + ♂	3.3 ± 1.1
♀ *vv* × *v* ♂	1.5 ± 1.2

* The amount of kynurenine is expressed in terms of arbitrary units of fluorescence. (Graf, 1957.)

Both the *rosy* and *maroon-like* mutants of *D. melanogaster* lack xanthine dehydrogenase activity, but only *maroon-like* shows a maternal effect: Crosses of ♀ *ma-l*$^+$/*ma-l* × *ma-l* ♂ give no progeny of *ma-l* phenotype, all *ma-l* animals having increased amounts of red pterin in the eyes compared to typical *ma-l* eyes. The maternal effects of *D. melanogaster* and *E. kühniella* concern processes initiated in the egg or in early development. The presence of small amounts of xanthine dehydrogenase activity in adult flies of *ma-l* progeny from the cross above indicates that the maternal effect in *ma-l* is effective at a much later time (Glassmann and Mitchell, 1959b). The substance transmitted through the egg is not yet known.

Another case of predetermination in connection with ommochrome synthesis, as well as a case of maternal inheritance, found in *w*-1, *brown*-1, and *brown*-2 mutants of *B. mori,* is reviewed extensively by Kikkawa (1953). In the case of *w*-1, the compounds responsible for predetermination were traceable in the eggs: in the cross ♀ *w*-1/*w*-1 × ♂ +/+ kynurenine was found but in the cross ♀ +/+ × ♂ *w*-1/*w*-1 3-hydroxykynurenine is present (Kikkawa, 1953).

Reciprocal crosses between +/*lem-l* and *lem/lem-l* in *B. mori* (see also Section IV,C) showed that if +/*lem-l* was used as mother, normal black-colored young larvae hatched, which became yellow at the first molt and died afterward, whereas the yellowish brown larvae derived from a *lem/lem-l* mother already died at the egg stage (Tsujita, 1955). Reciprocal transplantations of ovaries confirmed the suggestion that the body color of larvae as well as the date of death is not determined by the genotype of the embryo (+/*lem-l* or *lem/lem-l*), but by the mother in which the egg develops (Tsujita and Sakaguchi, 1958). Quantitative analysis of the pterins in the transplanted ovaries (Sakaguchi and Tsujita, 1955) leads to the conclusion that, in this case, the active principle found in the somatic cells of the mother and transmitted to the egg is indicated by the relative amounts of isoxanthopterin and "xanthopterin B" present (see also Section IV,C). However, this difference remains characteristic for the larval phenotype found later; it is possibly only a very early manifestation, as it was found in the eggs of mutant *a* and *bch* in *Ephestia* (Egelhaaf, 1956b).

VII. Modifications by External Factors

In some cases change of temperature which can result in either an increase or a decrease of the amount of pigment, acts only by either improvement or reduction in viability. Therefore a uniform quantitative change of all pterins present occurs. In other cases, temperature may act in a specific way on the synthesis of pterins and ommochromes. The mutants *white-mottled*-4 and *white-blood* of *D. melanogaster* will be used as examples.

In *w-bl*, which is a member of the *white*-pseudoallelic series, Ephrussi and Herold (1945) showed decreasing amounts of brown and red pigment with increase of temperature from 18°C to 25°C during a sensitive period (40–48 hours after pupation). *White-bl* implants, used to donate kynurenine to a *vermilion* host, affected ommochrome pigmentation of the host more strikingly at 30°C than at 19°C (cf. Ephrussi, 1942a). This indicates that the early steps in ommochrome formation are not influenced by the change in temperature and therefore more precursor is placed at the host's disposal, utilization of the kynurenine by the *w-bl* donor being inhibited at higher temperatures.

Flies raised at 18°C show a reduction in red pterin to about 50% of that found in the wild type. The amounts of yellow pterin and of HB-pterins (2-amino-4-hydroxypterin and biopterin, which indicate the amount of the hydrogenated biopterin derivative in living tissue) are twice to three times that of wild type. If the flies are raised at 25°C, all pterins decrease very markedly. Compared with wild type

flies, about one-tenth of the red pterin and about one-third of the other pterins remain (Ziegler, 1960b).

White-mottled-4—in contrast to *w-bl*—is the result of an inversion (cf. Lewis, 1950). As in *w-bl*, the red pterin is reduced to about one-third of the amount found in wild type while the yellow pterin, 2-amino-4-hydroxypterin, and biopterin are increased. In contrast to *w-bl*, for *w-m*-4 the temperature favorable for pterin synthesis is 25°C, and it responds to the shift to an unfavorable temperature (18°C) in a fundamentally different way: the amount of red pterin is reduced more strongly after raising the flies at the unfavorable temperature (18°C), but the yellow pterin, 2-amino-4-hydroxypterin and biopterin are not reduced concomitantly. These three pterins are accumulated in such a way that their average amount rises from about 150% at 25°C to about 300% at 18°C when compared with wild type flies (Ziegler, 1960b).

One might suggest therefore that in *w-m*-4, change in temperature changes the equilibrium between red pterin, on the one hand, and tetrahydrobiopterin compound and yellow pterin, on the other hand, whereas in *w-bl* temperature affects a point preceding these "eye pterins" in their respective biosyntheses.

We have seen above (Section III,C), that in *w-Calliphora* cold treatment can induce formation of the red-brown ommochrome pigment. It would also be interesting to know what happens to the pterins which are changed in their equilibrium between yellow pterin and the tetrahydrobiopterin compound in the ommochrome-less *w*-eyes, when the "cold-induced" ommochromes are formed.

In addition to these few examples from cases where the pterins and ommochromes are relatively well understood, the widespread occurrence of these two pigments among arthropods opens a wide area of questions concerning their role in phenomena such as modification by temperature, seasonal dimorphism, melanism, and phenocopy.

VIII. Pterin Mutants in Vertebrates

During the last decade pterins were found in the skin and in the pigment-epithelium layer of amphibians, fishes, and reptiles (cf. Ziegler, 1956b). The degradation products (e.g., 2-amino-4-hydroxypterin and pterincarbonic acid) are the same as in *Drosophila*, but besides these, the yellow pterin and the tetrahydrobiopterin derivative are identical with the compounds found in insects (Ziegler, 1960a).

For genetic studies, the intimate relationships to melanin synthesis may become important. Obika and Hama (1960), using inhibition of melanin synthesis by phenylthiourea, showed that in amphibians there is

a substantial connection between pterin synthesis and melanin formation in the melanophores. The fact that the very first appearance of fluorescent pterins in fishes [the pterins are later on found in the faint yellowish "pterinophores" (Ziegler, 1956c)] occurs within the melanophores supports this suggestion.

Those mutants in which melanin is formed, but secondarily disappears after hatching (like in *Guppy blond* or *Guppy golden*), have pterin patterns identical with wild type (Goodrich and Ziegler, unpublished data). Intergeneric, interspecific, and intraspecific matings yield offspring with normal or atypical cell growth or, in some cases, with melanomas (Gordon, 1950). These matings were made with the genus *Platypoecilus,* whose red pterin (described by Goodrich *et al.,* 1941) is very similar to or even identical with that of *Rana temporaria* and with the genus *Xiphophorus,* which accumulates yellow pterin and other—unidentified—pterins (Goodrich and Ziegler, unpublished data). Because of the obvious close relationships between melanin synthesis and pterins, an intensive reinvestigation of the effects of modifying genes on the pigments of the melanomas of the hybrids is called for.

In addition, studies on the distribution of carotenoid pigments in the Mendelian color varieties of *Platypoecilus, Xiphophorus, Oryzias, Macropodus,* and *Betta* should be extended to the pterin pigments which were unknown at the time the original studies (Goodrich *et al.,* 1941) were done.

IX. Taxonomic Questions and Concluding Remarks

In many cases, the patterns of pterins or of compounds connected with ommochrome metabolism as "phenes" of a certain genotype are reflections of the degree of relationship. Möhlmann (1958) found that all Nymphalidae (*Vanessa io, V. urtica, Araschnia levana,* etc.) have the same pattern of fluorescent substances, presumably pterins and ommochromes. Pieridae, Papilionidae, and Notodontidae lack xanthurenic acid, which is found in Sphingidae and Geometridae in high concentration. Only Noctuidae lack kynurenine. Close relationships with respect to the common occurrence of several fluorescent compounds, mostly pterins, were found between Pyralidae and Nymphalidae.

In fishes of the family of Cyprinidae, the pattern of pterins present in the skin reflects the relationships between different genera. In this way, the close relationship between *Squalius leuciscus, S. cephalus, Alburnus lucidus,* and *A. bipunctatus,* already shown by other criteria (Schutz, 1956), was demonstrated (Ziegler, 1956c). According to their pterin patterns, *Cyprinus carpio* and *Rhodeus amarus* again were found to

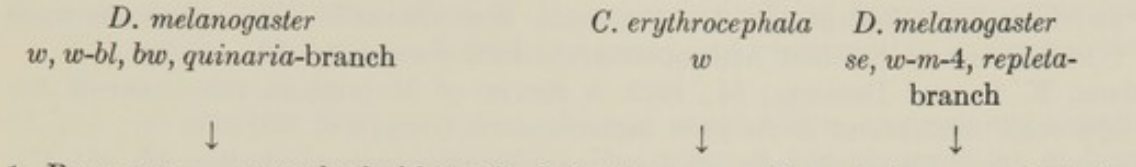
be at the "periphery" of the family of Cyprinidae. In these genera only the main spot, which is characteristic for all Cyprinidae, and which corresponds to the "ichthyopterin" is present. This "ichthyopterin" (Hüttel and Sprengling, 1943), found after chromatographic separation of the skin extract, which might correspond to "cyprino-pourpre (A2)" of Hama *et al.* (1960) seems to be 7-hydroxybiopterin (Kauffmann, 1959).

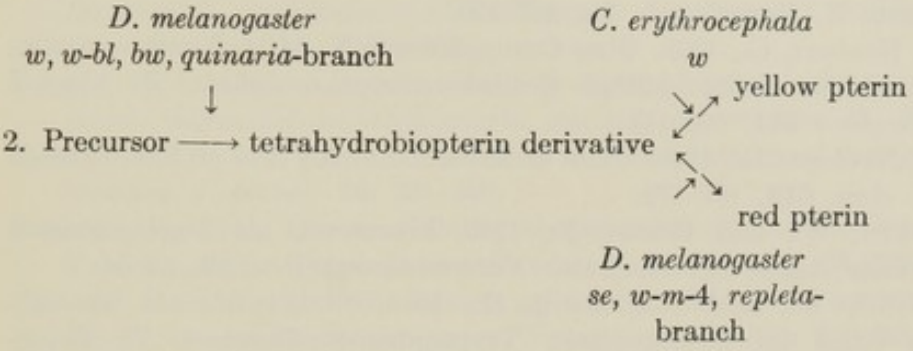
Recently a contribution to the problems of evolution in the genus *Drosophila* by study of the pterin pattern in the testis was given by Hubby and Throckmorton (1960). They showed that representatives from primitive groups (*virilis* group in the subgenus *Drosophila* and *obscura* group in the subgenus *Sophophora*) accumulate some pterins, including red pterin, in the testis. These compounds are reduced or even absent in representatives of derived groups, such as the *repleta—robusta*, the *cardini*, or the *melanogaster* group. The trend toward reduction of the pterins in the testis seems to occur independently in each one of these four major evolutionary lines studied. The phenotypic result of this trend—reduction or elimination of the red pterin—in each of the evolutionary lines is the same. Two mechanisms seem to result in the elimination of the red and yellow pterins: (1) Other pterins (2-amino-4-hydroxypterin, biopterin) are almost eliminated along with the red and yellow pterin as in the *quinaria*-branch. (2) The elimination of red pterin is accompanied by an accumulation of the three pterins, 2-amino-4-hydroxypterin, biopterin (which at least may be partially degradation products of the hydrogenated biopterin compound), and yellow pterin in the *repleta*-branch.

These results indicate that in the first case there is a block early in the pathway of pterin synthesis, whereas in the second case, there is a "terminal" block in synthesis, causing accumulation of already formed pterins.

The findings of Hubby and Throckmorton (1960) parallel the ones on the action of the genes *white-blood* and *white-mottled*-4 on the eye pterins of *D. melanogaster* (see Section VII) and also the action of other mutations. In one group (*quinaria*-branch, *w-bl*, *bw*) *all* pterins are reduced by the action of the gene; in the other group (*repleta*-branch, *w-m*-4, *se*) only the red pterin is eliminated while the yellow pterin and the tetrahydrobiopterin compound (2-amino-4-hydroxypterin and biopterin as degradation products) are accumulated.

In conclusion, our present state of knowledge offers two possibilities for the pathway of synthesis of "eye pterins"; a tentative outline of these pathways and the location of the gene effects in *D. melanogaster* and *C. erythrocephala* is offered:





References

Almeida, F. F. de, 1958a. Parabiosen zwischen verschiedenen Genotypen von *Plodia interpunctella* und *Ephestia kühniella*. *Z. Naturforsch.* **13b,** 617–621.

Almeida, F. F. de, 1958b. Über die fluoreszierenden Stoffe in den Augen dreier Genotypen von *Plodia interpunctella*. *Z. Naturforsch.* **13b,** 687–691.

Anders, A., 1956. Untersuchungen über die Pigmente einiger Augenfarbmutanten von *Gammarus pulex*. *Verhandl. deut. Zool. Ges., Zool. Anz.* **19,** 286–291.

Anders, G., 1955. Untersuchungen über das pleiotrope Manifestationsmuster der Mutante *lozenge-clawless* (lz^{cl}) von *Drosophila melanogaster*. *Z. Vererbungslehre* **87,** 113–186.

Anders, G., and Urpsrung, H., 1959. Bildung von Pigmentschollen im Auge von *Drosophila melanogaster* nach experimenteller Schädigung der Imaginalanlagen. *Rev. suisse zool.* **66,** 259–265.

Aslaksen, E., and Hadorn, E., 1957. Untersuchungen über eine Mutante (*red*) von *Drosophila melanogaster* mit roten Malpighischen Gefässen. *Arch. Julius Klaus-Stift. Vererbungsforsch. Sozialanthropol. u. Rassenhyg.* **32,** 464–469.

Autrum, H., 1955. Die spektrale Empfindlichkeit der Augenmutation *white-apricot* von *Calliphora erythrocephala*. *Biol. Zentr.* **74,** 515–524.

Autrum, H., and Langer, H., 1958. Photolabile Pterine im Auge von *Calliphora erythrocephala*. *Biol. Zentr.* **77,** 196–201.

Baglioni, C., 1959. Genetic control of tryptophan peroxidase-oxidase in *Drosophila melanogaster*. *Nature* **184,** 1084–1085.

Beadle, G. W., 1937. Development of eye colors in *Drosophila*: Fat bodies and Malpighian tubes in relation to diffusible substances. *Genetics* **22,** 587–611.

Beadle, G., and Ephrussi, B., 1935. Différenciation de la couleur de l'oeil *cinnabar* chez la *Drosophile* (*Drosophila melanogaster*). *Compt. rend. acad. sci.* **201,** 620–622.

Beadle, G. W., and Mitchell, H. K., 1947. Kynurenine as an intermediate in the formation of nicotinic acid from tryptophane by *Neurospora*. *Proc. Natl. Acad. Sci. U.S.* **33,** 155–158.

Becker, E., 1942. Über Eigenschaften, Verbreitung und die genetisch-entwicklungs-

physiologische Bedeutung der Pigmente der Ommatin- und Ommingruppe (Ommochrome) bei den Arthropoden. *Z. Vererbungslehre* **80,** 157–204.

Brehme, K. S., and Demerec, M., 1942. A survey of Malpighian tube color in the eye color mutants of *Drosophila melanogaster*. *Growth* **6,** 351–355.

Butenandt, A., 1959. Wirkstoffe im Insektenreich. *Naturwissenschaften* **46,** 461–471.

