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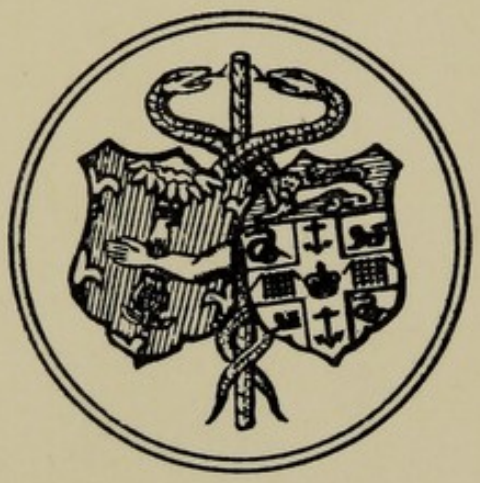

GENERAL CYTOGENETICS

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J. SYBENGA

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Wageningen

1972

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To the memory of Boukje Sybenga-Schepers

PREFACE

The field of cytogenetics is wide and diversified. The different specializations (Drosophila polytene cytology, human karyotyping, plant cytogenetics, etc.) seem to have only few contact points and tend to be treated as separate entities. Yet they all have one common basis: the chromosome, its behaviour and the genetic consequences of this behaviour. It is gradually becoming clear that the chromosomes of all higher organisms, in composition and behaviour are fundamentally equal. Studying chromosomes in plants is quite relevant for understanding chromosomes in man and vice versa. It is important, therefore, to try to treat the entire science of cytogenetics as one unit. While such an approach may already be difficult with a simpler subject, it is truly complicated with this particularly wide field, certainly when the book must be kept within pocket size. There are a few ways out: one can generalize and consider only the major aspects in a simplified way that makes them understandable to the uninitiated student without further help. Or one can make the coverage wider and introduce some detail, hoping that the student has a sound basic knowledge of genetics and can count on someone to help him out when he gets baffled by the complexities of cytogenetic thought. The latter alternative was chosen, but is should be understood that the coverage still had to be far from complete.

The book contains a reasonable amount of systematically arranged information that may be used for reading assignments accompanying a cytogenetics course, and may perhaps replace extensive note-taking during lectures.

Because of the suitability of plant chromosomes for experimental manipulation, their study has contributed considerably to the understanding of chromosome behaviour. It is for this reason that many examples have been taken from plant cytogenetics. This may perhaps help preventing unnecessary "rediscovery" in humans and other animals of phenomena that for decades have been known to occur in plants.

CONTENTS

Chapter 1

BACKGROUND

1.1. The material basis

1.1.1. *Self-reproduction*

An essential characteristic of living material is its capacity for self-reproduction. In its absence, life, once originated, would not escape extinction: there are numerous internal and external processes that carry out an efficient break-down. This self-reproduction must be extremely exact as the vital processes of even the simplest forms of life are so complicated that slight deviations from an established pattern, proven to be efficient, will almost certainly result in a reduction of fitness.

All living material, from virus to complex multicellular organism contains a single basic substance that combines two essential functions: (1) exact replication (self-reproduction) and (2) strict regulation of the vital processes. The two functions are carried out in sequence, not simultaneously. This basic substance is usually DNA (deoxyribonucleic acid). In some cases (plant viruses for instance) it is the related substance RNA (ribonucleic acid). The exact replication of the basic regulating substance is the root of the self-reproduction of the species.

1.1.2. *DNA, RNA*

DNA and RNA consist of long molecular chains of *nucleotides*, which are the mono-phosphate esters of *nucleosides*. Nucleosides contain a pentose sugar (deoxyribose in DNA and ribose in RNA) and one of four nitrogenous bases: adenine, guanine (both purines), thymine (only in DNA and substituted by uracil in RNA)

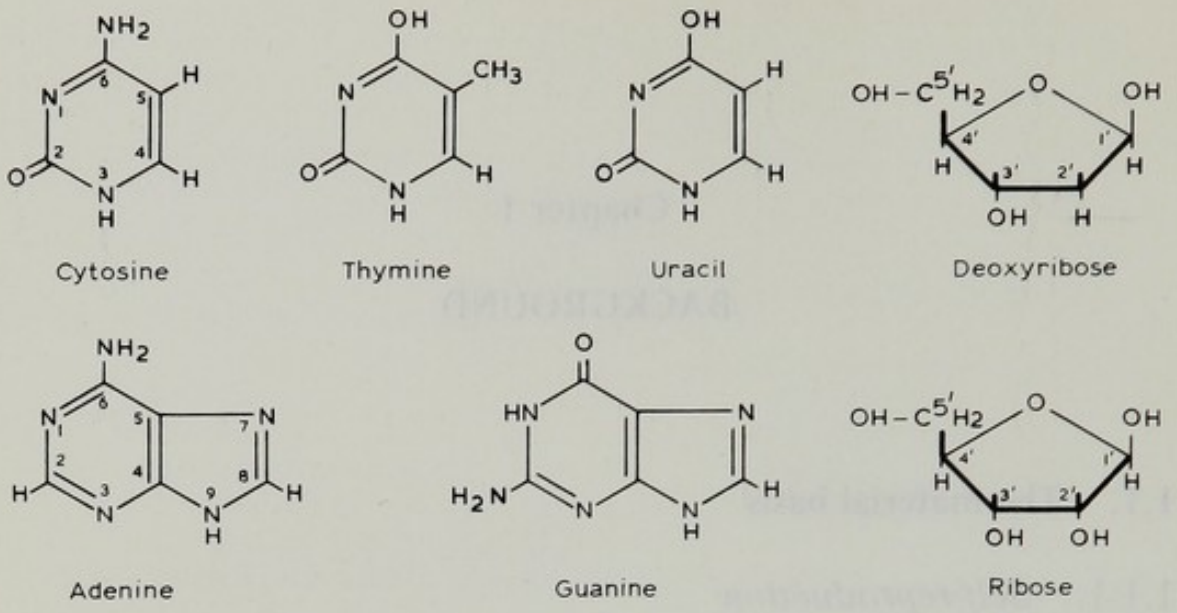


Fig. 1.1. The components of DNA and RNA. DNA contains the pyrimidines cytosine and thymidine, the purines adenine and guanine and the pentose sugar deoxyribose. RNA contains the pyrimidines cytosine and uracil, the purines adenine and guanine and the pentose sugar ribose.

and cytosine (all three pyrimidines) (see fig.1.1). Thus only four types of nucleosides occur: adenosine, guanosine, thymidine and cytidine in DNA; adenosine, guanosine, uridine and cytidine in RNA. As a consequence, DNA and RNA contain only four types of nucleotide. DNA and RNA principally differ only in respect to the pentose sugar and one of the four nitrogenous bases.

1.1.3. *Replication*

Polymerization of the nucleotides into the DNA (or RNA) chains occurs serially. The nucleotide to be built in is in triphosphate form, with its phosphate groups attached to the 5′C atom of the sugar. In the (enzymatic) process of attachment two of the three phosphate groups are removed and the remaining one binds to the 3′C atom of the previously attached nucleotide of the growing chain (fig.1.2). Thus the backbone of the DNA (and RNA) macromolecules is formed by successive (deoxy)ribose and phosphate groups, with the nitrogenous bases sticking out from the sides. The chain is not symmetrical: one end is the 3′C atom of the sugar, the other end the phosphate group at the 5′C atom: a

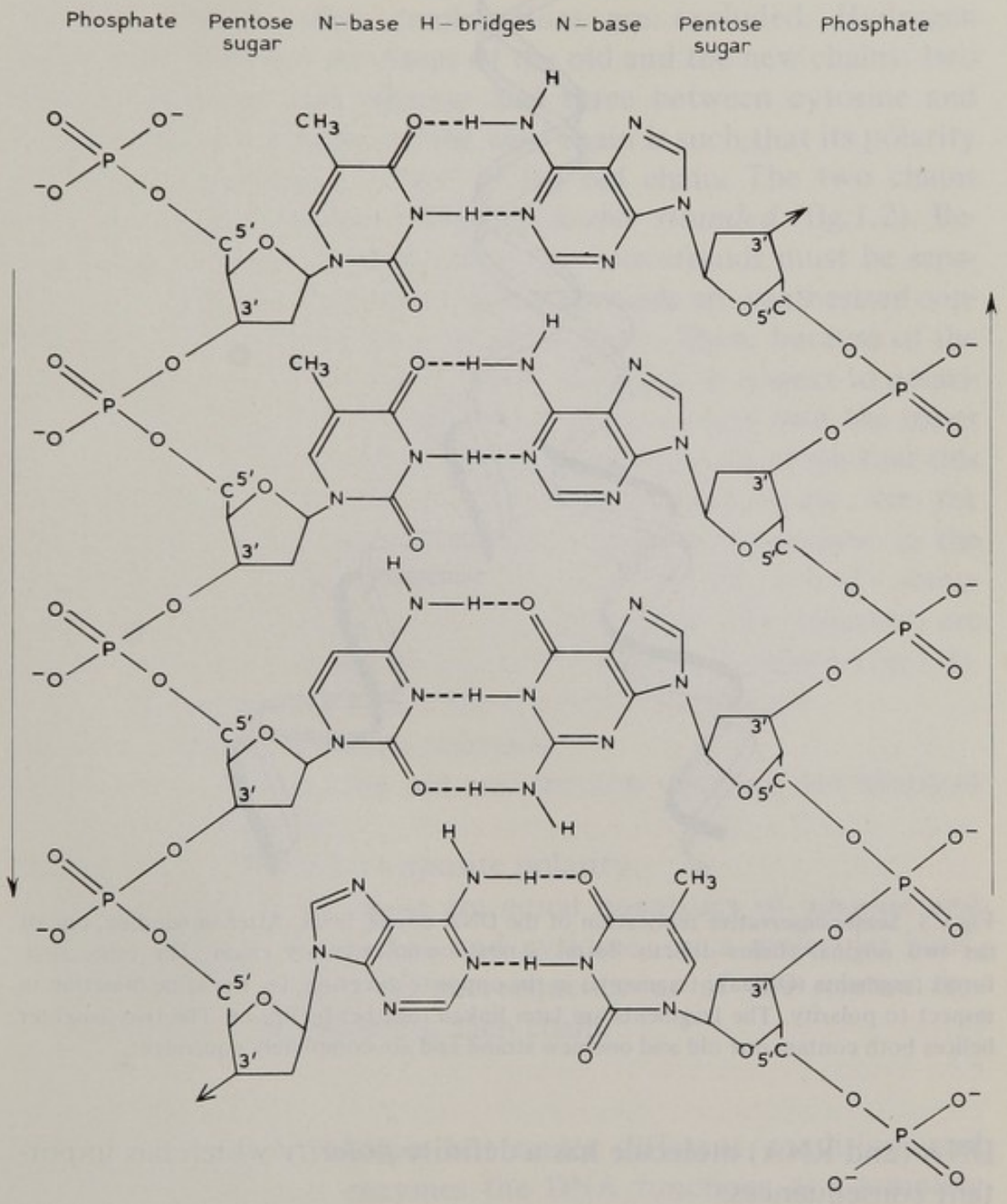


Fig.1.2. A fragment of a DNA double chain. The backbones of the two chains are formed by alternating sugar and phosphate groups. The polarity of the two chains is opposite. The nitrogenous bases are attached to the sugars and the complementary bases of the two chains are connected by hydrogen bridges. There are two between thymidine and adenine and three between cytosine and guanine. The double chain has the form of a helix with one revolution per 10 nucleotides.

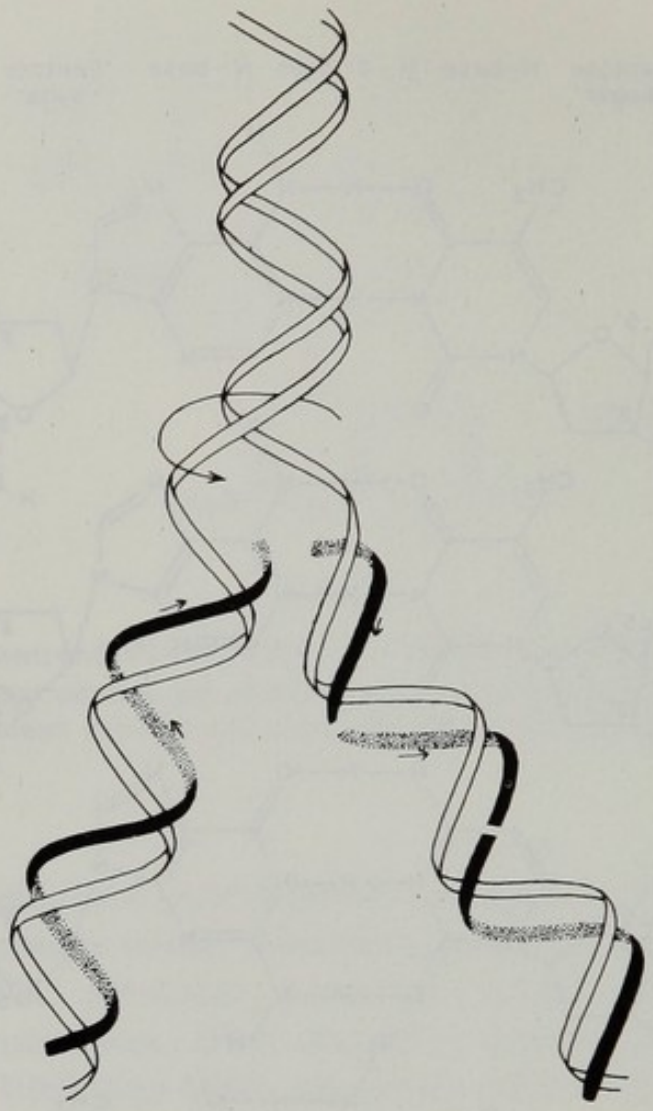


Fig.1.3. Semi-conservative replication of the DNA double helix. After unwinding, one of the two original chains directly forms a new, complementary chain. The other first forms fragments (Okazaki fragments) in the opposite direction, i.e. the same direction in respect to polarity. The fragments are later linked together by ligases. The two daughter helices both contain one old and one new strand and are completely equivalent.

DNA (and RNA) molecule has a definite *polarity* which has important consequences.

When DNA re(du)plicates, the new chain is laid down alongside an old chain, which functions as a template. There is a positive–negative relation between the two chains, in the sense that the nucleotides of the new chain are not identical to those of the old chain, but of specific complementary types. Opposite to thymine in the old chain, adenine is built into the new chain and vice versa.

Opposite to cytosine comes guanine (and vice versa). For stereochemical reasons other combinations are excluded. Hydrogen bonds form between the bases of the old and the new chains: two between thymine and adenine and three between cytosine and guanine. The orientation of the new chain is such that its polarity is reversed compared to that of the old chain. The two chains normally remain together: DNA is *double stranded* (fig.1.2). Before new synthesis can take place, the two strands must be separated. It might be expected that both strands are synthesized continuously starting from one initiation point. Then, because of the opposite polarity of the two strands, synthesis in respect to polarity would be into one direction in one strand and into the other direction in the other strand. There are strong indications that this is not the case: one strand is synthesized continuously, but the other strand is synthesized in sections (Okazaki fragments) in the same direction in respect to polarity, i.e. into the *opposite* direction when considered from the initiation point. The fragments are subsequently (enzymatically) linked together by ligases (fig.1.3).

Some important aspects of the structure of DNA are:

(1) DNA normally is double stranded;
(2) The two strands (the old and the new one) are not identical but complementary;
(3) The two strands have opposite polarity;
(4) In the total DNA there are equal quantities of adenine and thymine, and of guanine and cytidine, on a molecular basis. This is independent of the sequence or of the relative frequencies of nucleotides in each chain.

1.1.4. *Transcription*

In a comparable fashion but under different conditions and effected by different enzymes the DNA functions as a template for the formation of chains of RNA (transcription). After completion these RNA chains are released from the DNA without forming H-bonds. The RNA macro-molecule formed on the DNA is called *messenger* RNA (mRNA). It is carried to the cytoplasm where it is used as a template for the formation of polypeptide chains. This polypeptide synthesis is carried out by small bodies,

the ribosomes, that usually act in groups (polysomes), moving along the mRNA molecules in the process of polypeptide formation. The RNA moiety of the ribosomes (rRNA) consists of at least one small and two large RNA molecules that are transcribed on specific segments of the DNA. In the ribosome they are combined with proteins. The ribosomes pick up a third type of RNA: the *transfer* RNA (tRNA), that consists of much shorter chains, folded in a specific manner and often containing special base-types. These tRNAs are formed on their own DNA segments. The tRNA molecules have two recognition sites: on one an amino acid is attached enzymatically by a specific synthetase. The other site consists of a group of three nucleotides. Type and sequence of these nucleotides appear to be specific for the amino acid attached to the other site. Thus, for each amino acid there exists (at least) one specific tRNA characterized by a distinct group of three nucleotides. After having picked up a tRNA molecule with an amino acid, the ribosome first attaches the tRNA to the mRNA: the three specific tRNA nucleotides are fitted on a complementary set of three nucleotides in the mRNA. The first is placed on a specific initial site and the second follows on the next three nucleotides of the mRNA. The ribosome must select the correct tRNA for each site. For fitting the tRNA nucleotide triplets onto the mRNA triplets the same rules are followed as with transcription: adenine on uracil (which in RNA takes the place of thymine in DNA) etc. The row of amino acids is threaded together to a polypeptide chain and then the tRNA molecules are released from the mRNA and from the amino acids. Now both RNA forms are available for a new cycle, or are broken down. The sequence of amino acids in the polypeptide thus depends on the nucleotide sequence in the RNA, and consequently on that in the DNA, three nucleotides corresponding to one amino acid. This *triplet* of nucleotides in the mRNA contains the code for a specific amino acid and is named *codon*. Some codons have specific functions, such as interpunction. One amino acid may correspond to more than one codon: the code is degenerated. The codons UAA, UAG and UGA indicate the end of the polypeptide chain, the codons AUG and GUG the beginning.

For the replication of DNA both strands are used. For transcription only one is available: this prevents that two different polypeptide chains are derived from one DNA double strand. Which one is read off is determined by specific base sequences.

1.1.5. *Mutation*

Some polypeptide chains are built into structural proteins, but others are used to make enzymes. Small changes in the DNA, resulting from loss or from doubling of single nucleotides, can alter the entire pattern of transcription, since always three consecutive nucleotides form one codon. When the reading frame is shifted, all following codons are changed. A polypeptide may be formed, but it may not be expected to be functional as part of an enzyme. When no alternative intact DNA chain is available, a functional enzyme is not formed and the character conditioned by this enzyme is not expressed. Such a heritable change in a character is called a mutation, in this case a "frame shift" mutation.

Other changes in the DNA only locally affecting the reading frame such as inversion of a small segment or translocation from the original site to another location very nearby, or the replacement of one nucleotide by another, all will yield minor effects if the corresponding polypeptide segment is of minor importance. Whenever amino acid sequences in corresponding polypeptides have been studied in related species, small variations without great consequences have been detected. Occasionally, however, even simple amino acid substitutions, the consequence of single nucleotide replacements, have been found to have drastic effects. Both, harmless variations and drastic changes due to single amino acid substitution (sickle-cell anaemia for instance) have been found in haemoglobin and other components of the blood of man.

All such DNA changes (mutations) are transmitted with each cycle of DNA replication: they are heritable. In nature, mutations occur "spontaneously" (cause unknown) in a low frequency. They can be induced experimentally in high frequencies by chemicals (such as ethyl-methane-sulphonate, EMS, and other alkylating agents), by ionizing radiations, and by ultraviolet radiation. A high spontaneous mutation frequency would thoroughly disturb the

biochemical system of an organism. Therefore all forms of life have mechanisms that protect their DNA against a high mutation rate, and other mechanisms that can repair mutational damage. A few mutations, however, may result in a favourable mutant *phenotype* (the actual appearance of an individual, determined by a heritable component, the *genotype* and environmental factors). The individual in which this mutation is expressed, may have a reproductive advantage relative to the non-mutant individuals, or it may be able to maintain itself under unusual conditions. Mutations are the most important means of living material to improve itself even when it is at the expense of a large number of failures. Therefore, for no form or life is it desirable that mutation is precluded completely.

1.1.6. *Gene, cistron, operon, factor, allele*

For a long time geneticists have used the term "gene" for the hypothetical basic unit of function, of mutation and of recombination (see chapter 2). It has become clear that no single unit would simultaneously satisfy the criteria of these three categories. The unit of mutation may be a small group of atoms in one nucleotide. The unit of recombination, at least in lower organisms may be one nucleotide. The unit of function, however, can be quite large, consisting of a segment of DNA corresponding to at least one polypeptide chain, often to several.

Since 1961 when Benzer published his studies on complementation of different mutations of a single function in bacteriophage the term *cistron* has been in use as a unit of function. The term refers to specific differences in complementation when two mutations occur in the same DNA double strand (cis) and when the two occur in different double strands (trans) in the same cell. The cistron perhaps corresponds with one polypeptide chain. As complementation phenomena are more complicated than originally understood, the value of the experimental cistron is somewhat doubtful, but as a concept of functional unit it has survived.

Another unit of function, or rather of regulation is the *operon* (Jacob and Monod): a number of DNA segments coding for different enzymes, usually related to a single metabolic function in

bacteria, are sometimes found to lie in a row. They are preceded by a special regulating segment, the operator, that determines whether or not transcription of the succeeding segments will be initiated. The operator is activated by the promotor (or activator), in some instances perhaps also part of the complex. Functioning of the promotor is determined by the presence or absence of substances connected with the biochemical processes for which the operon supplies the enzymes. The term operon is used for the entire complex. It has so far been demonstrated with reasonable certainty only in micro-organisms.

A genetic unit of general applicability apparently does not exist and the best solution may be return to the *gene* as a working-concept, using it without further specifications. Especially in the field of cytogenetics, where the biochemical and functional analysis of the gene itself usually is not the subject of inquiry, it may well be used in its original sense: a unit of function, altered by mutation and generally behaving as a unit of recombination in most higher organisms. Another acceptable term is (*genetic*) *factor*, similarly used without specification.

Mutation can cause originally identical genes to occur in different states. These are called *alleles*: alternative forms of fundamentally the same gene.