Butenandt, A., and Albrecht, W., 1952. Bestimmung des Tryptophangehaltes verschiedener Rassen der Mehlmotte *Ephestia kühniella* als Beitrag zur Analyse der Genwirkungen. *Z. Naturforsch.* **7b,** 287–290.

Butenandt, A., and Neubert, G., 1955. Über Ommochrome. V. Mitt. Xanthommatin, ein Augenfarbstoff der Schmeissfliege *Calliphora erythrocephala*. *Z. physiol. Chem. Hoppe-Seyler's* **301,** 109–114.

Butenandt, A., and Neubert, G., 1958. Über Ommochrome. XVII. Zur Konstitution der Ommine, I. *Ann.* **618,** 167–172.

Butenandt, A., Weidel, W., and Becker, E., 1940. Kynurenin als Augenpigmentbildung auslösendes Agens bei Insekten. *Naturwissenschaften* **28,** 63–64.

Butenandt, A., Weidel, W., and Schlossberg, H., 1949. 3-Oxykynurenin als cn^+-Gen-abhängiges Glied im intermediären Tryptophan-Stoffwechsel. *Z. Naturforsch.* **4b,** 242–244.

Butenandt, A., Schiedt, U., and Biekert, E., 1954a. Über Ommochrome. III. Mitt. Synthese des Xanthommatins. *Ann.* **588,** 106–116.

Butenandt, A., Schiedt, U., Biekert, E., and Cromartie, R. J. T., 1954b. Über Ommochrome. IV. Mitt. Konstitution des Xanthommatins. *Ann.* **590,** 75–90.

Butenandt, A., Biekert, E., and Linzen, B., 1956. Über Ommochrome. VII. Mitt. Modellversuche zur Bildung des Xanthommatins *in vivo*. *Z. physiol. Chem. Hoppe-Seyler's* **305,** 284–289.

Butenandt, A., Biekert, E., and Linzen, B., 1958. Über Ommochrome. XIV. Mitt. Zur Verbreitung der Ommine im Tierreich. *Z. physiol. Chem. Hoppe-Seyler's* **313,** 251–258.

Caspari, E., 1933. Über die Wirkung eines pleiotropen Gens bei der Mehlmotte *Ephestia kühniella* Zeller. *Wilhelm Roux' Arch. Entwicklungsmech. Organ.* **130,** 353–381.

Caspari, E., 1936. Zur Analyse der Matroklinie der Vererbung in der *a*-Serie der Augenfarbmutationen bei der Mehlmotte *Ephestia kühniella* Z. *Z. Vererbungslehre* **71,** 546–555.

Caspari, E., 1946. On the effects of the gene *a* on the chemical composition of *Ephestia kühniella* Zeller. *Genetics* **31,** 454–474.

Caspari, E., 1950. Serological differences between a^+a^+ and *aa Ephestia*. *Genetics* **35,** 100–101.

Caspari, E., 1955. On the pigment formation in the testis sheath of *Rt* and *rt Ephestia kühniella* Zeller. *Biol. Zentr.* **74,** 585–602.

Caspari, E., 1958. The genetic control of riboflavin in the testis of *Ephestia*. *Proc. Intern. Congr. Genet., 10th Congr.* (*Montreal*).

Caspari, E., and Blomstrand, I., 1958. A yellow pigment in the testis of *Ephestia:* its development and its control by genes. *Genetics* **43,** 679–694.

Caspari, E., and Gottlieb, F., 1959. On a modifier of the gene *a* in *Ephestia kühniella*. *Z. Vererbungslehre* **90,** 263–272.

Caspari, E., and Richards, J., 1948a. Differences in protein constitution of a^+a^+ and *aa Ephestia*. *Genetics* **33,** 605–606.

Caspari, E., and Richards, J., 1948b. Genic action. *Yearbook Carnegie Inst.* **47,** 183–189.

Clayton, F. E., 1957. The effect of *lozenge* pseudoalleles on eye pigmentation in *Drosophila melanogaster*. I. Brown pigment and pigmentation in *lozenge* males. *Genetics*. **42,** 28–41.

Clayton, F. E., 1958. The effect of the *lozenge* pseudoallelic series on eye pigmentation in *Drosophila melanogaster*. II. Red pigment and pigmentation in *lozenge* compounds. *Genetics* **43,** 261–273.

Clayton, F. E., 1959. The effect of *lozenge* pseudoalleles on eye pigmentation in *Drosophila melanogaster*. III. Pigment development during pupal differentiation. *Genetics* **44,** 1041–1052.

Counce, S. J., 1957. A female sterile mutant (*deep orange*) of *Drosophila melanogaster* increasing isoxanthopterin content. *Experientia* **13,** 354–356.

Crew, F. A. E., and Lamy, R., 1932. A case of conditioned dominance in *Drosophila obscura*. *J. Genet.* **26,** 351–358.

Danneel, R., 1941. Die Ausfärbung überlebender *v*- and *cn-Drosophila*—Augen mit Produkten des Tryptophanstoffwechsels. *Biol. Zentr.* **61,** 388–398.

Danneel, R., 1955. Über die beiden gelbäugigen Mutanten *sev* und *secn* der Fruchtfliege *Drosophila melanogaster*. *Naturwissenschaften* **42,** 566.

Danneel, R., and Zimmermann, B., 1954. Über das Vorkommen und Schicksal des Kynurenins bei verschiedenen Drosophilarassen. *Z. Naturforsch.* **9b,** 788–792.

Danneel, R., and Eschrich-Zimmermann, B., 1957. Beziehungen zwischen dem Vorkommen von Harnsäure und verschiedenen Pterinen bei *Drosophila melanogaster*. *Z. Naturforsch.* **12b,** 730–732.

Dickler, H., 1943. White-eyed mutation in *Phormia regina* Meigen. *Am. Naturalist* **77,** 287–288.

Egelhaaf, A., 1956a. Die fluoreszierenden Stoffe in den Organen dreier Genotypen von *Ephestia kühniella*. *Z. Vererbungslehre* **87,** 769–783.

Egelhaaf, A., 1956b. Quantitativer Vergleich der Fluoreszenzmuster der Eier dreier Genotypen von *Ephestia kühniella*. *Naturwissenschaften* **43,** 165–166.

Egelhaaf, A., 1956c. Photolabile Fluoreszenzstoffe bei *Ephestia kühniella*. *Naturwissenschaften* **43,** 309.

Egelhaaf, A., 1957. Der Gehalt an freiem Tryptophan und Kynurenin bei den Genotypen a^+ und *a* von *Ephestia kühniella* während der Entwicklung. *Z. Naturforsch.* **12b,** 465–472.

Egelhaaf, A., 1958. Nachweis eines genabhängigen L-Tryptophan-oxydase-Systems bei *Ephestia kühniella*. *Z. Naturforsch.* **13b,** 275–279.

Ephrussi, B., 1942a. Analysis of eye color differentiation in *Drosophila*. *Cold Spring Harbor Symposia Quant. Biol.* **10,** 40–48.

Ephrussi, B., 1942b. Chemistry of "eye color hormones" of *Drosophila*. *Quart. Rev. Biol.* **17,** 327–353.

Ephrussi, B., and Chevais, S., 1937. Development of eye colors in *Drosophila:* relation between pigmentation and release of the diffusible substances. *Proc. Natl. Acad. Sci. US.* **23,** 428–434.

Ephrussi, B., and Herold, J. L., 1944. Studies of eye pigments of *Drosophila*. I. Methods of extraction and quantitative estimation of the pigment components. *Genetics* **29,** 148–175.

Ephrussi, B., and Herold, J. L., 1945. Studies of eye pigments in *Drosophila*. II. Effect of temperature on the red and brown pigments in the mutant *blood* (*w-bl*). *Genetics* **30,** 62–70.

Fingerman, M., 1952. The role of the eye-pigments of *Drosophila melanogaster* in photic orientation. *J. Exptl. Zool.* **120,** 131–164.

Forrest, H. S., and Mitchell, H. K., 1954a. Pteridines from *Drosophila*. I. Isolation of a yellow pigment. *J. Am. Chem. Soc.* **76,** 5656–5658.

Forrest, H. S., and Mitchell, H. K., 1954b. Pteridines from *Drosophila*. II. Structure of the yellow pigment. *J. Am. Chem. Soc.* **76,** 5658–5662.

Forrest, H. S., and Mitchell, H. K., 1955. Pteridines from *Drosophila*. III. Isolation and identification of three more pteridines. *J. Am. Chem. Soc.* **77,** 4865–4869.

Forrest, H. S., Glassmann, E., and Mitchell, H. K., 1956. The conversion of 2-amino-4-hydroxypteridine to isoxanthopterin in *Drosophila melanogaster*. *Science* **124,** 725–726.

Forrest, H. S., Hartfield, D., and Baalen, C. van, 1959. Characterization of a second yellow compound from *Drosophila melanogaster*. *Nature* **183,** 1269–1270.

Fujimori, E., 1959. Interaction between pteridines and tryptophan. *Proc. Natl. Acad. Sci. US.* **45,** 133–136.

Fujito, S., 1956. The relation between tryptophan metabolism and phototaxis in the *tan*-3 mutant of *Drosophila melanogaster*. *Idengaku Zasshi* **31,** 119–124.

Gilchrist, B. M., and Haldane, J. B. S., 1947. Sex linkage and sex determination in a mosquito, *Culex molestus*. *Hereditas* **33,** 175–190.

Glass, B., 1957. In pursuit of a gene. *Science* **126,** 683–689.

Glassmann, E., 1956. Kynurenine formamidase in mutants of *Drosophila*. *Genetics* **41,** 566–574.

Glassmann, E., and Mitchell, H. K., 1959a. Mutants of *Drosophila melanogaster* deficient in xanthine dehydrogenase. *Genetics* **44,** 153–162.

Glassmann, E., and Mitchell, H. K., 1959b. Maternal effect of *ma-l*$^{+}$ on xanthine dehydrogenase of *Drosophila melanogaster*. *Genetics* **44,** 546–554.

Goldschmidt, E., 1954. Fluorescent substances in chromatograms of a mutant stock of *Drosophila*. *Nature* **174,** 883–884.

Goldschmidt, E., 1958. Cryptic genetic factors changing pterine concentrations of *Drosophila* eyes. *Proc. Intern. Congr. Genet., 10th Congr. (Montreal)*, pp. 99–100.

Goodrich, H. B., Hill, G. A., and Arrick, M. S., 1941. The identification of gene controlled pigments in *Platypoecilus* and *Xiphophorus* and comparisons with other tropical fishes. *Genetics* **26,** 573–586.

Gordon, M., 1950. The origin of modifying genes that influence the normal and atypical growth of pigment cells in fishes. *Zoologica* **35,** 19–20.

Gottschewski, G., and Tan, C. C., 1938. The homology of the eye color genes in *Drosophila melanogaster* and *Drosophila pseudoobscura* as determined by transplantation. II. *Genetics* **23,** 221–238.

Graf, G. E., 1957. Biochemical predetermination in *Drosophila*. *Experientia* **13,** 107.

Graf, G. E., and Hadorn, E., 1959. Untersuchungen über fluoreszierende Stoffe der Hoden von *Drosophila Z. Naturforsch.* **14b,** 14–17.

Graf, G. E., Hadorn, E., and Ursprung, H., 1959. Experiments on the isoxanthopterin metabolism in *Drosophila melanogaster*. *J. Insect. Physiol.* **2,** 120–124.

Green, M. M., 1949a. Red eye pigment determination in the *lozenge* allelic series of *Drosophila melanogaster*. *Am. Naturalist* **82,** 188–192.

Green, M. M., 1949b. A study of tryptophan in eye color mutants of *Drosophila*. *Genetics* **34,** 564–572.

Green, M. M., 1952. Mutant isoalleles at the *vermilion* locus in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. US.* **38,** 300–305.

Green, M. M., 1954. Pseudoallelism at the *vermilion* locus in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. US.* **40,** 92–99.

Green, M. M., 1955. Homologous eye color mutants in the honeybee and *Drosophila*. *Evolution* **9,** 215–216.

Green, M. M., and Green, K. C., 1949. Crossing-over between alleles at the *lozenge* locus in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. US.* **35,** 586–591.

Hadorn, E., 1954a. Ontogenetische Änderungen im Gehalt an Isoxanthopterin bei verschiedenen Genotypen von *Drosophila melanogaster*. *Experientia* **10,** 483–484.

Hadorn, E., 1954b. Approaches to the study of biochemical and developmental effects of mutations. *Proc. Intern. Congr. Genet., 9th Congr., Bellagio, Italy,* pp. 326–337.

Hadorn, E., 1958. Contribution to the physiological and biochemical genetics of pteridines and pigments in insects. *Intern. Congr. Genet., 10th Congr. (Montreal)*, pp. 337–354.

Hadorn, E., and Egelhaaf, A., 1956. Biochemische Polyphänie und Stoffverteilung im Körper verschiedener Augenfarb-Genotypen von *Ephestia kühniella*. *Z. Naturforsch.* **11b,** 21–25.

Hadorn, E., and Graf, G. E., 1958. Weitere Untersuchungen über den nicht-autonomen Pterinstoffwechsel der Mutante *rosy* von *Drosophila melanogaster*. *Zool. Anz.* **160,** 231–243.

Hadorn, E., and Kühn, A., 1953. Chromatographische und fluorometrische Untersuchungen zur biochemischen Polyphänie von Augenfarb-Genen bei *Ephestia kühniella*. *Z. Naturforsch.* **8b,** 582–589.

Hadorn, E., and Kürsteiner, R., 1955. Unterschiede in Exkretstoffen bei verschiedenen Genotypen von *Drosophila melanogaster*. *Arch. Julius Klaus-Stift. Vererbungsforsch. Sozialanthropol. u. Rassenhyg.* **30,** 494–498.

Hadorn, E., and Mitchell, H. K., 1951. Properties of mutants of *Drosophila melanogaster* and changes during development as revealed by paper chromatography. *Proc. Natl. Acad. Sci. US.* **37,** 650–665.

Hadorn, E., and Schwinck, I., 1956. Fehlen von Isoxanthopterin und Nicht-Autonomie in der Bildung der roten Augenpigmente bei einer Mutante (*rosy*) von *Drosophila melanogaster*. *Z. Vererbungslehre* **87,** 528–553.

Hadorn, E., and Ziegler, I., 1958. Untersuchungen zur Entwicklung, Geschlechtsspezifität und phänogenetischen Autonomie der Augen-Pterine verschiedener Genotypen. *Z. Vererbungslehre* **89,** 221–234.

Hadorn, E., Graf, G. E., and Ursprung, H., 1958. Der Isoxanthopterin-Gehalt transplantierter Hoden von *Drosophila melanogaster* als nicht-autonomes Merkmal. *Rev. suisse zool.* **65,** 335–342.

Hama, T., Matsumoto, J., and Obika, M., 1960. Localization of pterins in the goldfish-skin melanophores and erythrophores. *Proc. Japan. Acad.* **36,** 217–222.

Hanser, G., 1948. Über die Histogenese der Augenpigmentgranula bei verschiedenen Rassen von *Ephestia kühniella* Z. und *Ptychopoda seriata* Schrk. *Z. Vererbungslehre* **82,** 74–97.

Hanser, G., 1959. Genphysiologische Untersuchungen an der *w*-Mutante von *Calliphora erythrocephala*. *Z. Naturforsch.* **14b,** 194–201.

Hoenigsberg, H. F., and Castiglioni, M. C., 1958. Biochemical differences between inbred and outbred lines of *Drosophila melanogaster* studied by paper partition chromatography. *Nature* **181,** 1404.

Howland, R. B., Glancy, E. A., and Sonnenblick, B. P., 1937. Transplantation of wild type and *vermilion* eye disks among four species of *Drosophila*. *Genetics* **22,** 434–442.

Hubby, J. L., and Throckmorton, L. H., 1960. Evolution and pteridine metabolism in the genus *Drosophila*. *Proc. Natl. Acad. Sci. U.S.* **46,** 65–78.

Hüttel, R., and Sprengling, G., 1943. Über Ichthyopterin, einen blau fluoreszierenden Stoff aus Fischhaut. *Ann. Chem. Liebigs* **554,** 69–82.