1.2. The organization of DNA. Chromosomes

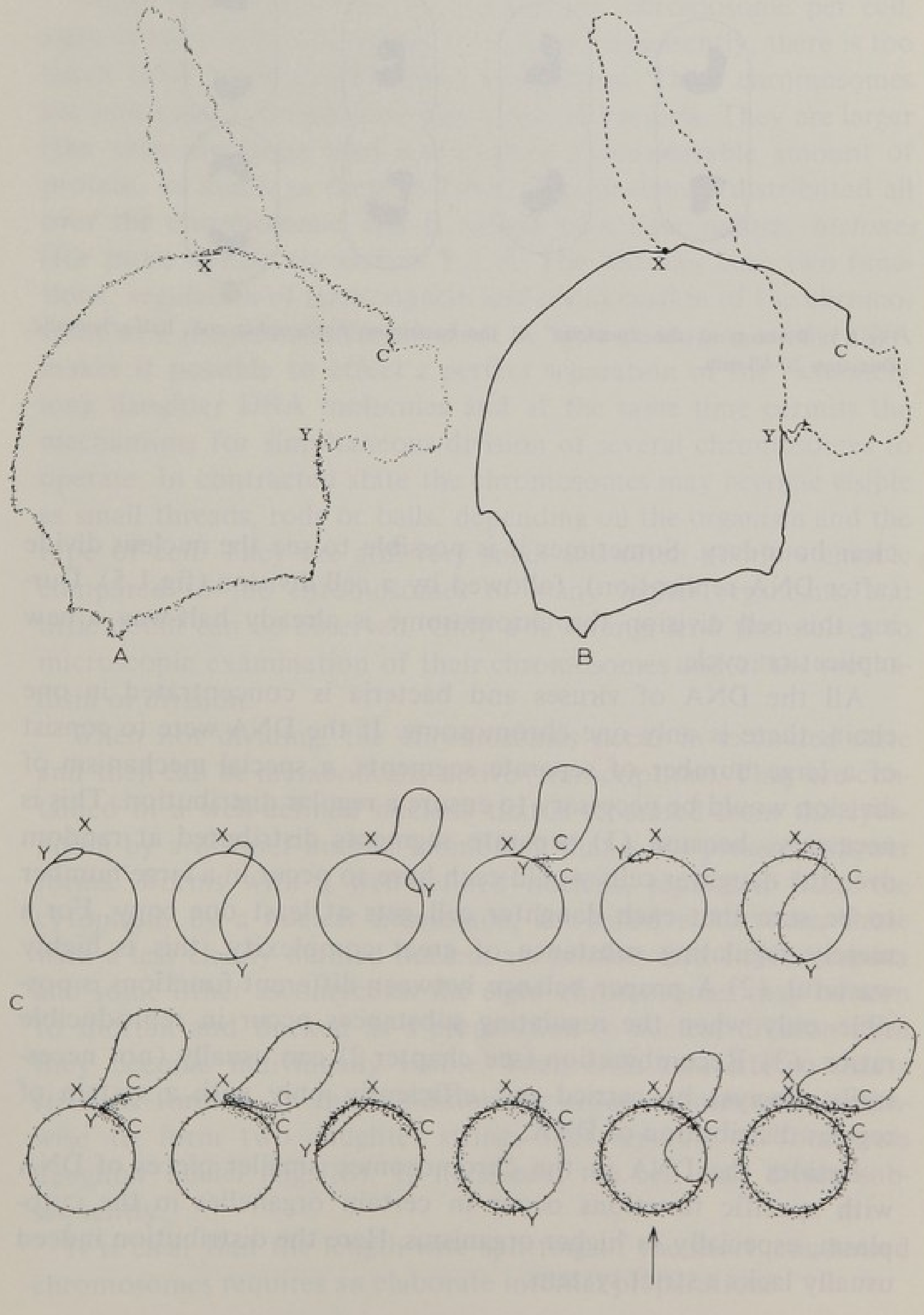
1.2.1. *Viruses and bacteria*

DNA double strands form a spiral (*helix*) with one revolution per ten nucleotides, and a length of 34 Å per revolution. The cross-section of the helix is 20 Å. The current model of the free DNA molecule was constructed by Watson and Crick in 1953, on the basis of X-ray diffraction and other data of themselves and others. During DNA replication the two strands are separated. Both have a new chain formed, so that two new double strands result, each identical to the old one. Both consist of one new and one old strand: DNA replication is said to be *semiconservative* (fig.1.3 and section 1.1.3). The unwinding of DNA during replication is hard to visualize. For long chains it should become imprac-

ticable, which should set a limit to the length of individual DNA strands. Yet this is hardly the case. All DNA of phage T4 of *Escherichia coli* occurs in one double strand of 20 Å cross section and approximately 60 μ in length. In *E. coli*, the DNA molecule is approximately 1000 μ in length, which with a diameter of 20 Å, is gigantic. To make it even more complicated, these helices under certain conditions have a ring shape, for instance during replication. DNA synthesis in the ring starts at a specific point and goes around the ring at a high speed; in 20 min 1000 μ of DNA must have replicated (fig.1.4). During the process the helix is unwound and thus, with a circular DNA molecule, a "swivel" is required, assumed to be located at the replication-initiation point. A hardly conceivable speed of thousands of revolutions per second is necessary. There still are many unsolved problems. The role of tiny fragments of protein deposited on the DNA chain has not yet entirely been cleared up.

The relatively simple, long DNA chains with protein and specific structures (such as the swivel), of viruses and bacteria are often called *chromosomes*, in analogy of the stainable bodies called chromosomes, that higher organisms use to wrap up their DNA (sections 1.2.2 and 1.2.3). The DNA of bacteria often assumes the shape of a microscopically visible small ball, the *nucleus*, without

Fig.1.4. Drawing of a microautoradiogram (A) of a spread-out ring chromosome of *Escherichia coli* during replication, after Cairns (1963). It is the second round of DNA synthesis since the addition of Tritium-marked thymidine to the culture medium, and this is one of the two sister chromosomes that were separated after completion of the last round of synthesis. DNA-synthesis is now at Y and proceeds in the direction of C. The stretch between Y and C has tritiated thymidine in only one of its two strands: it shows weak radioactivity, compare (C). In the process of DNA synthesis with marked thymidine the non-radioactive parent strand is combined with a marked strand and the resulting double strand again has weak activity (above Y). The radioactive parent strand is also combined with a radioactive new strand, and the resulting double strand has strong radioactivity (below Y). Because the first time tritiated thymidine was applied, DNA synthesis was at C, the entire chromosome segment from C to Y (over X) is double marked. X is the initiation and end point of DNA synthesis: when replication has arrived at X the two rings are completed and separate. One will be entirely double marked, the other single, as it still carries the original unmarked strand. Further explanation in B and C. The chromosome is normally folded in a "nucleus" but here spread by a special technique for autoradiography.


X
Y
C
A
B
C

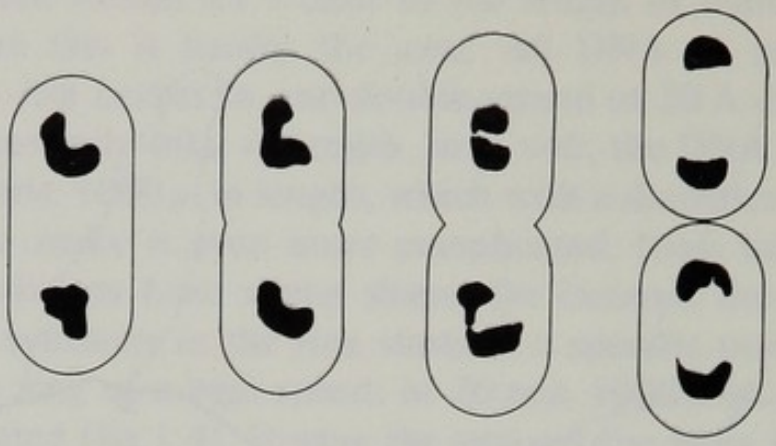


Fig.1.5. Division of the "nucleus" of the bacterium *Escherichia coli*, half-schematic. Duration 20-40 min.

clear boundary. Sometimes it is possible to see the nucleus divide (after DNA replication), followed by a cell division (fig.1.5). During this cell division the chromosome is already half-way a new replication cycle.

All the DNA of viruses and bacteria is concentrated in one chain; there is only one chromosome. If the DNA were to consist of a large number of separate segments, a special mechanism of division would be necessary to ensure a regular distribution. This is necessary, because (1) separate segments distributed at random over the daughter cells would each have to occur in a large number to be sure that each daughter cell gets at least one copy. For a merely regulating substance of great complexity, this is highly wasteful. (2) A proper balance between different functions is possible only when the regulating substances occur in reproducible ratios. (3) Recombination (see chapter 2) can usually (not necessarily always) be carried out efficiently only with a system of regular distribution of DNA.

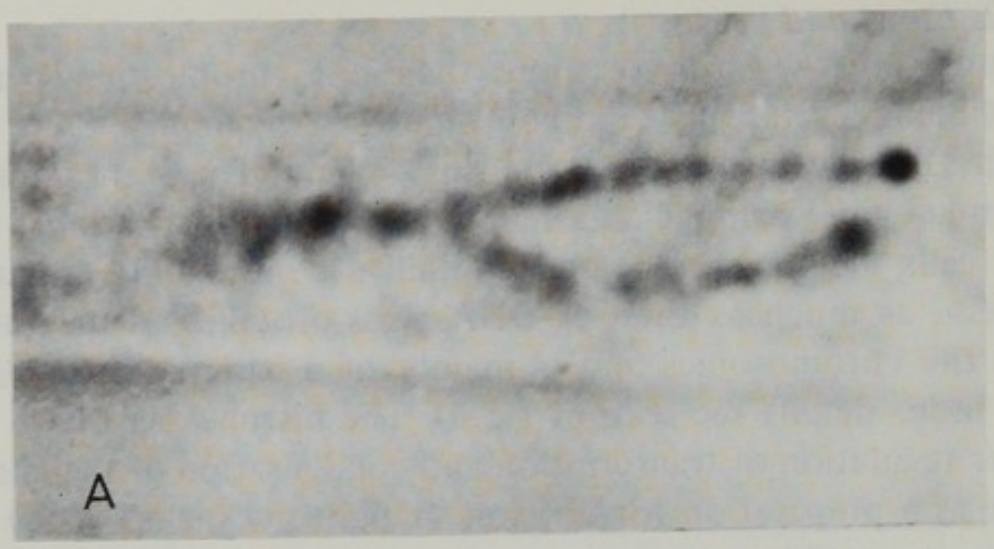
Besides the DNA in the chromosomes, smaller pieces of DNA with specific functions occur in certain organelles in the cytoplasm, especially in higher organisms. Here the distribution indeed usually lacks a strict system.

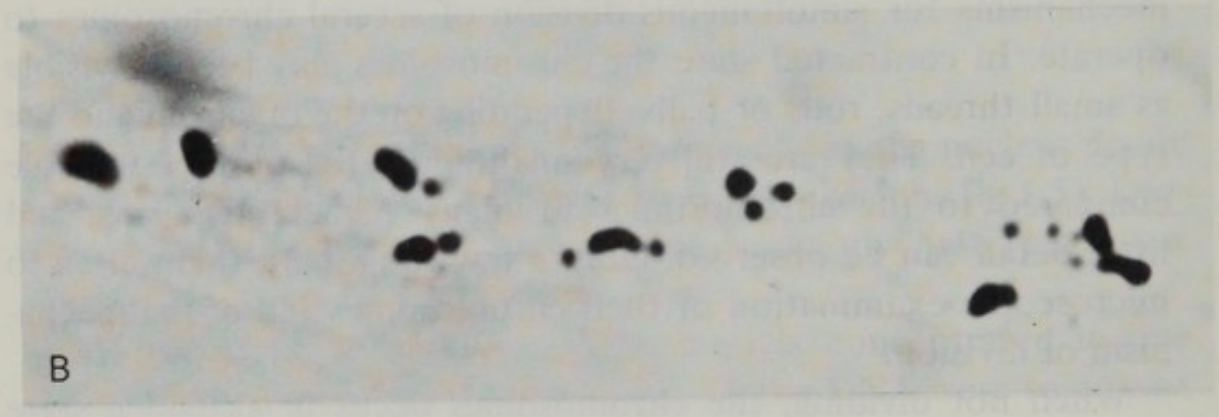
1.2.2. *Fungi and yeasts*

Fungi and yeasts have more than one chromosome per cell, eight being a relatively common number. Apparently, there is too much DNA to be handled as a single chain. These chromosomes are much more complicated than those of bacteria. They are larger (the cells are larger too) and contain a considerable amount of protein, as much as there is DNA. This protein is distributed all over the chromosomes and is mainly of a basic nature: *histones* (for more details see section 1.2.3). The histones have two functions: regulation of transcription and condensation of the chromosome as a preparation for division. A tight packaging of the DNA makes it possible to effect a perfect separation of the extremely long daughter DNA molecules and at the same time permits the mechanisms for simultaneous division of several chromosomes to operate. In contracted state the chromosomes may become visible as small threads, rods or balls, depending on the organism and the type of cell. They are still very small and often poorly stainable compared to the chromosomes of many higher organisms, and little detail can be observed. Only a few fungi lend themselves to microscopic examination of their chromosomes and of the mechanism of division.

When not dividing, the chromosomes occur in extended state and then can be metabolically active (transcription). They are contained in a well-defined nucleus that is separated from the cytoplasm by a distinct nuclear membrane that is not present in lower forms. Forms with a well-defined nucleus, separated from the cytoplasm by a nuclear membrane, are called *eukaryotes*, those with a less clearly defined nucleus are *prokaryotes*. In Neurospora and some other ascomycetes the eight chromosomes may be seen to shorten and thicken as a preparation to nuclear division until they become individually visible. Their ends associate so that a string is formed. In this condition the chromosomes split length-wise to form two daughter strings that separate to form two daughter nuclei (fig.1.6). If necessary, the cell may divide subsequently.

It is clear that the length-wise splitting of the short, condensed chromosomes requires an elaborate internal preparation.





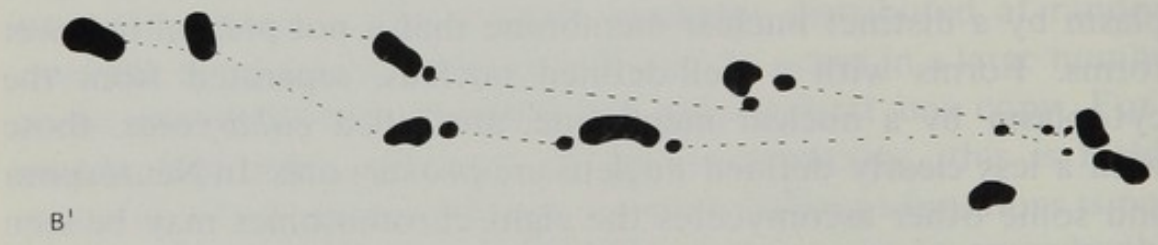


Fig.1.6. Nuclear and chromosome division in *Neurospora crassa*. (A) With phase-contrast the nucleus is visible as one chain, split into two halves at right, apparently still single at left. (B) At a somewhat later stage and with only the DNA stained, the individual chromosomes can be distinguished. Their connections are invisible but they can be seen to be situated in a row (compare drawing B'). At left the division is still incomplete but in the middle the two halves of each chromosome are clearly separated. Note the differences in shape. At right the situation is less clear (see Weyer et al., 1965).

1.2.3. *Higher organisms. Histones. Chromatin*

The chromosomes of higher organisms in general are larger, and also provided with protein all along the DNA chain. Each chromosome can contain enormous quantities of DNA: the relatively small nuclei of the fruit fly Drosophila, for instance, contain a total length of approximately 16,000 μ DNA, divided over eight chromosomes. Many organisms even have much larger chromosomes. Nevertheless, enzymatic break-down studies have revealed that the longitudinal continuity of the entire chromosome is determined by the DNA exclusively. Here again, the chromosomal proteins are predominantly histones (protamines in the sperm of most animals). They are attached to the phosphoric acid residues, which they neutralize. In viruses and bacteria these acid groups are neutralized by the metal ions of the cell plasma, in the free virus particles by the coat proteins. The histones, mainly by their electrostatic properties, affect the shape of the DNA–histone complex by coiling and folding. This complex, the deoxynucleo protein (DNP), in association with numerous other substances in quantitatively minor amounts (acidic proteins, RNA, lipids, divalent cations, enzymes, etc.) is called *chromatin.*

When histones and DNA are tightly associated, transcription of the DNA template is suppressed. Complete removal of the histone is no prerequisite for template activity. It is sufficient that the steric hindrance of RNA polymerase by the DNA-histone supercoil is removed. This is achieved probably mainly by acidic proteins, perhaps in association with some forms of RNA. During the metabolic state of the chromosomes a large proportion (perhaps 60-80%) is in an "open" state. Chromatin can be isolated without loosing its capacity of being transcribed. Such isolated chromatin has yielded valuable information on the role of histones and other substances on the regulation of transcription (see Bonner et al., 1968).

There are several different types of histone, but the majority can be classified into five or six main fractions. The same basic types occur in mammals, birds, fishes and plants. They differ in amino acid composition and in electrophoretic mobility: f1 is very lysine- and alanine-rich; f2a1 is high in arginine and glycine and

contains less lysine; f2a2 has slightly more lysine than arginine, it is also relatively rich in leucine; f2b is slightly lysine-rich and contains only half as much arginine as lysine, while it contains relatively much serine; f3 is high in arginine. (See further Georgiev, 1969.)

The histones occur more or less at random along the DNP strand. The lysine-rich histones are the most effective in inhibiting transcription as well as in the packing of the chromatin. The histones are not sufficiently specific to regulate cellular and organ differentiation, they merely act as a switch. The insertion of acid proteins that opens the switch is under the control of other regulatory systems, among which hormones are the best known, although not necessarily the most common.

In differentiated tissues the majority of the genes is blocked for transcription as only part of the potential functions may be expressed. This block appears to be rather definite and irreversible. In sperm all gene action must be blocked, but only in a transient way. This is achieved by replacing all histones by protamines. Protamines are also basic proteins, but of even simpler constitution than histones. While histones fit in the large groove of the DNA double helix, protamines fit in the narrow groove. They completely inhibit transcription and by replacing the histones, erase the "developmental history" of the chromatin. At the same time they cause a much tighter condensation, permitting an extreme streamlining of the sperm nucleus.

The role of histones in contraction is also quite important. The nucleus of a normal body cell of the fruit fly *Drosophila melanogaster*, for instance, with a total length of 16,000 μ of DNA in eight chromosomes may have a diameter of 5 μ while each chromosome in condensed state is only a few μ long. It is at least remarkable that in spite of this the linear arrangement of the DNA has been maintained and that the length-wise split of all this DNA is brought about by simple length-wise splitting of the chromosomes.

1.2.4. *Heterochromatin*

During certain stages of the chromosome division cycle maximal

condensation is required to permit proper separation of the two halves. At the same time gene transcription is repressed. After completion of the division decondensation usually follows, and genes that must function are available for derepression. Some chromosome segments, however, maintain the condensed state during most of the time that the majority of the chromatin is not condensed. While the chromatin that follows the normal condensation–decondensation cycle is called *euchromatin*, that with a deviating condensation system is called *heterochromatin*. Heterochromatin is often recognizable as small stainable bodies in rather diffuse nuclei. The phenomenon that chromosomal segments are visible because of contraction at a stage in which most of the chromatin is diffuse, is also called *heteropycnosis*. Negative heteropycnosis denotes a condition of absence or reduction of condensation in normally contracted chromosomes at the time of division. It is sometimes, but not necessarily always correlated with the occurrence of heterochromatin and can often be induced artificially. Heterochromatinization is regulated by special factors in the heterochromatic segment and can be variable in extent. All genes in the heterochromatic segment are repressed. Replication remains possible, but is delayed relative to the euchromatin. It occurs late in the synthesis (S) phase: *late replication*. Repressed and late replicating heterochromatic segments may occasionally fail to show typical condensation in some nuclei.

Two types of heterochromatin may be distinguished: (1) constitutive heterochromatin, a constant condition of the chromosome segment involved that is only slightly variable in expression, and (2) facultative heterochromatin that is expressed only under special conditions. Facultative heterochromatinization is an important means of genetic regulation (compare sex-chromatin, section 3.2.2). For more details see Frenster (1969), and Lima-de-Faria (1969).

1.2.5. *Replicons*

Although the condensation of the chromatin permits the chromosome to be handled as a mechanical entity in division, its DNA has such a large size that it can not be handled as a single unit of

replication with an average replication rate of 0.5–1.5 μ DNA/min. DNA synthesis does not start at one end of the chromosome to proceed to the other end. There are numerous fixed DNA replication initiation sites from where synthesis starts in a definite direction, and proceeds until an end point is reached. This end point either coincides with another initiation point or with another end point, depending on the direction of synthesis in adjacent segments. Thus the chromosome is partitioned into a number of units of replication: replicons.

DNA synthesis is usually studied by supplying a cell in the synthesis phase with a radioactive precursor of DNA, that can later be traced by autoradiography. Usually thymidine is used, containing one or more atoms of radioactive hydrogen (Tritium, ^3H): tritiated thymidine. Thymidine is specific for DNA and is not incorporated into RNA, which would need uridine in its place. Tritium emits a weak beta ray (a relatively slow electron) that will precipitate silver grains from a photographic emulsion in the immediate vicinity of the Tritium atom. The silver grains mark the presence of a radioactive atom and consequently of incorporated marked thymidine. Since the replicons do not replicate simultaneously, the moment of application of tritiated thymidine during S-phase determines which segments of the chromosome will become radioactive. By applying tritiated thymidine in short pulses at specific periods of the S-phase, the temporal pattern of DNA synthesis can be studied. This pattern appears to be irregular but constant, and specific for each chromosome. Heterochromatin always replicates late.

1.2.6. *Semi-conservative replication*

It has been observed that DNA replicated semiconservatively: each daughter double strand contains one new and one old strand (section 1.2.1). Taylor and others showed that the same is true for chromosomes. Roots of the broad bean, *Vicia faba*, were given tritiated thymidine during one cycle of DNA synthesis, and subsequently it was washed out. At the first separation of the two daughter halves of the chromosomes (the *chromatids*) both appeared to be radioactive (fig.1.7). When the