Jucci, C., 1932. Inheritance of cocoon color and other characters in silkworm. *Intern. Congr. Genet., 6th Congr.*, pp. 377–379.

Kalmus, H., 1943. The optomotor responses of some eye mutants of *Drosophila*. *J. Genet.* **45,** 206–213.

Kanehisa, T., 1956. Eye-colour genes and tumor incidence. *Idengaku Zasshi* **31,** 144–146.

Kauffmann, Th., 1959. Notiz über die Konstitution des Ichthyopterins. *Ann.* **625,** 133–139.

Kikkawa, H., 1941. Mechanism of pigment formation in *Bombyx* and *Drosophila*. *Genetics* **26,** 587–607.

Kikkawa, H., 1953. Biochemical genetics in *Bombyx mori*. *Advances in Genet.* **5,** 107–135.

Knox, W. E., and Mehler, A. H., 1950. The conversion of tryptophan to kynurenine in liver. I. The coupled tryptophan peroxidase system forming formylkynurenine. *J. Biol. Chem.* **187,** 419–430.

Krebs, E. G., and Norris, E. R., 1949. The competitive inhibition of xanthine oxidation by xanthopterin. *Arch. Biochem.* **24,** 49–54.

Kühn, A., 1941a. Über eine Gen-Wirkkette der Pigmentbildung bei Insekten. *Nachr. Akad. Wiss. Göttingen, Math-. physik. Kl., 1941,* p. 231.

Kühn, A., 1941b. Zwei neue Pigmentierungsmutationen (schwarze Raupe und schwarze Intercostalfelder der Puppe) bei *Ptychopoda seriata* Schrk. *Biol. Zentr.* **61,** 225–241.

Kühn, A., 1956. Versuche zur Entwicklung eines Modells der Genwirkungen. *Naturwissenschaften* **43,** 25–28.

Kühn, A., 1957. Eine neue Mutation in der Ommochrombildungskette bei *Ephestia kühniella*. *Z. Naturforsch.* **12b,** 728–730.

Kühn, A., and Becker, E., 1942. Quantitative Beziehungen zwischen zugeführtem Kynurenin und Augenpigment bei *Ephestia kühniella* Z. *Biol. Zentr.* **62,** 303–317.

Kühn, A., and Berg, B., 1955. Zur genetischen Analyse der Mutation *biochemica* von *Ephestia kühniella*. *Z. Vererbungslehre* **87,** 25–36.

Kühn, A., and Berg, B., 1956. Über die Kombination von *wa* (weissäugig) und *biochemica* bei *Ephestia kühniella*. *Z. Vererbungslehre* **87,** 335–337.

Kühn, A., and Egelhaaf, A., 1955. Zur chemischen Polyphänie bei *Ephestia kühniella*. *Naturwissenschaften* **42,** 634–635.

Kühn, A., and Egelhaaf, A., 1959a. Über die Mutation *br* (braunäugig) bei *Ephestia kühniella*. *Z. Vererbungslehre* **90,** 244–250.

Kühn, A., and Egelhaaf, A., 1959b. Der rote Augenfarbstoff von *Ephestia* und *Ptychopoda* ein Pterinpigment. *Z. Naturforsch.* **14b,** 654–659.

Kühn, A., and Henke, K., 1930. Eine Mutation der Augenfarbe und der Entwicklungsgeschwindigkeit bei der Mehlmotte *Ephestia kühniella*. *Wilhelm Roux' Arch. Entwicklungsmech. Organ.* **122,** 204–212.

Kühn, A., and Plagge, E., 1937. Prädetermination der Raupenpigmentierung bei *Ephestia kühniella* Z. durch den Genotypus der Mutter und durch arteigene und artfremde Implantate. *Biol. Zentr.* **57,** 113–126.

Kühn, A., and Schwartz, V., 1942. Über eine weissäugige Mutante (*wa*) von *Ephestia kühniella*. *Biol. Zentr.* **62,** 226–230.

Lewis, E. B., 1950. The phenomenon of position effect. *Advances in Genet.* **3,** 73–112.

Mackerras, M. J., 1933. Note on the occurrence of a white-eyed mutant race of *Lucilia cuprina* Wied. *Australian J. Exptl. Biol. Med. Sci.* **11,** 45–47.

Mainx, F., 1938. Analyse der Genwirkung durch Faktorenkombination. Versuche mit den Augenfarbenfaktoren von *Drosophila melanogaster*. *Z. Vererbungslehre* **75,** 256–276.

Mehler, A. H., and Knox, W. E., 1950. The conversion of tryptophan to kynurenine in liver. II. The enzymatic hydrolysis of formyl kynurenine. *J. Biol. Chem.* **187,** 431–438.

Mitchell, H. K., Glassmann, E., and Hadorn, E., 1959. Hypoxanthine in *rosy* and *maroon-like* mutants of *Drosophila melanogaster*. *Science* **129,** 268–269.

Möhlmann, E., 1958. Chromatographische Untersuchungen an verschiedenen Genotypen von *Plodia interpunctella* im Vergleich mit anderen Schmetterlingsarten. *Z. Vererbungslehre* **89,** 651–674.

Morita, T., and Tokuyama, T., 1959. Pigment in *white*-locus alleles. *Drosophila Inform. Serv.* **33,** 148.

Nawa, S., Sakaguchi, B., and Taira, T., 1957. Genetical and biochemical studies in the metabolism of pteridines in insects. *Ann. Rept. Natl. Inst. Genet.* (*Japan*) **7,** 32–35.

Nolte, D. J., 1950. The eye-pigmentary system of *Drosophila:* The pigment cells. *J. Genet.* **50,** 79–99.

Nolte, D. J., 1952. The eye-pigmentary system of *Drosophila*. III. The action of eye-colour genes. *J. Genet.* **51,** 142–186.

Nolte, D. J., 1954. The eye-pigmentary system of *Drosophila*. V. The pigments of the light and dark groups of mutants. *J. Genet.* **52,** 127–139.

Nolte, D. J., 1955. The eye-pigmentary system of *Drosophila*. VI. The pigments of the ruby and red groups of mutants. *J. Genet.* **53,** 1–10.

Obika, M., and Hama, T., 1960. The effect of melanophore inhibition upon the pterin synthesis in amphibians. *Proc. Japan Acad.* **36,** 151–155.

Plagge, E., 1939. Genabhängige Wirkstoffe bei Tieren. *Ergeb. Biol.* **17,** 105–150.

Reisener-Glasewald, E., 1956. Über die Entwicklung des Bestandes an fluoreszierenden Stoffen in den Köpfen von *Ephestia kühniella* in Abhängigkeit von verschiedenen Augenfarbgenen. *Z. Vererbungslehre* **87,** 668–693.

Sakaguchi, B., 1955. Biochemical and genetical studies on wild silkworm. II. On the nature of the pigments in the epidermal tissues of the Chinese tussar silkworm, *Antheraea pernyi*. *Ann. Rept. Natl. Inst. Genet.* (*Japan*) **5,** 34–35.

Sakaguchi, B., and Tsujita, M., 1955. Genetical and biochemical studies on the yellow lethal silkworm. IV. Changes in the quantity of pterin compounds in explanted ovaries. *Ann. Rept. Natl. Inst. Genet.* (*Japan*) **6,** 33–34.

Schutz, F., 1956. Vergleichende Untersuchungen über die Schreckreaktion bei Fischen und deren Verbreitung. *Z. vergleich. Physiol.* **38,** 84–135.

Sturtevant, A. H., 1947. White-eyed mutants of *Diptera*. *Nature* **160,** 754–755.

Sturtevant, A. H., and Novitski, E., 1941. The homologies of the chromosome elements in the genus *Drosophila*. *Genetics* **26,** 517–541.

Taira, T., and Nawa, S., 1958. No direct metabolic relation between pterines and uric acid, flavins or folic acid in *Drosophila melanogaster*. *Idengaku Zasshi* **33,** 42–45.

Taira, T., and Nawa, S., 1959. A note on pigmentation in the eye. *Drosophila Inform. Serv.* **33,** 167.

Tate, P., 1947a. A sex-linked and sex-limited white-eyed mutation of the blow-fly (*Calliphora erythrocephala*). *J. Genet.* **48,** 176–191.

Tate, P., 1947b. The effect of cold upon the development of pigment in a white-eyed mutant of the blow-fly (*Calliphora erythrocephala*). *J. Genet.* **48,** 192–193.

Tatum, E. L., 1939a. Nutritional requirements of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. U.S.* **25,** 490–497.

Tatum, E. L., 1939b. Development of eye-colors in *Drosophila:* Bacterial synthesis of v^+ hormone. *Proc. Natl. Acad. Sci. U.S.* **25,** 486–490.

Tatum, E. L., and Haagen-Smit, A. J., 1941. Identification of *Drosophila* v^+ hormone of bacterial origin. *J. Biol. Chem.* **140,** 575–580.

Tsujita, M., 1955. On the relation of the *"lethal yellow"* gene to the *"lemon"* in *Bombyx mori,* with special reference to the maternal inheritance of *lethal yellow. Idengaku Zasshi* **30,** 107–117.

Tsujita, M., and Sakaguchi, B., 1955. Genetical and biochemical studies of *yellow lethal* larvae in the silkworm. (1) On the nature of pterin obtained from the *yellow lethal* strain. *Idengaku Zasshi* **30,** 83–88.

Tsujita, M., and Sakaguchi, B., 1957. Genetical and biochemical studies on the *yellow lethal* silkworm. VI. Chitin production and melanin metabolism. *Ann. Rept. Natl. Inst. Genet. (Japan)* **7,** 31–32.

Tsujita, M., and Sakaguchi, B., 1958. Studies on the maternal inheritance of the *lethal yellow* in the silkworm *Bombyx mori.* II. Ovarian transplantations *Idengaku Zasshi* **33,** 210–215.

Ursprung, H., 1959. Nonautonomy of the eye-color mutant *bronzy. Drosophila Inform. Serv.* **33,** 174.

Ursprung, H., Graf, G. E., and Andres, G., 1958. Experimentell ausgelöste Bildung von rotem Pigment in den Malpighischen Gefässen von *Drosophila melanogaster. Rev. suisse zool.* **65,** 449–460.

Viscontini, M. E., and Möhlmann, E., 1959. Fluoreszierende Stoffe aus *Drosophila melanogaster.* 12. Mitt. Die gelb fluoreszierenden Pterine: Sepiapterin und Isosepiapterin. *Helv. Chim. Acta* **42,** 836–841.

Viscontini, M. E., and Weilenmann, H. R., 1959. Über Pteridinchemie. 2. Mitt. Rückoxydation des 2-Amino-6-hydroxy-7,8,9,10-tetrahydropteridins an der Luft. *Helv. Chim. Acta* **42,** 1854–1862.

Viscontini, M. E., Scholler, M., Loeser, E., Karrer, P., and Hadorn, E., 1955. Isolierung fluoreszierender Stoffe aus *Drosophila melanogaster. Helv. Chim. Acta* **38,** 397–401, 1222–1224, 2034–2035.

Viscontini, M. E., Kühn, A., and Egelhaaf, A., 1956. Isolierung fluoreszierender Stoffe aus *Ephestia kühniella. Z. Naturforsch.* **11b,** 501–504.

Viscontini, M. E., Hadorn, E., and Karrer, P., 1957. Fluoreszierende Stoffe aus *Drosophila melanogaster:* die roten Augenfarbstoffe. *Helv. Chim. Acta* **40,** 579–585.

Wagner, R. P., and Mitchell, H. K., 1955. "Genetics and Metabolism." Wiley, New York.

Ward, C. L., and Hammen, C. S., 1957. New mutations affecting tryptophan-derived eye pigments in three species of insects. *Evolution* **11,** 60–64.

Wettstein, D. von, 1957. Genetics and the submicroscopic cytology of plastids *Hereditas* **43,** 303–317.

Weygand, F., and Waldschmidt, M., 1955. Über die Biosynthese des Leukopterins, untersucht mit C-14-markierten Verbindungen am Kohlweissling. *Angew. Chem.* **67,** 338.

Wieland, H., and Liebig, R., 1944. Ergänzende Beiträge zur Kenntnis der Pteridine. *Ann.* **555,** 146–156.

Wolfram, R., 1948. Die Ommochrom-Menge in den Malpighischen Gefässen bestimmende Allele der Mehlmotte, *Ephestia kühniella*. *Z. Naturforsch.* **3b,** 291–293.

Yagi, N., and Saitoh, K., 1955. Studies on the gynandromorphs of *Colias erate* (*hyale*) *poliographus* M., concerning genetic components in relation to the form of pterin pigment in the scales of the wing. *Japan J. Zool.* **11,** 345–352.

Zeutschel, B., 1958. Entwicklung und Lage der Augenpigmente bei verschiedenen Drosophilamutanten. *Z. Vererbungslehre* **89,** 508–520.

Ziegler, I., 1956a. Untersuchungen über die photolabilen Pterine in der Haut der Amphibien und in den Augen von *Drosophila melanogaster*. *Z. Naturforsch.* **11b,** 493–500.

Ziegler, I., 1956b. Pterine: Pigmente und Wirkstoffe in Tierreich. *Biol. Revs. Cambridge Phil. Soc.* **31,** 313–348.

Ziegler, I., 1956c. Untersuchungen über die Purin- und Pterinpigmente in der Haut und in den Augen der Weissfische. *Z. vergleich. Physiol.* **39,** 163–189.

Ziegler, I., 1960a. Tetrahydrobiopterin-Derivat als lichtempfindliche Verbindung bei Amphibien und Insekten. *Z. Naturforsch.* **15b,** 460–465.

Ziegler, I., 1960b. Zur genphysiologischen Analyse der Pterine im Insektenauge. *Z. Vererbungslehre* (in press).

Ziegler, I., 1960c. Zur Feinstruktur der Augengranula bei *Drosophila melanogaster*. *Z. Vererbungslehre* **91,** 206–209.

Ziegler, I., and Hadorn, E., 1958. Manifestation rezessiver Augenfarb-Gene im Pterininventar heterozygoter Genotypen von *Drosophila melanogaster*. *Z. Vererbungslehre* **89,** 235–245.

Ziegler, I., and Jaenicke, L., 1959. Zur Wirkungsweise des *white*- Allels bei *Drosophila melanogaster*. *Z. Vererbungslehre* **90,** 53–61.

AUTHOR INDEX

Numbers in italics indicate the page on which the references are listed.

A

Abe, M., 130, *151*
Acton, A. B., 187, 197, *212*
Adams, M. H., 150, *151*
Adler, J., 93, *151, 152*
Aé, A. S., 166, 186, *212*
Albrecht, W., 360, *396*
Albuquerque, R. M., 27, *51*
Alexander, H. E., 65, 66, 70, 71, 74, 75, 84, 86, 87, 90, 91, 94, 95, *151, 158, 163*
Alexander, P., 40, *58*
Alloway, J. L., 63, *151*
Almeida, F. F. de, 354, 356, 363, 370, *395*
Alper, T., 3, 4, 5, 8, *51, 53*
Ambrose, E. J., 49, *51*
Amy, R. L., 336, *343*
Anders, A., 366, *395*
Anders, G., 362, 381, 382, *395, 402*
Anderson, R. L., 298, 314, *343, 348*
Anderson, T. F., 64, 142, *151*
Armstrong, J. A., 17, *51*
Armstrong, J. M., 232, *249*
Arrick, M. S., 393, *398*
Aslaksen, E., 362, *395*
Assejeva, T., 238, *249*
Atwood, K. C., 10, 11, *59,* 322, 324, 332, *343*
Auerbach, C., 30, *51,* 91, *151,* 340, *343*
Austrian, R., 69, 70, 71, 81, 94, 144, *151, 152*
Autrum, H., 355, 370, 387, *395*
Avery, O. T., 63, 70, 72, 73, 74, 81, 92, 94, 95, 96, *152, 159*

B

Baalen, C. van, 351, *398*
Baglioni, C., 361, 367, *395*
Bains, G. S., 228, 230, 233, *249*
Balassa, R., 66, 69, 70, 71, *152*
Balbinder, E., 65, 67, *154*
Bamford, R., 225, *250, 255*
Banic, S., 65, 67, *154*
Banks, H., 17, *52*
Barclay, R. K., 78, *156*
Barnett, L., 143, *153*
Bateman, K. G., 261, 264, 265, *290*
Bauch, R., 17, *51*
Baud, C. A., 26, *51*
Beadle, G. W., 143, *152,* 314, *343,* 356, 358, 361, *395*
Beamish, K. I., 233, *249*
Beardmore, J. A., 278, *290*
Beatty, A. V., 3, 4, 7, 13, 14, 15, 16, 35, 46, *51, 53, 57*
Beatty, J. W., 13, 14, 15, 16, 35, 46, *51*
Becker, E., 350, 358, 359, 364, 366, 384, *395, 396, 400*
Beers, R. F., 17, *52*
Begg, M., 258, *291*
Beiser, S. M., 71, 91, 92, *152*
Beiser, W. C., Jr., 341, *344*
Belling, J., 224, *249*
Belozerskii, A. N., 66, *157*
Bendich, A., 91, 92, *152*
Benzer, S., 133, *152*
Berg, B., 369, 382, 383, 386, *400*
Berg, R. L., 284, *291*
Berger, R. E., 28, *52*
Bergeron, J. A., 49, *58*
Bernheimer, H. P., 71, 144, *151, 152*
Bessman, M. J., 93, *151, 152*
Biekert, E., 350, 378, 382, *396*
Biesele, J. J., 28, *52*
Binder, M., 65, 67, *163*
Bishop, D. W., 318, *343*
Bitter, G., 217, 218, 241, 242, *249*
Black, W., 224, *249*
Blakeslee, A. F., 224, *249*
Bleier, H., 229, *249*
Blest, A. D., 180, 181, *212*
Blomstrand, I., 388, *396*
Blum, H. F., 17, *52*
Boam, T. B., 198, *216*

Boivin, A., 65, 66, 70, *152*
Booth, J., 28, *52*
Bostian, C. H., 300, 302, 329, *343*, *347*
Bowater, W., 171, 175, *212*
Boyland, E., 28, *52*
Bracco, R. M., 65, 66, 70, 148, *153*
Brachet, J., 49, *52*, 147, *153*
Braun, W., 92, *160*
Brehme, K. S., 269, *291*, 385, *396*
Brenner, S., 143, *153*
Bridges, C. B., 269, *291*
Brower, J. V., 178, 181, 182 ,183, 184, *212*, *213*
Brower, L. P., 178, 195, *213*
Brown, E. R., 68, *153*
Brown, G. B., 93, *153*
Brown, G. L., 77, 78, 91, 92, *153*, *156*
Brücher, H., 245, 247, 248, *249*
Bryan, B. E., 70, 120, 121, 122, 123, 128, *153*
Bukasov, S. M., 218, 219, 220, 223, 241, 242, 247, *249*, *250*, *252*
Bunker, H. P., 309, *344*
Buretz, K. M., 325, *344*
Burgi, E., 76, *156*
Burrous, J. W., 148, *159*
Butenandt, A., 350, 358, 359, 360, 378, 382, *396*
Butler, J. A. V., 80, 81, 91, *153*, *159*
Butler, L., 225, *254*

C

Cadman, C. H., 233, 234, 238, *250*
Callan, H. G., 26, *52*
Campos, F. F., 225, *250*
Cannon, W. B., 277, *291*
Carlson, E. A., 112, *153*
Carpenter, G. D. H., 181, 184, *213*
Carson, G. P., 223, 230, *250*
Case, M. E., 133, *153*
Caspari, E., 191, *213*, 356, 359, 360, *361*, 362, 367, 372, 377, 378, 382, 386, 388, 389, *396*
Caspari, S., 331, *347*
Castiglioni, M. C., 372, *399*
Catlin, B. W., 66, 70, 148, *153*
Cavalieri, L. F., 80, 89, 91, 105, 110, *153*, *161*
Chapman, V., 231, 239, *254*
Chargaff, E., 74, 77, 91, 108, *153*, *154*, *163*
Chase, M., 75, 82, 133, *154*, *156*
Chayen, J., 28, 44, 49, *52*, *55*
Cherry, W. B., 68, *153*
Chevais, S., 358, 379, *397*
Chittenden, F. H., 296, *344*
Choudhuri, H. C., 225, 228, 229, 230, *250*
Christensen, H. M., 225, *250*
Clare, N. T., 17, *52*
Clark, A. M., 320, 327, 328, 329, 330, 341, 342, *344*
Clark, C. F., 228, 233, 238, *253*
Clark, J. B., 22, *59*
Clark, L. B., 325, *345*
Clarke, C. A., 166, 185, 186, 187, 188, 189, 190, 197, 198, 199, *213*
Clarke, M., 28, *52*
Clayton, F. E., 367, 368, 381, *397*
Clayton, G. A., 283, *291*
Cobb, M., 40, *58*
Cockayne, E. A., 166, 192, *213*
Cockerham, G., 238, *250*
Cohn, N. S., 12, *52*
Cole, C. S., 238, *250*
Collins, C., 13, *51*
Colowick, M. S., 71, *151*
Cooper, D. C., 224, 225, 226, 227, 228, 229, 230, 233, 235, 236, 237, *249*, *250*, *253*
Cooper, J. P., 228, *250*
Corey, R. R., 66, 68, 70, *154*
Correll, D. S., 218, 222, 248, *250*
Cott, H. B., 178, *213*
Counce, S. J., 353, 386, *397*
Cousens, S. F., 40, *58*
Craig, D. L., 36, 37, *55*
Crampton, C. F., 91, *153*
Crane, H. R., 79, *158*
Crane, M. B., 232, *250*
Creed, E. R., 203, 204, 205, *213*
Crew, F. A. E., 350, *397*
Crick, F. H. C., 78, 79, 139, *163*
Croghan, P. C., 263, *291*
Crouse, H. V., 23, *52*

D

D'Amato, F., 50, *52*
Danneel, R., 350, 359, 369, 378, 382, 389, *397*
Darlington, C. D., 28, 29, 38, *52*, 228, 229, *250*

Davies, H. G., 28, 44, *52*
Davis, B. D., 64, 99, *154*
Davis, E. A., 29, *56*
Dawson, M. H., 63, *154*
DeBruyn, P. P. H., 17, *52*
Deeley, E. M., 28, 44, *52*
DeFilippes, F. M., 82, 83, *154, 156*
Delaunay, A., 65, *152*
Delbrück, M., 78, 133, *154*
Dellweg, H., 91, *163*
Demerec, M., 65, 67, 121, 123, *154*, 320, *344*, 385, *396*
Demerec, Z. E., 65, 67, *154*
de Ruiter, L., 179, *213, 214*
Deschner, E. E., 5, 16, *52*
Dewey, D. L., 5, 8, *52*
Dickler, H., 380, *397*
Dobzhansky, Th., 148, *154*, 197, 202, *214*, 277, 278, *290, 291*
Dodds, K. S., 228, 229, 243, *250, 251*
Doermann, A. H., 82, 133, *154*
Doty, P., 77, 80, 87, 88, 89, 92, 105, *154, 159, 161, 162*
Dowdeswell, W. H., 203, 204, 205, 206, 208, *213, 214*
Doyle, B., 84, *161*
Drew, R. M., 70, 82, *154, 155*
Dubow, R. J., 49, *58*
Dun, R. B., 280, *291*
Dunal, 217, *251*

E

Eagle, H., 95, *154*
Egelhaaf, A., 354, 360, *361*, 362, 363, 364, 369, 370, 378, 383, 384, 385, 390, 391, *397, 399, 400, 402*
Ehrenberg, A., 8, *58*
Ehrenberg, L., 8, *58*
Eigner, J., 80, 87, 88, *154*
Ellison, W., 228, 232, 233, 237, *251*
Emme, E. K., 226, 228, 229, 240, 244, *251*
Englesberg, E., 90, *154*
Ephrati-Elizur, E., 69, 91, 122, *154, 155, 162*
Ephrussi, B., 350, 356, 358, 379, 391, *395, 397*
Ephrussi-Taylor, H. E., (Taylor, H. E.), 70, 71, 72, 75, 78, 80, 82, 83, 84, 89, 90, 92, 94, 95, 96, 105, 106, 111, 117, 118, 122, 125, 128, 129, 130, 131, 136, 137, 145, 146, *155, 156, 157, 158, 159, 162*
Eschrich-Zimmermann, B., *see also* Zimmermann, B., 389, *397*
Evans, A. H., 70, 119, 128, 145, *156, 157*
Evans, H. J., 1, 6, 7, 15, 45, 47, 49, *52*

F

Fabergé, A. C., 10, *53*
Falconer, D. S., 278, *291*
Fano, U., 320, *344*
Farr, R. S., 17, *52*
Fingerman, M., 387, *397*
Fisher, R. A., 167, 168, 181, 184, 196, 206, *214*, 239, *251*
Fitzgerald, P. L., 65, 67, 74, *154, 163*
Flanders, E. E., 317, *344*
Fluder, Z., 65, 66, 70, 148, *160*
Fluke, D. J., 82, 84, *155, 159*, 320, *344*
Ford, C. E., 38, 39, *53*
Ford, E. B., 166, 167, 168, 174, 175, 178, 179, 181, 182, 184, 185, 186, 187, 188, 189, 190, 191, 193, 195, 197, 200, 202, 203, 204, 205, 206, 208, 211, *213, 214*
Ford, H. D., 200, 202, *214*
Forman, B., 204, *214*
Forrest, H. S., 351, 353, 373, *398*
Fox, M. S., 75, 97, 99, 103, 104, 105, 106, 109, 110, 125, 127, 128, 130, 136, *155*
Frandsen, N. O., 225, *256*
Fraser, A. S., 280, 283, *291*
Fraser, D., 107, *155, 159*
Fredga, K., 28, *53*
Fuerst, R., 8, *58*
Fujimori, E., 369, *398*
Fujito, S., 387, *398*
Fukuda, Y., 237, *251*

G

Gahan, P. S., 49, *55*
Garen, A., 140, *155*
Gell, P. G. H., 241, *251*
Genieys, P., 296, *344*
Gierer, A., 90, *155*
Gilchrist, B. M., 380, 387, *398*
Giles, N. H., 3, 4, 7, 15, *53, 57*, 133, *153*, 326, *344*
Gilles, A., 224, 226, 228, 231, 233, 239, *251*
Glancy, E. A., 364, *399*
Glanville, E. V., 65, 67, *154*

Glass, B., 181, *214*, 320, *344*, 386, *398*
Glassmann, E., 361, 373, 389, 390, *398*, *401*
Glover, S. W., 65, 67, *154*
Goldman, I., 65, 67, *154*
Goldschmidt, E., 371, *398*
Goldschmidt, R. B., 166, 188, *214*, 275, 276, *291*
Gollnick, K., 19, *57*
Goodgal, S. H., 70, 75, 77, 85, 86, 103, 104, 105, 116, 127, 128, 136, *156*, *161*, *162*
Goodrich, D. S., 258, *291*
Goodrich, H. B., 393, *398*
Gopal-Ayengar, A. R., 49, *51*
Gordon, M., 393, *398*
Gordon, M. A., 68, *153*
Gordon, S. A., 17, *53*
Gottlieb, F., 367, *396*
Gottschalk, W., 223, 225, 226, 233, 244, 246, 247, *251*
Gottschewski, G., 364, 366, *398*
Gowen, J. W., 325, *345*
Graf, G. E., 29, *53*, 362, 372, 373, 374, 375, 376, 377, 385, 386, 390, *398*, *399*, *402*
Gray, L. H., 5, 8, 16, 34, 35, *52*, *53*, *55*
Greb, R. J., 310, 314, *348*
Green, D. McD., 70, 82, 84, 123, 129, 130, 149, *156*
Green, F. O., 8, *53*
Green, K. C., 368, *399*
Green, M. M., 356, 359, 361, 366, 367, 368, 381, 386, *398*, *399*
Greer, S., 84, *163*
Griboff, G., 91, *163*
Griffith, F., 62, 66, *156*
Grosch, D. S., 299, 304, 305, 306, 317, 331, 337, 338, 339, 340, *344*, *345*, *346*
Gross, J. D., 65, 67, *154*
Grossman, L., 93, *158*
Guild, W. R., 82, 83, 99, *154*, *156*, *160*

H

Haagen-Smit, A. J., 358, *402*
Haas, F., 22, *59*
Hackett, D. P., 43, *53*
Haddox, C. H., 8, *58*
Hadorn, E., 350, 351, 352, 353, 354, 362, 368, 369, 372, 373, 374, 375, 376, 377, 378, 379, 383, 384, 385, 386, 388, 389, *395*, *398*, *399*, *401*, *402*, *403*
Hahn, E., 66, 71, 84, 101, 118, 148, *151*, *158*, *163*
Haldane, J. B. S., 239, *251*, 380, 387, *398*
Hall, C. E., 78, *156*
Hama, T., 392, 394, *399*, *401*
Hamilton, L. D., 78, *156*
Hammen, C. S., 364, 366, *402*
Hanser, G., 363, 370, 377, 378, 382, 383, *399*
Hansch, C., 30, *56*
Harrison, B. J., 210, *215*
Hartfield, D., 351, *398*
Hartman, P. E., 65, 67, *154*
Hase, A., 296, *345*
Hashimoto, K., 65, 67, *154*
Hawes, C. A., 8, *53*
Hawkes, J. G., 218, 219, 220, 221, 222, 223, 224, 237, 240, 241, 242, 243, 244, 246, 247, *251*, *252*, *254*
Hayes, W., 64, 103, 114, 142, *156*, *163*
Heidenthal, G., 317, 318, 319, 320, 324, 325, *345*
Hendley, D. D., 17, *52*
Henke, K., 356, *400*
Henschen, W., 333, *345*
Herold, J. L., 350, 391, *397*
Herr, E. B., Jr., 327, 342, *344*
Herriott, R. M., 70, 75, 77, 86, 89, 99, 103, 104, 105, 116, 128, 138, *156*, *161*, *162*
Hershey, A. D., 75, 76, 141, *156*
Hill, G. A., 393, *398*
Hoenigsberg, H. F., 372, *399*
Hogness, D. S., 66, 67, 109, 145, *157*
Horn, A. B., 303, *345*
Hotchkiss, R. D., 70, 71, 72, 74, 82, 87, 95, 96, 97, 98, 99, 101, 102, 103, 104, 105, 106, 109, 110, 112, 113, 115, 116, 119, 121, 122, 123, 124, 126, 127, 128, 129, 130, 131, 133, 137, 144, 145, 146, *152*, *155*, *156*, *157*, *159*
Hougas, R. W., 218, 224, 225, 226, 227, 228, 229, 230, 233, 235, 236, 237, 241, *249*, *252*, *253*, *254*, *255*
Howard, A., 28, 44, 49, *53*, *56*
Howard, H. W., 223, 224, 225, 226, 228, 230, 236, 238, 241, 246, *249*, *250*, *252*, *255*

Howard-Flanders, P., 3, 4, 5, 8, *51*, *53*
Howarth, T. G., 180, *214*
Howland, R. B., 364, *399*
Hubby, J. L., 385, 394, *400*
Hüttel, R., 394, *400*
Hughes, C., 30, *53*
Hughes, W. L., 76, *162*
Huijsman, C. A., 238, *252*, *255*
Hulanicka, E., *see* Hulanicka-Bankowska, E.
Hulanicka-Bankowska, E., (Hulanicka, E.), 65, 66, 70, 86, 148, *160*
Hutchinson, D. J., 70, *161*, *162*
Huxley, J. S., 287, *291*

I

Idelman, S., 49, *53*
Imshenetskii, A. A., 66, *157*
Inaba, F., 297, 298, 300, 302, 307, 342, *345*
Ionesco, H., 69, 71, 108, *161*
Ivanovskaja, E. V., 227, 233, 237, *252*
Iyer, V. N., 70, 120, 128, 129, 130, *161*