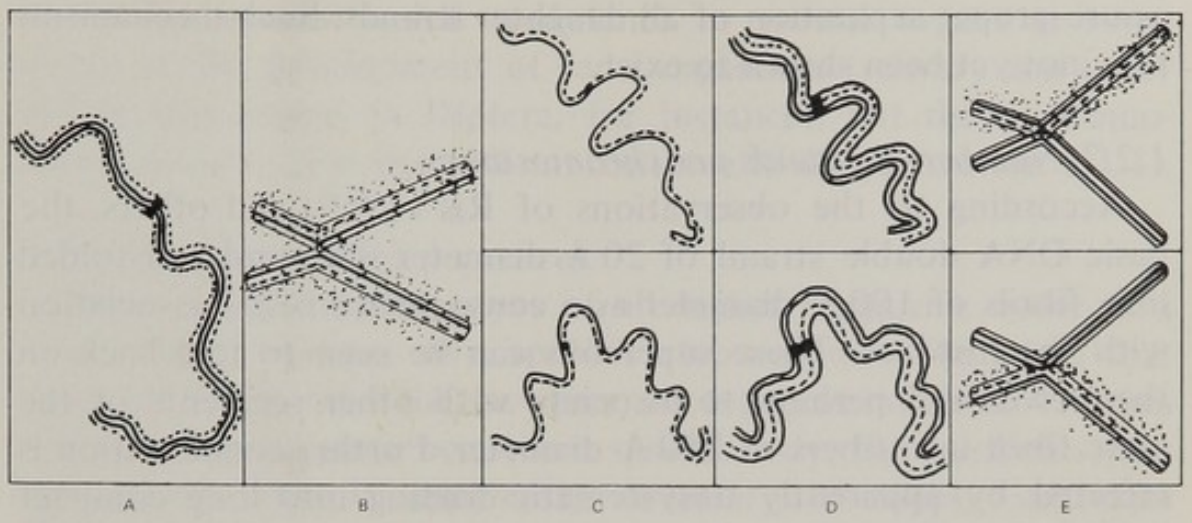


Fig.1.7. Semiconservative chromosome replication. Diagrammatically according to Taylor. At (A) a chromosome that has just completed replication in the presence of radioactive (tritiated) thymidine: there are two double strands and one strand of each is radioactive (stippled). At (B) the subsequent metaphase is shown after a pretreatment that separated the chromatids without splitting the centromere (colchicine, see section 1.4.2). In an autoradiogram both chromatids show the marker but each has one radioactive and one "cold" strand. After separation of the chromatids (C) a new round of DNA synthesis, now without radioactive thymine (D), results in one marked chromatid and one unmarked chromatid (E). This shows that indeed each chromatid contains two strands, one of which is derived from the parental chromosome and has served as a template for the newly formed strand.

cells were permitted to complete another cycle of chromosal division, now without tritiated thymidine, only one of the two chromatids of each chromosome appeared to be radioactive. This demonstrates that already at the initiation of DNA synthesis two strands must be available, each serving as a template for synthesis: after replication both contain new (radioactive) material. In the next division the radioactive strand synthesizes a new strand, the two together still being radioactive. The old strand also makes a new one, but these together are not radioactive. The radioactive and the "cold" chromatid, both containing a new and a template strand, can be distinguished by autoradiography.

The fact that both the DNA molecule and the complex chromosome replicate in a semi-conservative manner does not prove that a chromosome does not contain more than one DNA molecule. It may, but if it does not, special mechanisms must be available to

assure proper separation of all daughter strands. Such mechanisms have not yet been shown to exist.

1.2.7. *Number of strands per chromosome*

According to the observations of Ris (1969) and others, the basic DNA double strand of 20 Å diameter is wound and folded into fibrils of 100 Å diameter as a consequence of its association with the histones. These supercoils can be seen to fold back on themselves and perhaps to associate with other segments of the same fibril into fibers of 250 Å diameter. Further condensation is effected by apparently unsystematic folding into long compact rods that coil up into the chromosomes that can be observed microscopically during the contracted stages of division when the chromosomes split. Although it is possible that the basic unit of the 250 Å fibre is a single folded double DNA helix, the assumption that each chromosome (or chromatid) after splitting should always contain only a single double helix does not seem to be tenable. The fact that in lampbrush chromosomes (see section 3.1.2) probably only one DNA molecule forms the backbone of the chromosome, and further the fact that the pre-replication chromosome behaves as a single strand in respect to breakage and reunion after ionizing radiation, have led some authors to consider the chromosome to consist of fundamentally one strand and to consider observations to the contrary as artefacts. Multistrandedness (*polyteny*) of chromosomes indeed requires awkward hypotheses to explain observations such as those mentioned above and, for instance, semi-conservative replication, where the strands must be sorted out very carefully. Singlestrandedness, however, similarly requires complex explanations of some other phenomena, such as the clear doubleness of chromosomes after splitting (anaphase, section 1.3.1) in some organisms; continued doubling without DNA synthesis for several division cycles; doubleness in respect to breakage just prior to DNA synthesis; sub-chromatid breaks; etc. (see Wolff, 1969). The problem of chromosome strandedness clearly has not yet been solved. It is possible that often the chromosome is multistranded but also (for instance in meiosis, sections 2.4; 3.1.2) may be singlestranded. Some chromosomes are un-

doubtedly multistranded (polytene) in special organs at some stages in the development of some organisms (salivary and other glands and tissues in Diptera, for instance), but these chromosomes occur in cells that will not continue to divide (section 3.1.1).

1.2.8. *Repetitive DNA sequences*

Most of the normal genes of all higher organisms occur as a single DNA sequence in a specific chromosome. The genes that code for ribosomal RNA, however, are repeated hundreds of times and in extended state occur in a special body, the *nucleolus* (section 3.2.6). This is apparently necessary to supply sufficient rRNA. In addition, many other types of repetitive DNA sequences occur, the purpose of which is still practically unknown.

Britten and Kohne (1969) analysed the reassociation kinetics of isolated single strand DNA and concluded that 60% of the DNA of higher organisms occurred as repeated sequences, each in hundreds of thousands of copies. These repeated sequences are scattered throughout the genome. When DNA is precipitated in the ultracentrifuge, in a caesium chloride gradient, the bulk appears as a broad density band but some 10-15% of the total may appear as smaller (satellite) bands, distinctive from the bulk: *satellite DNA* that may be either lighter or heavier than the bulk of the DNA. This satellite DNA appears to be even more repetitive than that mentioned above. The light satellite DNA of the house mouse contains 10^6 copies of a single sequence of 400 nucleotides per set of chromosomes; in the guinea pig there are some 10^5 copies in the satellite DNA. They are not all identical but the degree of homogeneity is high. According to Southern (1970) the basic unit is 6 nucleotide pairs rather than 400 which makes a total of 10^7 short repeated sequences in the guinea pig. These are not identical: random mutations have introduced numerous alterations, but the basic unit remains recognizable. It appears that the repeated sequences of the satellite DNA are evolutionarily new: related species often have widely diverged patterns. Their role is still mysterious. Pardue and Gall in 1970 succeeded in hybridizing radioactive labelled satellite DNA of the mouse with denatured DNA in cytological preparations. The label appeared to localize near the centromere, indica-

ting that the repetitive satellite DNA of the mouse is concentrated in the heterochromatin associated with the centromere. In general, satellite DNA seems to be restricted to constitutive heterochromatin. Other repetitive DNA may be "spacer DNA" between genes.

1.3. The division of the nucleus of higher organisms

1.3.1. *Mitosis, karyokinesis*

Normally, chromosome division in higher organisms is followed by a nuclear division. In exceptional cases this is not so, but then the number of chromosomes per nucleus doubles with each division (*endomitosis*). It occurs only where further growth by cell division is not required or not desirable, but where great biochemical activity takes place. Examples are endomitotic nuclei in specialized tissues of many animals and some plants and the macronucleus of ciliate protozoa (fig.1.8).

The common nuclear division is called mitosis or karyokinesis (fig.1.9). In a normal (uninucleate) cell that is preparing for division, the nucleus is generally situated in a central position. Usually there is no vacuole and the cell is relatively small. The nucleus is ball-shaped or ellipsoidal and surrounded by a *nuclear membrane* that is related to the endoplasmic reticulum, and that separates the nucleus from the cytoplasma. Selective transport of many substances is possible across the nuclear membrane. The nucleus is colourless but can be stained after the proper preparations, usually killing and fixation. The chromosomes in the metabolic nucleus are in a rather extended state and even after staining visible only as a dotted, sometimes slightly threadlike mass. In a number of organisms some more spiralized and therefore more strikingly stained parts are visible: the heterochromatic (section 1.2.4) chromosome segments or *prochromosomes*. In this "resting" nucleus a number of nucleoli can be seen, often fused into one. They are formed at specific locations (*nucleolar organizers*) on specific chromosomes (section 3.2.6). The nucleoli contain repetitive DNA segments in a very extended state (section 1.2.8) that code for ribosomal RNA (rRNA). They are usually ball-shaped and their

Fig. 1.8. High degree of polyploidy ($4n = 960$) as a result of endomitosis (endopolyploidy) in the short-lived endosperm of *Cucurbita pepo*. Note the rows of identical daughter chromosomes derived from the same mother chromosome in a series of replications that were not followed by nuclear division (Varghese, 1971).

size is typical for the organism in which they occur and can vary from hardly visible to about 1/3 of the nuclear volume. On the outer surface of the nuclear membrane of animals and some lower organisms a small body can be found, often in the shape of a cylinder: the *centriole*. It has a function in the mechanism of the nuclear division and splits into two halves prior to mitosis. The two halves move to opposite positions on the nucleus at the beginning of mitosis.

When the nucleus prepares to divide, the chromosomes condense until they are clearly visible but still thin threads (*prophase*) with the heterochromatic segments still distinguishable. With further condensation the double character of the chromosomes becomes visible: each consists of two, closely associated, longitudinal halves, the chromatids. In early prophase the chromosome somewhat resembles a string of beads with thinner and thicker parts: the *chromomeres*. In late prophase these disappear and the chromosome has a more even surface. Then also the difference between euchromatin and heterochromatin becomes unclear.

At late prophase each chromosome generally shows one prominent constriction, the *primary constriction* or *centromere*, also (actually better) called *kinetochore*. It is the point that at the succeeding stages directs the movements of the chromosomes during nuclear division. The primary constriction divides the chromosome in two *arms*. Usually at or near the place where the nucleolus is attached to its chromosome, another constriction can be observed: the *secondary constriction*. Its location varies from species to species, and consequently the size of the segment terminal from this constriction varies in size. This segment is called the *satellite*. The name is merely descriptive and has nothing to do with satellite DNA. In man the satellites are very small. In general, in many species the secondary constriction tends to be rather distally located.

Occasionally, especially after cold-treatment, heterochromatic or other segments fail to condense and form very weakly stainable constrictions (*tertiary constrictions*; negative heteropycnosis, section 1.2.4). If these occur in heterochromatin, this apparently decondenses locally.