J

Jackson, S., 118, *157*
Jackson, T. H. E., 182, *216*
Jacob, F., 64, 71, 133, 142, *151*, *157*, *161*, *163*
Jaenicke, L., 378, 379, 382, *403*
James, D. W. F., 80, *153*
Jinks, J. L., 282, *291*
Jockey, P., 4, 8, *53*
Johnstone, F. E., 230, *253*
Josse, J., 144, *157*
Jucci, C., 389, *400*
Juzepczuk, S. W., 218, 219, 220, 241, *252*

K

Kaiser, A .D., 66, 67, 109, 145, *157*
Kalckar, H. M., 28, *54*
Kaleta, B. F., 8, *56*
Kalmus, H., 387, *400*
Kameraz, A. Ja., 220, 247, *250*
Kanazir, D., 65, 67, *154*
Kanehisa, T., 386, *400*
Kao, P., 331, *347*
Karrer, P., 351, 352, 353, *402*
Kauffmann, Th., 394, *400*
Kawakami, K., 236, *252*
Kelly, E. M., 329, *344*
Kellenberger, G., 141, *156*
Kelner, A., 85, *157*
Kenworthy, W., 327, *345*
Kettlewell, H. B. D., 171, 173, 174, 175, 177, 178, 180, 190, 191, 209, *214*, *215*
Kihlman, B. A., 1, 5, 6, 7, 8, 14, 15, 17, 18, 19, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 38, 39, *54*, *57*
Kikkawa, H., 358, 361, 389, 390, *400*
Kindred, B. M., 280, *291*
King, E. D., 22, 45, *54*, *57*
Kirby-Smith, J. S., 36, 37, *55*
Klein, D. T., 66, 68, 71, *157*
Klein, R. M., 66, 68, 71, *157*
Knox, W. E., 361, *400*, *401*
Koch, A. L., 28, *55*
Kohiyama, M., 94, *157*
Koller, P. C., 38, *52*
Komarov, V. L., 237, *252*
Koopmans, A., 226, 228, 229, *252*
Kornberg, A., 93, 144, *151*, *152*, *157*
Kornberg, S. R., 144, *157*
Kostoff, D., 218, *252*
Kotval, J. P., 34, 35, *55*
Koukides, M., 331, *347*
Koukol, J., 71, 145, *162*
Krantz, F. A., 239, 248, *252*
Krauss, M. R., 65, 66, 70, 71, 101, 118, *153*, *157*, *159*, *162*
Krebs, E. G., 373, 389, *400*
Krombein, K. V., 296, *346*
Kühn, A., 354, 356, 359, 363, 364, 369, 370, 378, 382, 383, 384, 386, 388, 389, *399*, *400*, *402*
Kürsteiner, R., 385, *399*
Kurahashi, K., 145, *157*

L

LaChance, L. E., 322, 327, 339, *344*, *345*
Lacks, S., 71, 87, 122, 123, 129, 137, 145, *157*
LaCour, L. F., 23, 49, *55*
Lahr, E. L., 65, 67, *154*
Lamm, R., 223, 226, 228, 229, 233, 234, 237, *252*
Lamy, R., 350, *397*
Landauer, W., 276, *291*
Lane, C., 181, 210, *215*

Lane, D., 70, 87, 88, *159*
Lane, G. R., 38, *55*
Langer, H., 355, 370, *395*
Laschet, L., 108, *163*
Latarjet, R., 82, 83, 84, *155, 158*
Lawrence, W. J. C., 228, *252*
Lea, D. E., 2, *55*
Lederberg, E. M., 64, 124, 141, 145, 150, *158, 160*
Lederberg, J., 64, 99, 107, 108, 116, 124, 141, 142, 150, *158, 160, 163*
Lehman, I. R., 93, *151, 152*
Lehoult, Y., 65, 66, 70, *152*
Leidy, G., 65, 66, 70, 71, 74, 75, 84, 86, 87, 90, 91, 94, 95, 98, 101, 118, 148, *151, 158, 163*
Lerman, L., 70, 75, 81, 82, 83, 84, 85, 86, 91, 92, 103, 104, 110, *158*
Lerner, I. M., 277, 282, *291*
Leslie, I., 63, *158*
Levan, A., 22, 23, 34, *54, 55*
Levine, L., 93, *158*
Levinthal, C., 76, 79, 141, *158*
Levitan, M., 197, *215*
Lewis, D., 230, 237, *252*
Lewis, E. B., 392, *401*
Lewontin, R. C., 277, *291*
Liebig, R., 373, 389, *403*
Lilly, L. J., 21, 22, 43, *55*
Linzen, B., 350, 378, 382, *396*
Lipschitz, R., 91, *153*
Litman, R. M., 90, 91, *158, 159*
Litt, M., 89, 105, *159*
Lively, E. R., 64, *158*
Livermore, J. R., 230, *253*
Lockwood, L. A. P. M., 263, *291*
Loeser, E., 351, 353, *402*
Long, D. H., 229, 243, *251*
Longley, A. E., 228, 233, 238, *253*
Lorkiewicz, Z., 91, *162*
Lorković, Z., 195, *215*
Lotfy, T., 34, 38, *55, 57*
Lovelace, R., 36, 50, *55*
Loveless, A., 18, 22, 30, 38, 39, *55*
Lucy, J. A., 91, *159*
Lüning, K. G., 326, *345*
Luippold, H. E., 9, 10, 11, 12, 13, 43, 49, *59*
Lunden, A. P., 238, 239, *253*
Lunt, R., 70, *161, 162*
Luria, S. E., 140, 148, *159*

M

MacBride, D. H., 298, *345*
McCarty, M., 63, 70, 72, 73, 74, 81, 90, 92, 94, 95, 96, *152, 159*
McCrady, E., 317, *345*
McDonald, J. M., 275, *292*
McEwen, M. B., 77, 92, *153*
Mackerras, M. J., 380, *401*
McLeish, J., 25, 28, 29, 47, *52, 55*
MacLeod, C. M., 63, 65, 66, 70, 71, 72, 73, 74, 92, 95, 101, 118, *152, 153, 157, 159, 162*
McWhirter, K. G., 203, 204, 205, 206, 208, *213, 214, 215*
Magnus, D. B. E., 195, *215*
Magoon, M. L., 218, 224, 225, 226, 227, 228, 229, 230, 233, 236, *253, 255*
Mahler, H. R., 107, *155, 159*
Mainx, F., 350, *401*
Marks, G. E., 218, 228, 241, *253*
Marmur, J., 70, 71, 77, 80, 82, 84, 87, 88, 89, 92, 105, 112, 113, 115, 116, *154, 157, 159, 162*
Marquardt, H., 49, 50, *55*
Martin, A. V., 91, *153*
Marvin, D. A., 78, *156*
Mather, K., 45, *57*, 196, 210, *215*, 282, 283, *292*
Matsubayashi, M., 227, 236, *252, 253*
Matsumoto, J., 394, *399*
Maxwell, J., 314, 317, 318, *343, 345*
Maynard Smith, J., 281, *291, 292*
Mayr, E., 202, *215*, 287, *292*
Mazé, R., 142, *151*
Mazia, D., 49, *55*
Meder, W., 19, *57*
Mehler, A. H., 361, *400, 401*
Merz, T., 38, 39, *58*
Meselson, M., 76, 92, 140, 141, *159, 161*
Meurman, O., 229, 233, *253*
Michaelis, A., 7, 34, 41, 42, *55, 57*
Milkman, R., 261, *292*
Mills, G .T., 71, 144, *152*
Mirsky, A. E., 49, *55, 56*, 73, *160*
Mitchell, C. J., 305, 328, 329, *344, 346*
Mitchell, H. K., 351, 353, 361, 363, 368, 373, 375, 385, 389, 390, *395, 398, 399, 401, 402*

Mitchell, M. B., 133, *160*
Miyake, T., 65, 67, *154*
Mizuno, D., 130, *151*
Möhlmann, E., 351, 353, 362, 393, *401, 402*
Moh, C. C., 17, *56, 59*
Moody, M. D., 68, *153*
Morgan, D. T., Jr., 225, *250*
Morita, T., 379, *401*
Morris, J. A., 283, *291*
Morse, M. L., 141, *160*
Morthland, F. W., 17, *52*
Moser, H., 65, 67, *154*, 336, *346*
Moses, M. J., 45, 49, *56, 58*
Moutschen-Dahmen, J., 22, *56*
Moutschen-Dahmen, M., 22, *56*
Müller, K. O., 238, *253*
Muesebeck, C. F. W., 296, *346*
Muir, R. M., 30, *56*
Muller, H. J., 316, *346*
Müntzing, A., 228, 229, 231, 233, 239, *253*
Mundry, K. W., 90, *155*
Murakami, W. T., 93, *158*
Murphy, W. E., 320, 324, 326, *347*

N

Nathan, H., *352*
Naumenko, V. A., 288, *292*
Navashin, M., 233, *253*
Nawa, S., 374, 382, 388, 389, *401*
Nay, T., 280, *291*
Naylor, A. W., 29, *56*
Neary, G. J., 1, 6, 7, 15, 45, *52*
Neish, W. J. P., 28, *56*
Nester, E. W., 116, *160*
Neubert, G., 350, 359, *396*
Neumüller, O. A., 19, *57*
Nikolaeva, N., 238, *249*
Ninan, T., 218, *255*
Nolte, D. J., 381, *401*
Norick, A., 28, *56*
Norris, E. R., 373, 389, *400*
Novitski, E., 364, *401*
Nyman, P. O., 28, *53*

O

Obika, M., 392, 394, *399, 401*
Odaka, T., 95, *160*
Östergren, G., 50, *56*
Okamoto, M., 231, 239, *255*
Okuno, S., 228, 236, *253*
Opara-Kubinska, Z., 91, *162*
Overgaard-Hansen, K., 28, *54*

P

Pahl, H. B., 91, 92, *152*
Pakula, R., 65, 66, 70, 86, 148, *160*
Pal, B. P., 230, *253*
Pandey, K. K., 230, 241, *254*
Pape, M., 19, *57*
Pardee, A. B., 91, 107, *159, 160*
Pavlovsky, O. A., 202, *214*, 278, *290*
Pelc, S. R., 28, 44, 49, *53, 56*
Peloquin, S. J., 235, 237, *252, 254*
Perova, K. Z., 66, *157*
Peters, N., 223, 225, 226, 233, 244, 246, 247, *251, 254*
Phillips, D. M. P., 81, *153*
Phillips, J. H., 92, *160*
Piternick, L. K., 276, *291*
Plagge, E., 356, 359, 362, 364, 367, 384, 389, 390, *400, 401*
Plescia, O., 92, *160*
Pollard, E., 82, *155*
Pontecorvo, G., 133, *160*, 196, *215*
Powers, E. L., 8, *56*
Prakken, R., 224, 227, 229, 231, 239, 247, *253, 254*
Pratt, M. I., 77, 92, *153*
Prestidge, L. S., 107, *160*
Pritchard, R. H., 133, *160*
Proom, H., 68, *162*
Propach, H., 226, 227, 229, *254*
Pullman, A., 28, *56*
Pullman, B., 28, *56*
Pushkarnath, 230, *253, 254*

R

Ramanujam, S., 224, *253*
Ramshorn, K., 34, *55*
Rancken, G., 229, 233, *253*
Randolph, L. F., 231, 239, *251*
Rapaport, J. A., 90, *160*
Rappaport, H. P., 99, *160*
Ravin, A. W., 70, 71, 94, 95, 98, 101, 112, 116, 118, 119, 120, 121, 122, 123, 125, 126, 127, 128, 129, 130, 147, 150, *160, 161*

Read, J., 2, 8, 27, 35, 49, *56, 58,* 326, *346*
Redman, W., 65, 66, 70, *151*
Reeve, E. C. R., 282, 283, *292*
Reichmann, M. E., 77, *161*
Reisener-Glasewald, E., 370, 378, *401*
Rekemeyer, M. L., 332, 333, *346*
Remington, C. L., 166, 180, 185, 186, 189, 190, 192, 193, 211, *215*
Rendel, J. M., 281, *292*
Revell, S. H., 1, 2, 12, 23, 38, 39, 40, 44, 45, 46, 47, 48, 50, *55, 56, 57*
Rice, S. A., 77, *161*
Richards, J., 360, 377, *396*
Rick, C. M., 218, 225, *254*
Rieger, R., 7, 34, 41, 42, *55, 57*
Rieman, G. H., 235, 237, *250*
Riley, H. P., 3, 53, *57*
Riley, R., 231, 239, *254*
Ris, H., 49, *55, 56, 57*
Risman, G. C., 304, *346*
Ritz, E., 148, *161*
Robertson, A., 283, *291*
Robertson, F. W., 278, 282, *292*
Robertson, R. W., 232, *249*
Robson, J. M., 91, *151,* 340, *343*
Robson, H. H., 17, *59*
Roe, A. S., 65, 66, 70, 148, *153*
Rogers, R. W., 337, *346*
Rolfe, R., 92, *161*
Roll, P. M., 93, *153*
Roman, H., 133, *161*
Rosenberg, B. H., 105, 110, *161*
Ross, H., 225, *256*
Ross, R. W., 235, 237, *252*
Ross, W. C. J., 38, 39, 40, *57*
Rothschild, M., 181, 210, *215*
Rubin, M. A., 320, *344*
Rupert, C. S., 85, 86, *156, 161*
Rutishauser, A., 23, *55*
Rybin, V. A., 228, *254*
Rydberg, P. A., 218, *254*

S

St. Clair, J., 107, 108, *158*
Saito, H., 94, *157*
Saitoh, K., 371, *403*
Sakaguchi, B., 355, 374, 375, 386, 391, *401, 402*
Salaman, R. N., 218, 220, 221, 222, 247, *254*
Sang, J. H., 275, *292*
Sax, K., 9, 10, 22, 45, *54, 57*
Schaeffer, P., 69, 70, 71, 96, 98, 104, 108, 110, 120, 129, 148, 149, *161*
Schenck, G. O., 19, *57*
Schiedt, U., 378, *396*
Schildkraut, C., 80, 87, 88, *154*
Schlossberg, H., 358, 361, *396*
Schmalhausen, I. L., 273, *292*
Schmidt, G., 81, *161*
Schmidt, R. S., 183, 184, *215*
Schneiderman, H. A., 22, *54*
Schnell, L .O., 228, 233, *254*
Scholler, M., 351, 353, *402*
Schulman, H. M., 108, *154*
Schutz, F., 393, *401*
Schwartz, P. A., 227, *254*
Schwartz, V., 378, *400*
Schwinck, I., 353, 372, 385, 386, *399*
Sears, E. R., 231, 239, *255*
Sepeleva, E. M., 232, *255*
Serra, J. A., 27, *51*
Setlow, R., 84, *161*
Sexton, O. J., 183, *215*
Shapiro, H. J., 108, *154*
Shatoury, H. H., 286, *292*
Sheldon, B. L., 281, *292*
Sheppard, P. M., 166, 167, 168, 169, 170, 178, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 195, 197, 198, 199, 200, 209, 210, *213, 215, 216*
Shooter, K. V., 81, *153*
Shug, A. L., 107, *155*
Sia, R. H. P., 63, *154*
Simmons, N. S., 78, *156*
Simms, E. S., 93, *151, 152*
Simpson, G. G., 287, *292*
Sirotnak, F. M., 70, 105, 110, *161, 162*
Smith, E. E. B., 71, 144, *152*
Smith, H. H., 38, *57, 58*
Smith, P. A., 310, *346*
Snell, G. D., 302, *346*
Sondhi, K. C., 281, *292*
Sonnenblick, B. P., 320, *346,* 364, *399*
Sparrman, B., 8, *58*
Sparrow, A. H., 49, *58*
Speicher, B. R., 298, 300, 301, 302, 304, 307, 308, 309, 310, 314, 330, 336, 342, *344, 346, 348*

Speicher, K. G., 298, 299, 300, 301, 308, 342, *346*
Spizizen, J., 66, 68, 71, 96, 99, 107, 108, 116, 145, *162*
Spotkov, E. M., 333, *346*
Spragg, S. P., 30, *53*
Sprengling, G., 394, *400*
Srb, A. M., 38, *58*
Srinavasan, P. R., 122, *155*
Stacey, K. A., 40, *58*
Stahl, F. W., 76, 82, 140, *154*, *159*
Stancati, M. F., 317, 318, *346*
Starr, M. P., 66, 68, 70, *154*
Starrells, R., 310, *348*
Stebbins, G. L., 228, 230, 239, 240, *255*
Steffensen, D., 49, *58*
Stehr, G., 193, *216*
Steiner, R. F., 17, *52*
Stent, G. S., 78, 133, *154*
Stern, C., 271, 272, *292*
Sterne, M., 68, *162*
Stocker, B. A. D., 101, *162*
Stokes, A. R., 78, *163*
Stone, W. S., 8, 22, *58*, *59*
Stow, I., 237, *255*
Stride, G. O., 195, *216*
Sturtevant, A. H., 364, 380, *401*
Sulbha, K., 231, 239, *255*
Sullivan, R. L., 317, 338, 339, 340, *345*, *346*
Suoeka, N., 76, 87, 92, *154*, *162*
Surrey, K., 17, *53*
Swaminathan, M. S., 218, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 241, 242, 244, 245, 246, 247, 248, *252*, *254*, *255*
Swanson, C. P., 17, 23, 36, 38, 39, 45, *57*, *58*
Swift, H. H., 76, *162*
Szent-Györgyi, A., 51, *58*
Szybalski, W., 91, *162*

T

Taira, T., *352*, 374, 382, 388, 389, *401*
Talmadge, M. B., 99, *162*
Tan, C. C., 364, 366, *398*
Tate, P., 356, 363, *402*
Tatum, E. L., 358, 359, 361, *402*
Taylor, H. E., *see* Ephrussi-Taylor, H. E.
Taylor, J. H., 45, 49, *56*, *58*, 76, *162*
Tebb, G., 282, *292*
Thoday, J. M., 2, 8, 21, 35, 43, 45, 46, *55*, *58*, 197, 198, 199, 200, *216*, 278, 282, *292*, 326, *346*
Thomas, C. A., Jr., 77, 107, *155*, *161*
Thomas, P. T., 233, *255*
Thomas, R., 65, 67, 72, 75, 80, 82, 85, 95, 96, 97, 101, 108, 110, 138, *162*
Throckmorton, L. H., 385, 394, *400*
Tinbergen, L., 181, *216*
Tokuyama, T., 379, *401*
Tolmach, L. J., 70, 75, 81, 82, 83, 84, 85, 86, 91, 103, 104, 110, *158*
Tomlin, S. G., 26, *52*
Tonkinson, S. M., 1, 45, *52*
Toole, M. G., 225, *255*
Torvik, M. M., *see* Torvik-Greb, M. M.
Torvik-Greb, M. M., (Torvik, M. M.), 298, 300, 306, *346*
Townes, H. K., 296, *346*
Toxopeus, H. J., 238, *255*
Tsujita, M., 355, 375, 386, 391, *401*, *402*

U

Udaka, S., 71, 145, *162*
Ursprung, H., 362, 372, 373, 374, 375, 376, 382, *395*, *398*, *399*, *402*

V

Van der Plank, J. E., 220, 222, *255*
Van Someren, V. G. L., 182, *216*
Van Vunakis, H., 93, *158*
Vavilov, N. I., 241, *255*
Vendrely, R., 65, 66, 70, *152*
Vennesland, R., 71, 145, *162*
Viscontini, M. E., 351, 352, 353, 354, 369, 370, *402*
Voll, M. J., 127, 128, 136, *162*
von Borstel, R. C., 85, *162*, 310, 311, 322, 324, 328, 332, 333, 336, 337, 340, 341, *346*, *347*
von Olah, L., 226, 229, *255*
von Wangenheim, K. H., 225, *255*, *256*

W

Wacker, A., 91, 108, *162*, *163*
Waddington, C. H., 257, 258, 260, 261, 262, 264, 266, 268, 270, 271, 276, 278, 279, 281, 282, 283, 286, 287, *292*, *293*

Wagner, R. P., 8, *58*, 363, *402*
Wakonig, T., 50, *56*
Walczak, W., 65, 66, 70, 86, 148, *160*
Waldschmidt, M., 388, *402*
Walker, M. C., 308, *347*
Ward, C. L., 364, 366, *402*
Watanabe, T., 95, *160*
Watson, J. D., 78, 79, 139, *163*
Watson, M., 91, *153*
Webb, R. B., 8, *56*
Weidel, W., 358, 359, 361, *396*
Weigle, J. J., 76, 141, *156*, *159*
Weil, A. J., 65, 67, *163*
Weilenmann, H. R., 352, *402*
Weil-Malherbe, H., 28, *59*
Weinstein, A., 133, *163*
Weiss, L., 28, *52*
Wenstrup, E. J., 313, *348*
Went, F. W., 248, *256*
Westover, W. E., 65, 67, *154*
Wettstein, D. von, 381, *402*
Weygand, F., 91, *163*, 388, *402*
Whiting, A. R., 298, 299, 300, 306, 311, 313, 320, 321, 322, 323, 324, 325, 326, 329, 331, 332, 334, 339, 340, *343*, *347*
Whiting, P. W., 296, 297, 298, 299, 300, 301, 302, 303, 306, 309, 310, 311, 312, 313, 314, 316, 317, 318, 320, 321, *347*, *348*
Wieland, H., 373, 389, *403*
Wilkins, M. H. F., 78, *156*, *163*
Williams, E. J., 237, *256*
Williamson, M. H., 168, *216*
Wilson, H. R., 78, *156*, *163*
Windecker, W., 181, *216*
Withrow, R. B., 19, *56*, *59*
Wolff, S., 9, 10, 11, 12, 13, 17, 43, 49, *59*, 85, *162*, 328, *347*
Wolfram, R., 384, *403*
Wollman, E. L., 64, 142, *151*, *157*, *163*
Woods, P. S., 76, *162*
Wright, S., 167, *216*
Wright, S. T. C., 241, *251*
Wyss, O., 22, *59*

Y

Yagi, N., 371, *403*
Yamamoto, N., 18, *59*
Yost, H. T., 17, *59*
Yura, T., 65, 67, *154*

Z

Zaitseva, T. A., 66, *157*
Zamenhof, S., 69, 74, 75, 84, 86, 87, 90, 91, 122, *154*, *155*, *162*, *163*
Zeutschel, B., 382, *403*
Zichichi, M. L., 141, *156*
Ziegler, I., 350, 351, 352, *352*, 353, 355, 369, 370, 371, 376, 377, 378, 379, 380, 382, 388, 392, 393, *399*, *403*
Zimmerman, S. B., 93, 144, *152*, *157*
Zimmermann, B., *see also* Eschrich-Zimmermann, B., 359, *397*
Zinder, N. D., 64, 99, 140, *155*, *158*, *163*

SUBJECT INDEX

A

Acquired characters, *see also* Assimilation,
  definition of, 258–259
Acridine orange,
  visible light, chromosomal breakage and, 17–21, 31, 33, 42–43
Adenosine triphosphate, chromosome rejoining and, 12
African swallowtail butterfly, *see Papilio dardanus*
Agrobacterium radiobacter, transformation in, 66, 68, 71
Agrobacterium rubi, transformation in, 66, 68, 71
Agrobacterium tumefaciens, transformation in, 66, 68, 71
Albumin, transformation and, 95, 96–97
Alcohol, *see* Ethyl alcohol.
Alleles,
  recessive, pterin formation and, 376–377
  transforming agents and, 111–112
Allium cepa,
  β-propiolactone and, 38, 39
  chromosome breakage and, 12, 22, 23
Alpha radiation,
  Habrobracon juglandis and, 320, 337
  Tradescantia bracteata and, 34–35
  Vicia faba and, 8, 35
Amauris niavius, mimetic evolution and, 188
2-amino-4-hydroxypteridine,
  chemical structure of, 352
  Drosophila melanogaster and, 351, 353
2-amino-4-hydroxypteridine-6-carboxylic acid, *see* Pterincarboxylic acid
Anal papillae, genetic assimilation and, 262–263, 271, 273, 274
Ancestry,
  Solanum tuberosum,
    diploid ancestor and, 243–247
    origin center and, 240–241
    tetraploid species and, 242–243
Androgenesis, *Habrobracon juglandis* and, 299
Anolis carolinensis, Batesian mimicry and, 183
Anthereae pernyi, pterins in, 375
Antibiotic,
  pseudoresistance, transformation and, 131
  resistance, transformation and, 119–122, 124, 125, 127–129, 130, 131
Antibodies, transformation and, 95
Antigenicity, deoxyribonucleic acid, 92–93
Apis mellifica, homologous eye color mutants and, 365
Argynnis paphia,
  courtship and, 195
  sex-controlled inheritance and, 192–193, 194
Assimilation,
  genetic,
    adaptive modifications and, 274
    anal papillae and, 262–263, 271, 273, 274
    autoregulation and, 273
    Baldwin effect and, 287–289
    bithorax and, 262, 266, 267, 268, 269, 271, 285, 286
    callosities and, 272, 284–285
    canalization and, 269–273, 279–287
    crossveinless and, 260–261, 264, 267, 268–269, 271, 274, 275, 279–280, 285–286
    definition of, 259
    developmental threshold and, 260–262
    dominance and, 265, 270, 271, 272
    Drosophila melanogaster and, 260-269, 271, 274, 275, 278, 279, 281 282
    dumpy and, 265–266, 267, 268
    epistasis and, 270, 271, 272
    genetic analysis and, 264–268

genetic variation origin and, 268–269
homeostasis and, 277–278
morphoses and, 274
non-threshold and, 262–263
phenocopies and, 274–276
selection and, 264
Autoregulation, canalization and, 273

B

Bacillus anthracis, transformation in, 68
Bacillus megaterium, nitric oxide and, 8
Bacillus subtilis, transformation in, 66, 68–69, 71, 91, 93, 94, 96, 99, 108, 116, 122, 145
Bacteria, *see also* specific species, evolution of, 147–151
Bacteriophage,
infection and, 75–76, 82
mutations in, 91, 143–144
transduction and, 64, 65, 140–142
transformation and, 67–68
Bacteriophage T3, photodynamic reaction and, 18
Baldwin effect, genetic assimilation and, 287–289
Bar, canalization improvement and, 281–282, 283
Barley, *see Hordeum sativum*
Basarthrum, Tuberarium and, 217
Battus philenor, Batesian mimicry and, 184, 185
Beta radiation, *Habrobracon juglandis* and, 317
Biopterin,
chemical structure of, 352
Drosophila melanogaster and, 351, 353
Biston betularia,
dominance and, 190, 191
industrial melanism and, 170–177
Biston betularia var. *carbonaria,*
distribution of, 172–173
frequency of, 171–173, 174, 175–176, 177
Biston betularia var. *insularia,* frequency of, 173–174
Bithorax, genetic assimilation and, 262, 266–267, 268, 269, 271, 285, 286
Black hairstreak moth, *see Strymonidia pruni*
Bombyx mori,
homologous eye color mutants and, 365
melanin and, 388
pterin-ommochrome synthesis, physiological processes and, 386–387
pterins in, 355, 375
purines and, 389
riboflavin and, 389
tryptophan metabolism and, 361–362
β-propiolactone,
Allium cepa and, 38, 39
Vicia faba and, 38, 39
Bracon brevicornis, see Habrobracon brevicornis
Bracon hebetor, see Habrobracon juglandis
Brassica campestris var. *toria,* autotetraploid and, 231
Broad bean, *see Vicia faba*
5-bromouracil, deoxyribonucleic acid and, 91
Brucella bronchiseptica, transformation and, 110
Butterflies, *see* specific species *and* Lepidoptera

C

Ca^{45}, *Habrobracon juglandis* and, 339
Caffeine, *see* 1,3,7-trimethylxanthine
Calliphora erythrocephala,
autonomous mutants, tryptophan metabolism and, 363
homologous eye color mutants and, 365
pterin-ommochrome synthesis, physiological processes and, 387
pterins in, 355, 370–371
white eye mutant and, 370–371, 380
Callosities, genetic assimilation and, 272, 284–285
Canalization,
adaptive modifications and, 274
autoregulation and, 273
evolution of, 284–285
genetic assimilation and, 269–273, 279–287
genetic basis,
canalized phenocopies and, 282–284
disruption and, 280–281

evolution of, 284–285
improvement and, 281–282
"tuning" pathways and, 285–287
wild type-variation and, 279–280
homeostasis and, 277–278, 282
morphoses and, 274
phenocopies and, 274–276
plants and, 284
polydactyly and, 286
Capsicum annum, haploid in, 225
Carbon monoxide, chromosomal aberrations and, 5–6, 12, 14, 15
Cells,
Habrobracon juglandis, size and, 304–306
human tissue culture, x-ray sensitivity of, 5, 8
mouse ascites tumor, nitric oxide and, 8
Cepaea sp., polymorphism and, 196–197
Chicks, Batesian mimicry and, 183–184
Chilo simplex, Habrobracon pectinophorae and, 297
Chinese character moth, *see Cilix glaucata*
Chinese tussar silkworm, *see Anthereae pernyi*
Chironomus dorsalis, super-genes and, 197
Chironomus tetans, polymorphism and, 187
Chloramphenicol, *see* Chloromycetin
Chloromycetin, transformation competence and, 99, 110, 126, 127
Choristoneura fumiferana, sex-controlled inheritance and, 193
Choristoneura pinus, sex-controlled inheritance and, 193
Chromosomes,
breakage,
breakage-first hypothesis and, 47
exchange hypothesis and, 47, 48–51
oxygen-dependence and, 2–34, 41, 42–43, 47
oxygen-independence and, 34–40, 41–42
rejoining, x-rays and, 9–17
Solanum tuberosum, 218
behavior and, 237
diploid species and, 229–230
haploid meiosis and, 224–225
morphology and, 232–233
pairing and, 233–235
pollen fertility and, 236–237
secondary association and, 228–229
secondary balance and, 229
seed setting and, 236–237
structure of, 49
Cilix glaucata, protective coloration and, 179, 180
Cinnabar moth, *see Hypocrita jacobaeae*
Cleora repandata, industrial melanism and, 174–175
Colchicine, *Habrobracon juglandis* and, 342
Colias philodice, sex-controlled inheritance and, 192–193, 194
Colias spp. polymorphism and, 166
Coloration,
protective,
Batesian mimicry and, 182–188
cryptic and, 177–180, 211
eyespots and, 180–181, 211
Müllerian mimicry and, 181–182
warnings and, 181
Competence, transformation and, 94–100, 108, 110
Conjugation,
deoxyribonucleic acid and, 64
Escherichia coli and, 63–64
evolution and, 147, 148, 150
recombination and, 140–142
Conversion, *see* Assimilation
Corn borer, *see Pyrausta nubilalis*
Crithidia fasciculata,
drosopterin and, 352
neodrosopterin and, 352
Crossing-over,
deoxyribonucleic acid and, 79, 117, 124, 131–142
polymorphism and, 196–197
Crossveinless, genetic assimilation and, 260–261, 264, 267, 268–269, 271, 274, 275, 279–280, 285–286
Culex molestus,
pterin-ommochrome synthesis, physiological processes and, 387
white eye mutant and, 380
Cupferron, *Vicia faba* and, 5, 6, 14–15, 17–21, 30–33

Cycnia mendica, dominance and, 189
Cytogenetics,
Solanum andigena and, 223–224
Solanum tuberosum and, 223–224
Cytology, *Habrobracon juglandis* and, 306–309