The chromosomes in early prophase exhibit a wide coil,

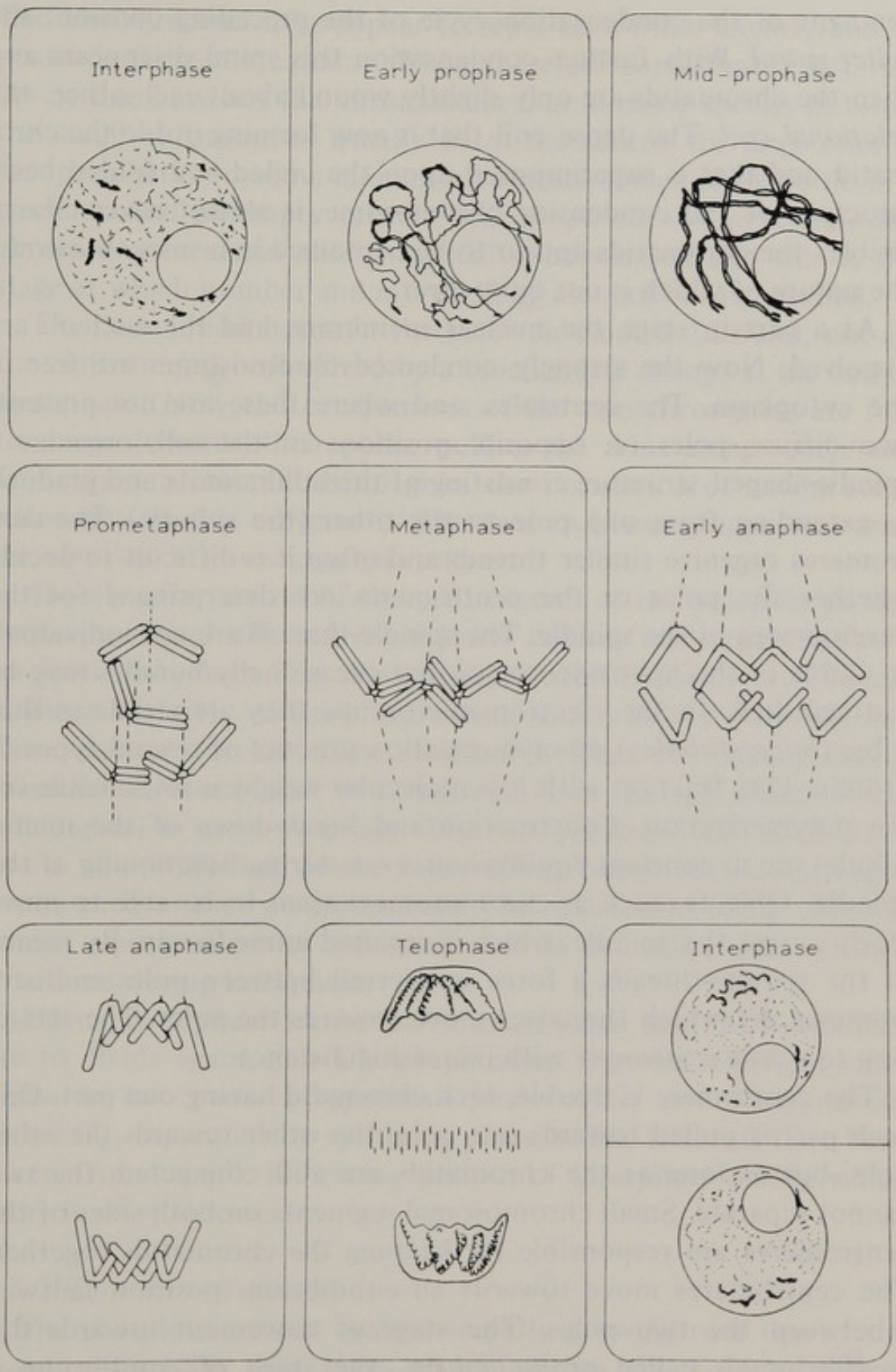


Fig.1.9. Mitosis. See section 1.3.1.

remnant of the condensation cycle of the preceding division: the *relict spiral*. With further condensation this spiral disappears and then the chromatids are only slightly wound about each other: the *relational coil*. The dense coil that is now forming inside the chromatid and that is superimposed upon the coiled and folded basic structure of the condensing chromosome, is almost never clearly visible: the chromatids appear to be contained in a smooth *matrix*, the nature of which is not quite clear.

At a certain stage the nuclear membrane and the nucleoli are dissolved. Now the strongly condensed chromosomes are free in the cytoplasm. The centrioles, and where these are not present, two diffuse poles on opposite positions in the cell, organize a spindle-shaped structure consisting of threadlike units and gradually extending from one pole to the other (the *spindle*). The centromeres organize similar threads and often it is difficult to decide whether the poles or the centromeres are determinant for the development of the spindle. The spindle-threads are not individually visible in the light microscope but occasionally bundles may be distinguished. In the electron microscope they are visible as thin tubes (*micro-tubules*), a polymerization product of several types of protein. One fraction with low molecular weight is responsible for the polymerization. Construction and break-down of the microtubules are in constant equilibrium, even during functioning of the spindle. Objects (such as chromosomes) seem to be able to move freely across the spindle, which is repaired immediately. By means of the spindle-threads a force is exerted between poles and centromeres, by which the latter move towards the poles. The attraction to a pole is stronger with increasing distance.

The centromere is double, each chromatid having one part. One such part is pulled towards one pole, the other towards the other pole, but as long as the chromatids are still connected, the two cannot separate. Small chromosomal segments on both sides of the centromeres are responsible for keeping the chromatids together. The centromeres move towards an equilibrium position halfway inbetween the two poles. The stage of movement towards this equilibrium is called *prometaphase*, the stage of equilibrium is *metaphase*. The centromeres are then in one plane: the equator.

The chromosome arms appear to repel each other slightly and to protrude from the equator. When there are considerable differences in size between the chromosomes, as in many species of birds, the small chromosmes usually lie at the centre, the large ones at the periphery. In some animals all chromosomes come to lie in a circle around an empty centre: *hollow spindle*. At metaphase the chromosomes attain their maximal condensation and this is the stage at which number and morphology are most easily studied.

The connection between the sister chromatids (around, not at, the centromere) is dissolved by a biochemical change at the end of metaphase. Now the centromeres of the two chromatids are free to move towards their poles, pulling the arms along: *anaphase*. Sometimes a chromatid-end gives the impression of having some autonomy in this movement. The two anaphase groups that arise are identical in chromosomal content.

Shortly after arrival of the centromeres at the pole, the groups contract, round off, and form a new nuclear membrane and new nucleoli: *telophase*. The chromosomal coils loosen up until finally nothing is visible of the chromosome contours and even the fine chromosomal threads seem to dissolve. That the original coil is maintained loosely, can be concluded from the appearance of the relict spiral in early prophase. The new prophase coil, however, is not a new stiffening of the relict spiral, but develops internally, inside each of the two chromatids. The orientation of the chromosomes: centromeres at one side, ends at the other side, remains unaltered during interphase.

During *interphase* transcription takes place, and for nuclei that are to divide again, also DNA replication. The latter divides interphase in two parts: presynthesis phase or G_1 and postsynthesis phase G_2, separated by synthesis phase S. The letters G have been taken from "gap", indicating periods of interphase during which there is no synthesis.

1.3.2. *Duration of mitosis*

The complete mitotic cycle in rapidly growing root tips of the broad bean *Vicia faba* takes on an average about 24 hr with a minimum of about 8 hr. The average duration of the S phase is

Table 1.1
Duration in minutes of mitotic stages in a number of organisms and tissues at different temperatures (several authors).

	Temp. °C	Prophase	Metaphase	Anaphase	Telophase	Together	Complete cycle
PLANTS							
Tradescantia sp.							
(stamen hairs)	10	–	–	–	–	135	
–	25	–	–	–	–	75	(340)
–	45	–	–	–	–	30	
(microspore)	30	–	–	–	–	1800	
Arrhenaterum sp. (grass)							
(stigma cells)	19	36–45	7–10	15–20	20–35	78–110	
ANIMALS							
Chortophaga sp. (insect)							
(embryo, neuroblast)	38	102	13	9	57	181	208
–	26	–	–	–	–	–	480
Gallus (chicken)							
(tissue cult., mesenchyma)	39	5–50 mostly >30	1–15 mostly 2–10	1–5 mostly 2–3	2–13*	70–180	
(embryo, eye tissue)	39	–	–	–	–	23–65	

* When end point is completed resting cell: 30–120 min.

7.5 hr, which is rather representative for many higher organisms (compare John and Lewis, 1969). G_1 and G_2 can vary considerably, even when actively dividing tissues are compared.

For a number of organisms the duration of the different stages, partly under different conditions, is shown in table 1.1. Compared with the division cycle of phage particles in bacteria (8-9 divisions in 20 min) even the division cycle of the chromosomes of Chortophaga is long. Phage particles, however, replicate only DNA, the protein coat is formed later. There is a correlation between the minimum mitotic cycle time and the volume of the interphase nucleus, which in its turn is correlated with DNA content.

1.4. Factors affecting nuclear division and chromosome morphology, number and structure

Internal and external factors have a great effect on the course of the nuclear division and on the chromosomes: genetic constitution of the organism, metabolic condition, water content, added chemicals, temperature (c.f. table 1.1), irradiation (ionizing and non-ionizing) etc., in general to be divided into biological, chemical and physical factors. Such factors and their effects will be considered here only as far as they can be used to improve the quality of the chromosomes for microscopical analysis, or for the induction of certain permanent alterations in respect to number and structure of the chromosomes.

1.4.1. *Alterations in the course of mitosis*

The *frequency* of stages that are expecially favourable for observing the chromosomes (for instance metaphase) may be increased by a number of measures. Some of these are: (1) Selection of growing conditions that increase the mitotic frequency. Generally this also brings the chromosomes in a condition that makes them favourable for observation. (2) Synchronization of mitosis, followed by fixation of the material at the moment the desired stage is reached. Cultures of leucocytes, that are an especially favourable material for the study of the chromosomes of higher animals, can

be brought to simultaneous division by adding a *phytohaemagglutinin*, usually prepared from certain varieties of kidney bean, Phaseolus. This substance has been in long use for blood group analysis where it intensifies the agglutination reaction. In the absence of haematocytes, it stimulates the leucocytes to simultaneous division, and consequently the cells reach metaphase almost at the same time. No such reactions are known in plants. Here growth substances like IAA, indole acetic acid, can stimulate cell division under certain conditions. Cytokinins (e.g. kinetine) have a somewhat stronger effect. Pronounced synchronization (also in animals) may sometimes be realized by carefully executed *temperature fluctuations*. More effective, under favourable conditions equalling the effect of phytohaemagglutinin on leucocytes, is 5-amino-uracil. It blocks DNA synthesis without preventing the preparatory stages. A large number of cells in a meristem can then be brought into the same stage, just preceding DNA synthesis. After the active substance has been thoroughly washed out, DNA synthesis is immediately resumed, and large numbers of cells pass through mitosis simultaneously. Rather soon, however, the cells are desynchronized again.

The size of the chromosomes at metaphase may be altered by a factor of two by the combination of the proper concentrations of nitrogen and phosphorus in the culture medium.

Quite a different category of factors are those that affect the functioning of the mitotic spindle. By blocking mitosis at metaphase, they may have a synchronizing effect, but for the study of chromosomes their major function is spreading and shortening the chromosomes. Heat shock at least several minutes at 35-40°C for plants of temperate regions, somewhat higher for most animals, interferes with the proper functioning of the spindle. The prometaphase and anaphase movements fail. After splitting of the centromeres all chromosomes may be brought together in a single nucleus: this *restitution nucleus* has twice the original chromosome number. The chromosomes remain in metaphase for a longer time, may become short and straight because of continued condensation and are spread about the cell without clear equator. This would facilitate their study if their structure did not deteriorate as a

result of the high temperature. In this respect a more extended *cold treatment* (many hours or even days a few degrees above 0°C) is more favourable for plants. The spindle function is interrupted and the chromosomes become short, straight and retain a good structure with clearly visible constrictions. Tertiary constrictions due to incomplete spiralization of specific segments may become visible in a characteristic pattern that can aid in the recognition of chromosal regions. The chromosomes do not collect in the equator but are evenly distributed throughout the cell: when the cell is squashed the chromosomes are readily spread out and flattened. The spindle is usually restored when a normal temperature follows and doubling of the chromosome number does not usually follow cold treatment.

A large group of chemicals has a similar effect, generally, however, with the exception of the induction of tertiary constrictions, while they can often cause a doubling of the chromosome number. Their application is usually simpler than cold treatment, while the effect can be more drastic and better predictable. The action of different chemicals on the spindle is rather divergent. Concentration, temperature, duration of treatment, condition and nature of the object also play a role. Different types of reaction can be distinguished (see also Eigsti and Dustin, 1955): *stathmokinesis* with complete blocking of mitosis at a specific stage and *merostathmokinesis* where mitosis is completed but in an abnormal way. Sometimes *merokinesis* is distinguished as a separate reaction type, where deviations are found only in the later stages of mitosis.