D

Datura stramonium, haploid in, 224–225
Deletions, transformation and, 123
Deoxyribonuclease, transforming DNA and, 81–82, 92, 96, 102, 103, 105, 108, 126
Deoxyribonucleic acid,
chromosomal,
Tradescantia paludosa and, 45–47
Vicia faba and, 44–45, 46, 47
conjugation and, 64
crossing-over and, 79, 117, 124, 131–142
molecular weight of, 77
mutation in, 69–72, 79, 88–89, 90, 93, 143–147
ploidy and, 76
reactivity, 79–80
antigenicity and, 92–93
chemical agents and, 90–91
depolymerization and, 81–82
fractionation and, 91–92
heat and, 86–89
intermolecular attractions and, 80–81
radiation and, 82–86
shearing and, 89–90
recombination and, 89, 93, 111, 114, 123–129, 131–142, 150
replication of, 78–79
stability of, 76
synapsis and, 79, 89, 111, 129, 138–139, 149, 150
transduction and, 64
transformation, 63, 64, 67–69, 72, 104, 111–112, 113–131
biosynthesis and, 93
essentialness and, 72–76
reactivity and, 79–93
structure and, 76–79
unwinding of, 78, 79
2,2′-dichloro-N-methyldiethylamine, *see* Nitrogen mustard
2,3,2′,3′-diepoxypropyl ether,
Tradescantia paludosa and, 38
Vicia faba and, 38, 39
Diethylnitrosamine, visible light-acridine orange and, 19, 31
1,2-dihydro-3,6-pyridazinedione, *see* Maleic hydrazide
Dimethylaniline, chromosomal aberrations and, 31
Dimethylnitrosamine, visible light-acridine orange and, 19, 31
1,3-dimethylxanthine, chromosomal aberrations and, 22
2,4-dinitrophenol,
ethyl alcohol and, 34, 42
maleic hydrazide and, 30
methylphenylnitrosamine and, 32
potassium cyanide and, 22
x-ray damage and, 7, 12
Diphenylnitrosamine, visible light-acridine orange and, 19, 20, 31
Diplococcus pneumoniae,
transformation,
antigenicity and, 93
chemical agents and, 90
competence and, 94, 95, 96, 98, 99
deoxyribonucleic acid and, 72–74, 80, 81, 82
extent and, 65, 66
genetic characters and, 70, 71
genetic integration and, 112, 123, 124, 125, 127, 129
heat and, 87, 88
heterocatalytic function mechanism and, 144–145, 146
history of, 62, 63
linkage and, 115–116, 117, 118, 119–122
penetration and, 101, 108, 109–110
phenotypic expression and, 130, 131
radiation and, 82, 83, 84, 86
recombination and, 136–137
Dominance,
evolution of, 188–192, 212
genetic assimilation and, 265, 270, 271, 272
Drosophila melanogaster,
adult incidence and, 319, 320
autonomous mutants, tryptophan metabolism and, 363

canalization in, 271, 274, 275–276, 278, 279–280, 281–282
disruptive selection and, 198, 199–200
egg hatchability and, 319, 320
eye-color genes, other organ pigmentation and, 385
eye color "hormones" and, 356–359
eye pigments and, 314, 350
genetic assimilation in, 260–269, 271, 274, 275, 278, 279, 281, 282
homologous eye color mutants and, 364, 365, 366
isoxanthopterin and, 375–376
mosaics and, 311
ommochrome synthesis, multiple alleles and, 367–368
phenocopies and, 275–276
pigment-carrying granules and, 378–382
precursor accumulation and, 359
pseudoalleles and, 367–368, 381
pterin-ommochrome synthesis, physiological processes and, 385–386, 387–388
pterins in, 353, 368–369, 371–372, 373–375, 376–377
riboflavin and, 388
selection and, 210
Drosophila pseudoobscura,
homologous eye color mutants and, 364, 365
pterin-ommochrome synthesis, physiological processes and, 387
Drosophila simulans, homologous eye color mutants and, 364
Drosophila subobscura, pterin-ommochrome synthesis, physiological processes and, 387
Drosophila spp.,
evolution and, 165–166
pterin pattern, evolution and, 394
Drosophila virilis,
homologous eye color mutants and, 364
vermilion mutant in, 361
Drosopterin, *Drosophila melanogaster* and, 351, 353
Dumpy, genetic assimilation and, 265–266, 267, 268

E

Eggs, *Habrobracon juglandis,* radiation and, 321–325
Embryos, lethal-bearing, *Habrobracon juglandis* and, 331–333
Ephestia kühniella,
autonomous mutants, tryptophan metabolism and, 363–364
dominance and, 191
eye-color genes, other organ pigmentation and, 384–385
eye color "hormones" and, 356–359
eye pigments and, 314
Habrobracon brevicornis and, 297
Habrobracon juglandis and, 295
homologous eye color mutants and, 365
ommochrome-pterin pleiotropism and, 383–384
ommochrome synthesis, multiple alleles and, 366–367
pigment-carrying granules and, 377–378
precursor accumulation and, 359–361
pterin-ommochrome synthesis, physiological processes and, 386
pterins in, 354, 369–370
riboflavin and, 388–389
Epistasis, genetic assimilation and, 270, 271, 272
Erisilkworm, pterins in, 375
Escherichia coli, bacteriophage DNA, 109, 140, 145, 5-bromouracil and, 91
conjugation in, 63–64
deoxyribonucleic acid and, 88, 109
phage T3, photodynamic reaction and, 18
photoreactivation and, 86
protoplasts and, 107, 108
transformation in, 65, 66, 67, 70
8-ethoxycaffeine,
chromosomal aberrations and, 23–28. 33, 42, 47
visible light-acridine orange and, 19
Ethyl alcohol, chromosomal aberrations and, 34, 42
Ethylenediaminetetraacetic acid, radiation, *Habrobracon juglandis* and, 327–328

Ethylenes, substituted, visible light-acridine orange and, 19
Euphydryas aurinia,
polygenically controlled characters, evolution and, 202
population size fluctuations and, 201, 202
wing pattern variability and, 201–202
Eupithecia centaureata, protective coloration and, 179, 180
Evolution,
bacterial and, 147–151
Batesian mimicry and, 188
canalization and, 284–285
canalized pathway "tuning" and, 285–287
dominance and, 188–192, 212
Drosophila spp. and, 165–166, 394
Euphydryas aurinia and, 202
super-genes and, 196–197
Eye spots, Lepidoptera and, 180–181, 211

F

Females, *Habrobracon juglandis* and, 299–300
Firefly, *see Photinus pyralis*
Fl1, *see* Drosopterin
Fl4, *see* 2-amino-4-hydroxypteridine *and* Biopterin
Fl5, *see* Yellow pterin
Fl13, *see* Isoxanthopterin
Formaldehyde, deoxyribonucleic acid, 90–91

G

Gaillardia aristata, Rhododipsa masoni and, 178–179
Gamma radiation, *Habrobracon juglandis* and, 339
Gammarus pulex, ommochrome elimination and, 366
Genes,
pleiotropic action,
eye color and, 384–385
heterocyclic compounds and, 388–389
ommochrome-pterin and, 383–384
physiological processes and, 385–388
pigment-carrying granules and, 377–382
Genetic drift, selection and, 167, 174, 202
Gonodontis bidentata, industrial melanism and, 175, 176
Grouse locust, *see Paratettix* sp.
Gynandromorphs, *Habrobracon juglandis* and, 312–313

H

Habrobracon brevicornis, cytology and, 308
diploid males in, 298
Ephestia kühniella and, 297
Pyrausta nubilalis and, 296
Habrobracon juglandis,
androgenetic males and, 299
colchicine and, 342
cytology and, 306–309
Ephestia kühniella and, 295
homologous eye color mutants and, 365
impaternate females and, 299–300
mosaics,
autonomous phenotypes and, 313–314
eye pigments and, 314
gynandromorph behavior and, 312–313
non-autonomous phenotypes and, 313–314
origin and, 310–312
mutants of, 309–310
nitrogen mustard and, 340–342
radiation, 316–317
adult effects and, 317
embryonic lethals and, 331–333
genome number and, 328–331
host irradiation and, 339–340
injury and, 334–337
isotope ingestion and, 337–339
mature sperm effects and, 317–320
modifying agents and, 326–328
sensitivity and, 328–331, 341
unlaid eggs and, 321–325
sex determination,
method and, 301
selection and, 302–303

sex-linkage and, 301–302
theory and, 303–304
sex types,
females and, 299–300
males and, 297–299
taxonomy of, 296–297
Habrobracon pectinophorae, see also Habrobracon juglandis,
Chilo simplex and, 297
sex-linkage and, 302
cytology and, 307
diploid males in, 298, 300
mutants and, 309, 310
HB-1, *see* 2-amino-4-hydroxypteridine
HB-2, *see* Biopterin
Heat, transforming DNA and, 86–89
Hemophilus ducreyi, transformation and, 110
Hemophilus influenzae, transformation in, 65, 66, 70, 71, 74–75, 80, 82, 84, 85–86, 89, 95, 96, 98, 99–100, 104, 110, 116, 118, 127, 129, 138, 148, 150
Hemophilus parainfluenzae, transformation in, 65, 66, 110, 148
Hemophilus pertussis, transformation and, 110
Hemophilus suis, transformation in, 65, 66, 110
Heterocatalysis, transformation and, 142–147
Heterodera rostochiensis, Solanum andigena and, 238
Homeostasis, canalization and, 277–278, 282
Hordeum sativum, chromosomal aberrations in, 22
Host, radiation, *Habrobracon juglandis* and, 339–340
Hybridization,
interspecific,
Lepidoptera and, 166, 190
potato and, 218, 226–228, 229
Hydrogen peroxide, oxygen effect and, 8
3-hydroxykynurenine, ommochrome precursor and, 356–359
Hyloicus pinastri, protective coloration and, 178, 179
Hyperbasarthrum, Tuberarium and, 217
Hypocrita jacobaeae, Müllerian mimicry and, 181

I

Inactivation, transforming DNA and, 80–92
Infrared radiation, x-rays, chromosome damage and, 17
Inheritance, Batesian mimicry and, 185–188
sex-controlled, Lepidoptera and, 192–195
Inherited characters, definition of, 258–259
Inhibition, transformation and, 104
Injury, radiation, *Habrobracon juglandis* and, 334–337
Integration, genetic, transformation and, 111–129
Iodine, visible light-acridine orange and, 19
Isodrosopterin, *Drosophila melanogaster* and, 351, 353
Isoxanthopterin,
chemical structure of, 352
Drosophila melanogaster and, 351, 353, 375–376
secondary sex character and, 375–376

J

Jack pine budworm, *see Choristoneura pinus*

K

Kynurenine, ommochrome precursor and, 356–359

L

Lasiocampa quercus, industrial melanism and, 177
Lepidoptera, *see also* specific species
cryptic coloration and, 177–180, 211
eye spots and, 180–181, 211
interspecific hybridization and, 166
polymorphism and, 166–167, 168, 178, 184, 185, 187, 188, 190, 191–192, 193, 194, 211–212
sex-controlled inheritance and, 192–195
warning coloration and, 181
Leptostemonum, Solanum tuberosum and, 217

Lesser yellow underwing moth, *see Triphaena comes*
Lethals,
dominant, *Habrobracon juglandis* and, 317, 318, 320, 322–323, 325, 327, 328, 340
recessive, *Habrabracon juglandis* and, 318–319, 320, 322–323, 324–325
Leucopterin, chemical structure of, 352
Limenitis archippus archippus, dominance and, 189
Limenitis archippus floridensis, dominance and, 189
Limenitis sp., mimetic pattern inheritance and, 185, 186
Lime-speck pug moth, *see Eupithecia centaureata*
Linkage,
maps, *Habrobracon juglandis* and, 315–316
sex, *Habrobracon juglandis* and, 301–302
transformation and, 114–123, 138–140
Lucilia cuprina, white eye mutant and, 380
Lycopersicon esculentum,
haploid in, 225
interspecific hybridization of, 218

M

Maleic hydrazide,
chromosomal aberrations and, 25, 28–30, 33, 42, 47
visible light-acridine orange and, 19, 21
Males, *Habrobracon juglandis* and, 297–299
Maniola jurtina,
polygenically controlled characters, natural selection and, 207–209
spot-frequency distribution and, 203–207
selection and, 203–209
Marsh fritillary butterfly, *see Euphydryas aurinia*
Meadow brown butterfly, *see Maniola jurtina*
Mediterranean flour moth, *see Ephestia kühniella*
Meiosis, *Habrobracon juglandis* and, 299–300
Melanin, pterin-ommochrome synthesis and, 388
Melanism, industrial, Lepidoptera and, 170–177, 190–191, 211
Metabolism, tryptophan, ommochrome synthesis and, 356–368
Methylphenylnitrosamine, visible light-acridine orange and, 19, 20, 31–33, 42
Mice,
homeostasis and, 278
selection and, 280–281
Micrococcus sp., deoxyribonucleic acid of, 87
Microorganisms, *see also* specific species, x-ray sensitivity of, 3–4, 8
Mimicry,
Batesian,
evolution and, 188
inheritance and, 185–188
laboratory effectiveness and, 182–184
wild effectiveness and, 184–185
Müllerian and, 181–182
Modifications, adaptive, canalization and, 274
Monte Carlo, canalization determination and, 283–284
Morphoses, canalization and, 274
Mosaics, *Habrobracon juglandis* and, 310–314
Moths, *see* specific species and Lepidoptera
Mottled beauty moth, *see Cleora repandata*
Musca domestica, homologous eye color mutants and, 364, 365, 366
Muslin moth, *see Cycnia mendica*
Mutation,
autonomous, pterins and, 368–371
deoxyribonucleic acid and, 69–72, 79, 88–89, 90, 93, 143–147
Habrobracon juglandis and, 309–310
homologous, ommochrome synthesis and, 364–366
pterin-ommochrome synthesis, physiological processes and, 385–388

visible, *Habrobracon juglandis* and, 318
Myleran, chromosomal aberrations and, 41

N

N^{15}, transforming DNA and, 106
Neisseria meningitidis, transformation in, 65, 66, 70
Neisseria sicca, transformation in, 65, 66
Neodrosopterin, *Crithidia fasciculata* and, 352
Neurospora crassa,
hydrogen peroxide and, 8
cryptophan metabolism and, 361
Neutron radiation, *Habrobracon juglandis* and, 320
Nitric oxide, x-ray effects and, 8–9, 18–21
Nitrogen mustard,
Habrobracon juglandis and, 340–342
transforming DNA and, 84, 91
Vicia faba and, 38–39, 40
visible light-acridine orange and, 19, 21
Nitrous acid, deoxyribonucleic acid and, 90
N-nitroso-N-methyl urethan, *Vicia faba* and, 38, 39
N-nitrosophenylhydroxylamine, *see* Cupferron

O

Ocelli, canalization improvement and, 281
Ommochromes,
external factors and, 391–392
3-hydroxykynurenine and, 356–359
kynurenine and, 356–359
predetermination and, 389–391
synthesis,
homologous mutants and, 364–366
modifiers and, 366–368
multiple alleles and, 366–368
tryptophan metabolism and, 356–368
tryptophan metabolism and, 359–362
Onion, *see Allium cepa*
Oxygen,
chromosomal breakage,
ethyl alcohol and, 34, 41
maleic hydrazide and, 28–30, 41
N-methylated oxypurines and, 22–28, 41
phenylnitrosamines and, 30–34, 41
potassium cyanide and, 21–22, 41
visible light-acridine orange and, 17–21, 41
x-rays and, 2–9, 41
chromosomal rejoining, x-rays and, 9–17
radiation, *Habrobracon juglandis* and, 326
Oxypurines, *see also* specific compounds, chromosomal aberrations and, 22–28, 42

P

P^{32},
Habrobracon juglandis and, 338–339
transforming DNA and, 103, 104, 106, 110
Pachystemonum, Solanum tuberosum and, 217
Pale brindled beauty moth, *see Phigalia pilosaria*
Panaxia dominula,
gene-frequency change and, 167–170
genetic drift and, 167
polygenically controlled characters and, 209–210
Panaxia dominula var. *bimacula,* gene-frequency change and, 167–170
Panaxia dominula var. *medionigra,* gene-frequency change and, 167–170
Papilio brevicauda,
Batesian mimicry and, 185–186
dominance and, 190
Papilio dardanus,
disruptive selection and, 198–199
dominance and, 188–189, 190, 191
mimetic pattern inheritance and, 187–188
polymorphism and, 184, 187–188, 194
protective coloration and, 179
super-genes and, 196, 197
Papilio demodocus, protective coloration and, 180
Papilio demoleus, protective coloration and, 180
Papilio eurymedon, movement of, 187

Papilio glaucus, Batesian mimicry and, 186, 187
courtship and, 195
Papilio hospiton, dominance and, 189
Papilio machaon, disruptive selection and, 200
dominance and, 189
Papilio memmon,
sex-controlled inheritance and, 192–193
super-genes and, 197
Papilio multicaudatus, movement of, 187
Papilio nitra, Batesian mimicry and, 185–186
Papilio polytes,
sex-controlled inheritance and, 192–193
super-genes and, 197
Papilio polyxenes,
dominance and, 190
mimetic pattern inheritance and, 185, 186
Papilio polyxenes, var. *machaon,* Batesian mimicry and, 185
Papilio rutulus, movement of, 187
Papilio troilus, Batesian mimicry and, 185, 185
Paratettix sp., polymorphism and, 196–197
Pea, *see Pisum sativum*
Penetration, transformation and, 100–111
Peppered moth, *see Biston betularia*
Periplaneta americana, homologous eye color mutants and, 365, 366
Phage, *see* Bacteriophage
Phenocopies, canalization and, 274–276
Phenotypes,
autonomous, *Habrobracon juglandis* and, 313–314
expression, transformation and, 111, 129–131
non-autonomous, *Habrobracon juglandis* and, 313–314
Phigalia pilosaria, Y-linkage and, 186
Phleum pratense, autotriploid and, 231
Phormia regina,
homologous eye color mutants and, 365, 366
white eye mutant and, 380
Photinus pyralis, Batesian mimicry and, 183
Photoperiodism,
Solanum andigena and, 223
Solanum tuberosum and, 222–223
Photoreactivation, transformation and, 85–86
Phryne fenestralis,
autonomous mutants, tryptophan metabolism and, 364
homologous eye color mutants and, 365, 366
Pieris bryoniae, Y-linkage and, 186
Pieris napi, Y-linkage and, 186
Pine hawk moth, *see Hyloicus pinastri*
Pisum sativum, chromosomal aberrations in, 22
Plants, canalization and, 284
Pleiotropism, ommochromes-pterins and, 377–389
Plodia interpunctella,
autonomous mutants, tryptophan metabolism and, 363
pterins in, 354, 370
tryptophan metabolism and, 362
homologous eye color mutants and, 365
Ploidy, deoxyribonucleic acid and, 76
Pneumococcus, *see Diplococcus pneumoniae*
Polydactyly, canalization and, 286
Polymorphism,
Cepaea sp. and, 196–197
crossing-over and, 196–197
Ephestia kühniella and, 191
Lepidoptera and, 166–167, 168, 178, 184, 185, 187, 188, 190, 191–192, 193, 194, 211–212
Polynucleotide, transforming DNA and, 103
Polyphosphate, transformation and, 94
Polyploidy,
Solanum tuberosum,
basic chromosome number and, 224–230
tetraploidy and, 230–240
Potassium cyanide,
chromosomal aberrations and, 5–6, 12, 21–22, 30–32, 33, 42–43

Habrobracon juglandis and, 342
ommochrome formation and, 378
Potato, *see Solanum tuberosum*
Predetermination, ommochrome synthesis and, 389–391
Protein, synthesis, chromosome rejoining and, 12
Protoplast, localized, transformation penetration and, 107, 108–109
Pseudacraea eurytus, Batesian mimicry and, 184
Pseudoalleles, *Drosophila melanogaster* and, 367–368, 381
Pterincarbonic acid,
chemical structure of, 352
Drosophila melanogaster and, 351, 353
Pterins, *see also* specific chemical,
autonomous mutants,
Calliphora erythrocephala and, 370–371
Colias erate poliographus and, 371
Drosophila melanogaster and, 368–369
Ephestia kühniella and, 369–370
Plodia interpunctella and, 370
Ptychopoda seriata and, 370
Bombyx mori epidermal tissue and, 355
Calliphora erythrocephala eyes and, 355
Drosophila melanogaster eyes and, 353
Ephestia kühniella eyes and, 354
external factors and, 391–392
non-autonomous mutants,
Anthereae pernyi and, 375
Bombyx mori and, 375
Drosophila melanogaster and, 372–375
Erisilkworm and, 375
Plodia interpunctella eyes and, 354
Ptychopoda seriata eyes and, 354
purines and, 389
recessive alleles and, 376–377
riboflavin and, 388–389
synthetic pathways and, 394–395
vertebrates and, 392–393, 394
Pteronidea ribesii, cytoplasmic buds and, 308
Ptychopoda seriata,
melanin and, 388
pterins in, 354, 370
Purines, pterins and, 389
Pyrausta nubilalis, Habrobracon juglandis and, 296
Pyrophosphate, transformation and, 94

Q

Quinones, visible light-acridine orange and, 19

R

Radiation,
Habrobracon juglandis, 316–317,
adult effects and, 317
embryonic lethals and, 331–333
genome number and, 328–331
host irradiation and, 339–340
injury and, 334–337
isotope ingestion and, 337–339
mature sperm effects and, 317–320
modifying agents and, 326–328
sensitivity and, 328–331, 341
unlaid eggs and, 321–325
Receptor, enzymatic, transformation penetration and, 107, 109
Recombination,
conjugation and, 140–142, 150
deoxyribonucleic acid and, 89, 93, 111, 114, 123–129, 131–142, 150
transduction and, 140–142, 150
Red pterin, *see* Drosopterin
Rhizobium, sp., transformation in, 66, 69, 70, 71
Rhodopipsa masoni, protective coloration and, 178–179
Riboflavin, pterins and, 388–389
Ribonucleic acid, protein synthesis and, 147
Rice plant pest, *see Chilo simplex.*

S

S^{35}, *Habrobracon juglandis* and, 339
Saccharomyces cerevisiae, x-ray sensitivity of, 4
Salmonellae,
deoxyribonucleic acid of, 87
transduction in, 64, 140

Salmonella typhimurium, transformation in, 65, 67
Salts, paramagnetic, visible light-acridine orange and, 19
Sawfly, *see Pteronidea ribesii*
Scalloped hazel moth, *see Gonodontis bidentata*
Scarlet tiger moth, *see Panaxia dominula*
Scute, canalization disruption and, 281
Selection,
  disruptive and, 197–200, 212
  Drosophila melanogaster and, 210
  fluctuations and, 167–170
  genetic drift and, 167, 174, 202
  industrial melanism and, 170–177
  Maniola jurtina and, 203–209
  mice and, 280–281
  natural, genetic assimilation and, 262, 263, 264, 284–285
  sex determination and, 302–303
Sensitivity, radiation, *Habrobracon juglandis* and, 328–331, 341
Sepia pterin, *see* Yellow pterin
Sesia, spp. mimetic pattern inheritance and, 185
Sex character, secondary, isoxanthopterin and, 375–376
Sex determination,
  Habrobracon juglandis,
    method and, 301
    selection and, 302–303
    sex-linkage and, 301–302
    theory and, 303–304
Shearing, transforming DNA and, 89–90, 104
Shigella dysenteriae, deoxyribonucleic acid of, 88, 140
Shigella flexneri, x-ray sensitivity of, 4
Shigella paradysenteriae, transformation in, 65, 67
Snail, *see Cepaea* sp.
Sodium azide,
  chromosomal aberrations and, 5–6
  Habrobracon juglandis and, 342
  maleic hydrazide and, 30
  methylphenylnitrosamine and, 32
Sodium nitrite, visible light-acridine orange and, 19, 20
Solanum acaule, triploid and, 226, 227
Solanum ajanhuiri, Solanum tuberosum ancestry and, 243, 244
Solanum ajuscoense, triploid and, 226
Solanum andigena,
  cytogenetic behavior and, 223–224
  leaf shape and, 222–223
  photoperiodic response of, 223
  secondary balance and, 229
  Solanum tuberosum, relationship between, 219–224
Solanum andigenum, see Solanum andigena
Solanum chacoense,
  triploid and, 226, 227
  Solanum tuberosum ancestry and, 245
Solanum chaucha,
  secondary balance and, 229
  secondary chromosomal association and, 229
  triploids and, 226
Solanum commersonii,
  genetic studies and, 229
  secondary balance and, 229
  triploids and, 226
Solanum curtilobum, secondary balance and, 229
Solanum demissum, polyhaploid and, 228
Solanum ehrenbergii, genetic studies and, 230
Solanum fonckii, Solanum tuberosum ancestry and, 242
Solanum goniocalyx, Solanum tuberosum ancestry and, 243, 244
Solanum henryi, secondary balance and, 229
Solanum herrerae, Solanum andigena ancestry and, 242
Solanum kurtzianum, Solanum tuberosum ancestry and, 245, 246
Solanum leptostigma, Solanum tuberosum ancestry and, 242, 243
Solanum longipedicellatum, triploid and, 227
Solanum lycopersicoides, interspecific hybridization of, 218
Solanum maglia, Solanum tuberosum ancestry and, 243
Solanum molinae, Solanum tuberosum ancestry and, 242

Solanum neohawkesii, triploid and, 227
Solanum pennellii, interspecific hybridization of, 218
Solanum phureja,
Solanum tuberosum ancestry and, 243, 244, 245
triploid and, 227
Solanum pinnatisectum, genetic studies and, 230
Solanum polyadenium,
chromosome pairing and, 233, 234
haploid in, 224
meiosis in, 226
Solanum polytrichon, triploid and, 227
Solanum rybinii,
genetic studies and, 229, 230
meiosis in, 225–226
triploid and, 226, 227
Solanum simplicifolium, genetic studies and, 230
Solanium sparsipilum, Solanium tuberosum ancestry and, 247
Solanum stenotomum,
secondary balance and, 229
secondary chromosomal association and, 229
Solanum tuberosum ancestry and, 243, 244–247
trivalent in, 225
Solanum sucrense, Solanum tuberosum ancestry and, 242
Solanum tuberosum,
chromosomes of, 218, 224–240
classification of, 217–218
cytogenetic behavior of, 223–224
photoperiodic response of, 222–223
polyploidy,
basic chromosome number and, 224–230
tetraploidy and, 230–240
probable ancestor of, 240–247
relationships of, 217–224
secondary balance and, 229
Solanum andigena, relationship between, 219–224
triploid and, 226, 227
Solanum wittmackii, Solanum tuberosum ancestry and, 242
Spiderwort, *see Tradescantia paludosa*
Spruce budworm, *see Choristoneura fumiferana*
Sr^{89}, *Habrobracon juglandis* and, 339
Staphylococcus aureus, transformation in, 66
Streptococcus, hemolytic strain *Challis.* transformation in, 65, 66
Streptococcus pneumoniae, see Diplococcus pneumoniae
Streptococcus sbe, transformation in, 65, 66
Streptococcus sp., deoxyribonucleic acid of, 87–88
Streptococcus viridans, transformation in, 65, 66, 70
Strymonidia pruni, protective coloration and, 179
Super-genes, evolution of, 196–197
Synapsis, deoxyribonucleic acid and, 79, 89, 111, 117, 129, 138–139, 149, 150
Synchrony, division, transformation competence and, 97

T

Temperature, chromosomal aberrations and, 15–17, 29, 31, 33
Tenebrio molitor, Batesian mimicry and, 183
Tetrahydrobiopterin derivative, *Drosophila melanogaster* and, 352, 353
1,3,7,9-tetramethyluric acid, chromosomal aberrations and, 22, 24–28
Tetraploidy,
Solanum tuberosum, 230–232,
behavior and, 237
chromosome morphology and, 232–233
chromosome pairing and, 233–236
genetic results and, 238–240
morphology and, 237–238
pollen fertility and, 236–237
seed setting and, 236–237
Theophylline, *see* 1,3-dimethylxanthine
Thymus, calf, deoxyribonucleic acid of, 87–88, 110
Tobacco mosaic virus, nitrous acid and, 90
Tradescantia bracteata, alpha radiation and, 34–35

Tradescantia paludosa,
chromosomal deoxyribonucleic acid and, 45–47
2,3,2′,3′-diepoxypropyl ether and, 38
mature pollen, ultraviolet radiation and, 35–38, 44
microspores, chromosome breakage in, 1, 3, 12, 13–17
pollen tubes, chromosome breakage and, 6–7, 35–38
Transduction,
bacteriophage and, 64, 65
deoxyribonucleic acid and, 64
recombination and, 140–142, 150
Salmonellae and, 64
Transformation,
allelic agents and, 111–112
bacteriophage λ dg and, 67–68
competence and, 94–100, 108, 110
crossing-over and, 79, 117, 124, 131–142
deletions and, 123
deoxyribonucleic acid, 63, 64, 67–69, 72, 104, 111–112, 113–131
biosynthesis and, 93
esssentialness and, 72–76
reactivity and, 79–93
structure and, 76–79
evolution and, 147, 148–151
extent,
bacterial species and, 65–69
genetic characters and, 69–71
genetic heterogeneity and, 112–114
genetic integration in, 111–129
heterocatalytic function mechanism and, 142–147
history of, 62–65
independent determinants and, 112–114
inhibition of, 104
linkage and, 114–123, 138–140
linkage map and, 118–119
penetration and, 100–111
phenotypic expression in, 129–131
photoreactivation and, 85–86
recombination and, 89, 93, 111, 114, 123–129, 131–142
reverse and, 111–112, 122–123
1,3,7-trimethylxanthine, chromosomal aberrations and, 22
Triphaena comes, dominance and, 190
Triploid, potato and, 226–228
Tryptophan, metabolism, ommochrome synthesis and, 356–368
Tuberarium, Pachystemonum and, 217, 218
Tumors, mouse ascites, x-ray sensitivity of, 4–5

U

Ultrasonics, transforming DNA and, 83, 89–90
Ultraviolet light,
conjugation and, 142
Habrobracon juglandis and, 328, 336–337
Tradescantia paludosa and, 35–38
transduction and, 140
transforming DNA and, 82, 84, 85, 90

V

Vertebrates, pterin mutants and, 392–393, 394
Vicia faba, alpha rays and, 8, 35
β-propiolactone and, 38, 39
chromosomal aberrations in, 1, 2–3, 10–12, 22, 23, 24–28, 29, 30–34, 35, 38–40
chromosomal deoxyribonucleic acid and, 44–45, 46, 47
cupferron treatment and, 5, 6, 14–15, 17–21, 30–33
2,3,2′,3′-diepoxypropyl ether and, 38, 39
nitrogen mustard and, 38–39, 40
N-nitroso-N-methyl urethan and, 38, 39, 44
Virus, *see* Bacteriophage *and* Tobacco mosaic virus
Visible light, acridine orange, chromosomal breakage and, 17–21, 31, 33, 42–43

X

Xanthommatin,
Calliphora erythrocephala and, 350
chemical structure of, 351
Xanthomonas phaseoli, transformation in, 66, 68, 70
Xanthopterin,
chemical structure of, 352

Drosophila melanogaster and, 351, 353
X-pterin, *Drosophila melanogaster* and, 351, 353
X-rays,
chromosomal aberrations,
cupferron and, 33
nitric acid and, 8–9, 18–21
oxygen and, 2–9
chromosome breakage,
nitric oxide and, 8–9, 18–21
oxygen and, 2–9, 42–43
dose rate, chromosomal exchanges and, 9–14
Habrobracon juglandis and, 316–317, 319, 321–336, 339, 341, 342
transforming DNA and, 82, 83
visible light-acridine orange and, 19

Y

Yellow pterin, *Drosophila melanogaster* and, 352, 353

Z

Zea mays, chromosomal aberrations in, 29

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