1.4.2. *Colchicine*

The frequently used chemical colchicine gives results that vary depending on temperature, duration of treatment, concentration, and condition and nature of the object. It binds to the low molecular weight protein-fraction that is responsible for the polymerization of the microtubuli of the spindle (see section 1.3.1). Consequently spindle (re)construction is partially or totally inhibited. With relatively high concentrations (e.g. 0.1-1.0%) and long duration (several hours), mitosis is blocked at metaphase and the typical colchicine metaphase (*c-metaphase*) is observed: the chromo-

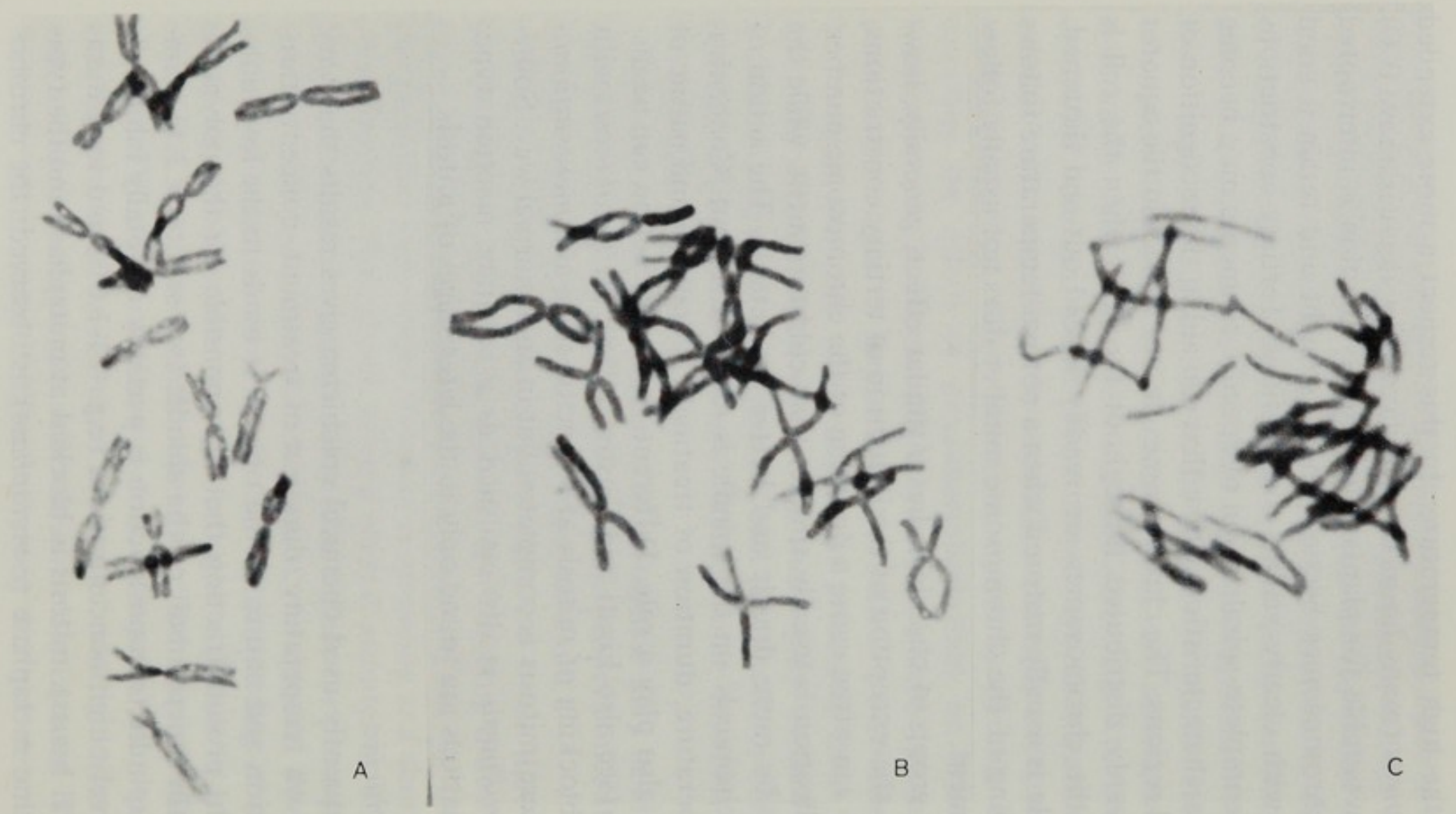


Fig. 1.10. The effect of colchicine on mitosis in the root tips of *Allium cepa* (onion). (A) At metaphase the chromosomes have not congressed at the equator; they are straight and relatively contracted, showing several of the details that are used for their recognition: length, location of centromere, etc. (B) At late colchicine-metaphase the chromatids separate except at the centromeric regions (the centromere itself may often be seen to be split at this stage). (C) Without regular anaphase the chromatids separate and the double number may be included into one restitution nucleus.

somes are relatively short, straight, clearly split into two chromatids, which are connected on both sides of the centromere, and they are spread over the cell (figs.1.10, 1.21). Prophase is not suppressed and an accumulation of metaphase stages results.

Variants of c-metaphase are: *ball-metaphase* (chromosomes without special orientation clumped together in the centre of the cell); *star-metaphase* (centromeres in one focus in the centre of the cell with the chromosome arms extending into the cytoplasm); *exploded metaphase* (chromosomes at the perifery of the cells, single or in small groups) and a special form of this last type: *reductional metaphase* (two groups with approximately the same number of chromosomes, each on one side of the cell). Colchicine is the most effective agent for the doubling of chromosome numbers, especially in plants (fig.1.10 C). Often, but not always, lower concentrations (e.g. 0.05%) and longer duration (e.g. 24 hr) at room temperature are better for chromosome doubling than high concentrations and short duration. Colchicine is generally used in chromosome studies of human and animal tissues, especially leucocyte cultures. Here the derivative colcemid is sometimes considered more effective. In combination with the phytohaemagglutinin method very large numbers of metaphase cells with short well-spread chromosomes can be obtained (fig.1.21).

For chromosome studies in plants other chemicals with comparable action are also in use: α-bromonaphtalene, paradichlorobenzene, 8-oxyquinoline and derivatives, while some insecticides, such as Lindane also have strong effects. Their usefulness is their low price, sometimes lower toxicity and occasionally better result. Oxyquinoline especially reveals many details of the chromosomes, but it fails to accumulate metaphase stages. 5-Azacytidine induces tertiary constrictions (segment extension).

1.4.3. *Polyploidy and polysomy*

Colchicine can induce a doubling of the chromosome number in plant cells. For animals colchicine in effective concentrations is too toxic for survival. Other chemicals, such as laughing-gas (N_2O) are promising but have yielded few results. Temperature-shock, pricking of fertilized or unfertilized eggs and other physical agents

have yielded occasional positive results: it is no problem to obtain for instance salamanders with doubled chromosome numbers. Warm-blooded animals are more resistant. Occasionally doubling occurs spontaneously as a result of the failure of normal division. Indirect induction by breeding experiments with spontaneous deviants have been successful in axolotl and silkworm, the latter in combination with (transient) genetical parthenogenesis (Astaurov, 1969). See also chapter 5.

When the chromosome number of a large number of cells has been doubled in an otherwise diploid individual, an entire sector with doubled number can maintain itself. The organism is a *mosaic*, from which progeny may be isolated that have the doubled number homogeneously in all their cells. In plants it is often possible to raise an entire organism with the doubled chromosome number from a mosaic, by means of vegetative reproduction, without the necessity of an extra generative generation. In plants, the term *chimaera* is frequently used as an equivalent of mosaic. In animals chimaera is generally restricted to cases of fusion of different individuals, usually at a very early stage of development.

1.4.4. *Terminology of chromosome-number variants*

A single, complete set of chromosomes is a *genome*. When there is only one genome per cell, the organism is *haploid*. The number of chromosomes of a genome, the haploid number, is generally designated by n. When there are two genomes, the organism is *diploid* ($2n$). For reasons that originally related to recombination of the genetic material, but that also have to do with gene action, the normal number of genomes in the body of higher organisms is two: they are diploid. When the degree of ploidy is higher than two, one speaks of *polyploidy*. With three genomes per cell the organism is *triploid* ($3n$), with four *tetraploid* ($4n$); and so on with pentaploid ($5n$), hexaploid ($6n$), heptaploid ($7n$), octoploid ($8n$). With higher multiples the Greek numeral indications are often replaced by the corresponding Arabic figures: 9-ploid ($9n$), 12-ploid ($12n$) etc. The number of genomes is the *degree of (poly)ploidy*. When all genomes are of the same kind, there is *autopolyploidy*; when they are identical: *pan-autoplyploidy*. This is a scarce pheno-

menon since except in a few cases (self-fertilizers) the original two genomes of the diploid will tend to be genetically different. Chromosome doubling of a heterozygous diploid leads to *hemi-autopolyploidy*. The differences between the genomes, of course, can vary from slight to large. In *allopolyploids* the genomes are quite different, having been derived from different taxonomic species or even different genera. Such different but corresponding genomes from related species are said to be *homoeologous* in contrast to like genomes that are *homologous* genomes. Usually the chromosome number of allopolyploids is designated as $2n$ like in a diploid, the *di-haploid* number being n. The *basic* number of the separate constituting genomes is then represented by x. The chromosome number of an allohexaploid can be represented by $2n = 6x$. Polyploidy, especially allopolypoidy, has played an important role in the evolution of plants and is important also for plant breeding. In animals it is much less common, only a limited number of natural autopolyploids being known. Examples of undisputed allopolyploids are scarce. Probably, polyploidy has played an important role in the early stages of mammalian evolution, but the genomes have differentiated to such an extent that no recognizable similarities have remained and practically all species can be considered functionally to be diploids.

The lack or excess of individual chromosomes is called *aneuploidy*; *hyperploidy* when there is an excess, *hypoploidy* when one or more are lacking. When more than two chromosomes of one specific type are present there is *polysomy* for this chromosome. A diploid that has a single extra chromosome has this chromosome three times and is therefore called *trisomic*. If it occurs four times, it is *tetrasomic*. When two different chromosomes occur three times, there is double trisomy. Although the number of chromosomes in double trisomy is the same as in tetrasomy, the situation is clearly different. With a certain chromosome occurring only once in stead of twice, the organism is *monosomic*; if a chromosome is totally absent, the organism is *nullisomic*.

The phenotypic expression of variations in chromosome number will be considered in chapter 5, where the complications of polyploidy and polysomy in generative reproduction will receive special attention.

1.4.5. *Chromosome-structural aberrations*

A number of chemical and physical factors can induce mutations through alterations in the DNA. Sometimes the effect is drastic enough to induce lesions in the structure of the chromosomes. Such lesions are usually readily repaired but when two or more occur close enough together to interact, combined repair may result in deviations from the original situation, which have important consequences for the genetic system. The most efficient agents in the induction of permanent chromosomal aberrations are the ionizing radiations. Although the course of events with breakage and reunion in chromosomes is not well understood, something is known about the way the primary action takes place, and also of the differences in action and effect of different types of ionizing radiation.

1.4.6. *Ionizing radiations*

Broadly speaking, two types may be distinguished:

(1) Non-corpuscular radiation (electromagnetic radiation) with mass 0 and velocity equal to that of light. It is emitted in units: *photons*. The wavelength determines the energy of the photon. Above a certain energy photons are capable of freeing electrons from their molecules, which thereby loose a unit of negative charge and become positive ions (ionization). The electron may attach to another molecule and give it a negative charge. Examples: X-rays and gamma rays.

(2) Corpuscular radiation with mass larger than zero and velocity lower than that of light. Velocity and mass determine the energy. There are charged particles (examples: alpha rays, beta rays, accelerated protons and other accelerated ions) and uncharged particles (examples: neutrons, mesons). The charged particles free electrons from atoms by means of their charge and can cause nuclear reactions in exceptional cases. Uncharged particles act indirectly, often through a nuclear reaction that liberates direct acting particles and electromagnetic radiation.

Electromagnetic radiations. There are three possibilities for producing ionizations: (1) Photo-electric effect: one electron per photon is ejected from the atom or molecule. The electron gets all

the energy of the photon minus its binding energy. (2) Compton-effect: an electron is ejected from an atom or molecule, with only part of the energy, the remaining energy goes into a new photon (with larger wavelength). (3) At energy levels above 1.02 MeV and interaction with heavy atoms, there is electron-positron formation.

Artificial radiations (X-rays) have a continuous energy spectrum; natural radiations (gamma) have one or a few spectral lines.

Large numbers of *secondary ionizations* are caused by the energy-rich primary electrons. The path of the primary (and of possibly secondary) electrons is short: the negative charge and relatively large mass give them a great probability of interaction with matter. Each ionization uses 33.5-35.0 eV.: energy-rich photons and their primary and secondary electrons yield thousands of ionizations. With decreasing energy (=velocity) the electron remains longer in the vicinity of other charged particles and the probability of interaction increases: at the end of the track the ionizations occur in clusters. The influence of atomic nuclei bends the tracks: there is scattering. The slower the electron, the more scattering.

Corpuscular radiation. Beta rays are fast electrons, originating from spontaneous or induced nuclear desintegration, and artificially in accelerators. Natural beta rays have a continuous energy spectrum, artificial beta rays a single spectral energy line: this situation is opposite to that with electromagnetic radiations. In air, beta rays penetrate several meters, in a fluid or solid a few millimeters. Upon entering an object, close to the surface the ionization is relatively dispersed, but at a specific depth a peak in ionization density occurs close to the ends of the tracks (Bragg-peak). The peak is not very discrete: electrons with a continuous energy spectrum differ in energy which causes the track ends to occur at different depths, and the tracks are not straight because of scattering.

Alpha rays are fast moving Helium ions, charge +2, mass 4, mono-energetic or with a limited number of spectral (energy) lines. They originate from spontaneous or induced nuclear disintegration. With low speed, electrons are absorbed, and consequently the ionizing capacity decreases. Because of the large mass there is relatively little scattering. The Bragg-peak is more discrete than with electrons. In air the penetration is a few centimeters, in fluids

and solids fractions of millimeters. Because of large mass and charge the energy is rapidly dispersed: the *ionization density* is high. The main track has clear side-tracks of secondary electrons of appreciable energy: *delta rays.*

Fast protons originate from nuclear desintegration, from interaction between fast neutrons and H atoms or from accelerators; charge +1, mass 1. In properties they resemble alpha rays but they have a lower ion density and better penetration.

No charge; *mass*: primarily, neutrons and mesons.
They originate from nuclear disintegration on a large scale in nuclear reactors and nuclear detonation, and from interaction of accelerated heavy particles with special atoms. When originating from nuclear reactions they are often contaminated with gamma rays.

Fast neutrons interact (by collision) in organic material mainly with H atoms that are converted into fast protons. The relatively low probability of interaction results in a high penetration and in the origin of ionization tracks (by protons) throughout the objects, especially in substances with low molecular weight.

Slow neutrons (= thermal neutrons with a velocity in the range of that of the temperature movements of molecules: n_T or N_T) remain sufficiently long in the vicinity of atomic nuclei to be absorbed. After absorbtion, the nucleus is labile: after a shorter or longer period of time disintegration occurs. Light nuclei then yield mostly alpha-, beta- and gamma radiation and fast protons; heavy nuclei break up into large fission products. In both cases the ion density is high. The probability of interaction is expressed as the cross section of the nucleus in "barn" units. It is not simply proportional to atomic weight. The most frequently occurring isotope of Borium, for instance, a relatively small atom, has a large cross section for N_T. The probability of interaction in organic material is relatively small: the penetration is high and the track origins are dispersed.

Dosimetry. The number of ionizations in a specific volume of gas can be measured from the reduction of a static potential between two electrodes in an ionization chamber. The number of ionizing particles (photons, e^-, He^{++}, etc.) can be counted directly

in a Geiger–Müller counter: the produced ion-pairs are accelerated in a strong electric field and the cataract of secondary ionizations is recorded as a weak electric pulse. Simultaneous ionizations are counted together. Further methods: quantitative determination of chemical reactions in special compounds; blackening of photographic plates. The flux of thermal neutrons is often determined from the radioactivity induced in gold foil.

Units. The oldest unit, the *röntgen* (r), equals the amount of X- or gamma radiation that causes 1.61×10^{12} ion pairs (equivalent to 87 ergs) to be formed in one gram of dry air. For other radiations the *rep* (röntgen-equivalent-physical) is the amount of radiation that releases in one gram of matter (air, water, tissue) an amount of energy equivalent to one r (for dry air this is 87 ergs). The *rem* (röntgen-equivalent-man) induces an equal amount of biological damage in man as does 1 r. To find the dose in rem the *relative biological effectiveness* (RBE) of the radiation in comparison to X-rays must be known. The unit most generally used presently, is the *rad*: the amount of radiation that releases 100 ergs per gram in the material irradiated. In most biological material 1 rad is close to 1 r. For further details see Bacq and Alexander (1961).

LET. The amount of energy released per unit of track length (linear energy transfer, LET) is of great importance for the effectiveness of the radiation. Alpha rays have a higher LET than beta rays.

Primary effect. Ionizations in essential structures can by themselves have a drastic effect. In addition, they give rise to radicals. A water molecule, for instance, upon ionization yields OH radicals ($OH^- \rightarrow OH + e^-$), that are very reactive and attack biologically important structures either directly or after formation of hydrogen peroxide ($OH + OH \rightarrow H_2O_2$). For this and other reasons the water content of the irradiated object is important for the radiation effect. Organic peroxides originate in a similar way. Presence of oxygen in the object increases the primary radiation effect, among other reasons by affecting peroxide formation. With atmospheric pressure the plateau of the oxygen effect is reached at 20% O_2 in the gaseous environment (fig.1.11A). An increase in the radiation effect by a factor of 2-3 is common, sometimes this factor

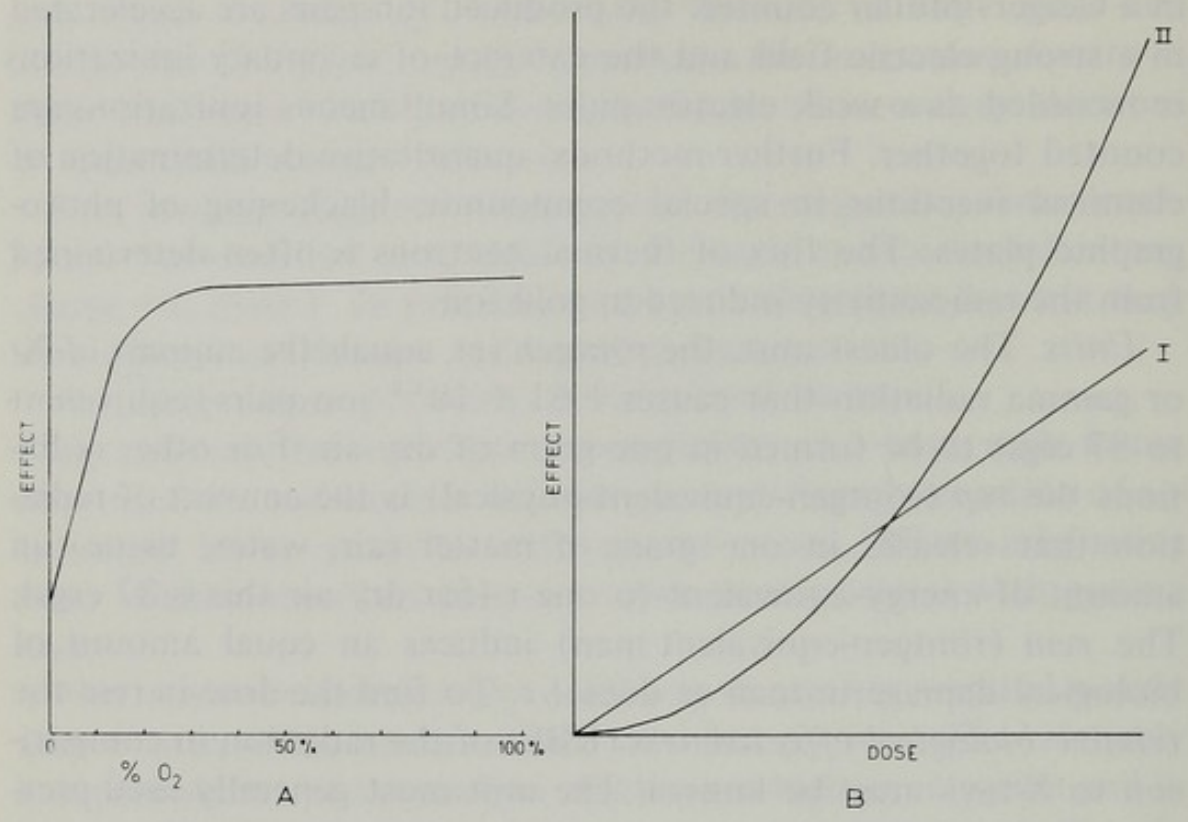


Fig.1.11. (A) The approximate relation between atmospheric oxygen content and the effect of ionizing radiation. (B) Idealised dose-effect curves for acute ionizing radiations. I linear. II exponential with exponent 2.

can be even higher. On the other hand, oxygen is required for the repair processes during and after irradiation: here absence of oxygen has an enhancing effect on the radiation damage, as has the presence of substances that interfere with respiration (see e.g. Sobels, 1968). Some chemicals have a *protective* effect: they often have reducing properties. Some, cysteine for instance, have sulphydrylgroups (-SH) that preferentially absorb radicals: they are *radical scavengers*. Till now the most effective of these protective substances seems to be 1,4-dithiotreitol. Such substances occur in nature and can be artificially introduced into the system. Side-effects can make them harmful in concentrations that are effective for protection against high doses of radiation. There are many chemical and physical modifying factors that affect the final radiation effect when applied before, during or after irradiation.

Other differences in sensitivity are inherent to the object. Important for instance is the DNA content of the nucleus and con-

sequently nuclear size: the more DNA, the more sensitive, as has been demonstrated by Sparrow and others. Polyploidy, on the other hand, although increasing the amount of DNA per nucleus, has a buffering effect: aberrations are tolerated better because of compensation as the chance is great that at least one copy of each gene remains unaffected. Per chromosome, however, the frequency of aberrations is generally the same as in the corresponding diploid. Different stages of mitosis have different sensitivities. For instance G_2 is more sensitive than G_1, one reason being that there are twice as many chromatids that can be affected and interact in G_2 as there are chromosomes in G_1. The biochemical conditions also are different.

With high LET the ionizations occur so close together that the local concentration of protective substances and the first repair processes are insufficient. In addition, there is more opportunity for interaction. Furthermore damage is drastic and consequently less readily repaired. Therefore, radiations with high LET often have a high RBE. The oxygen effect is for similar reasons usually reduced or even absent. Since for chromosomal rearrangements rather many ionizations are required, the densely ionizing radiations are usually the most efficient radiations for the induction of such aberrations.

1.4.7. *Radiomimetic substances*

Chemical substances of several kinds, among which many alkylating agents, have an effect that in many respects resembles that of ionizing radiations: radiomimetic substances. The first were discovered in 1943 by Auerbach and by Oehlkers: mustard gas and derivatives. Some of the radiomimetic substances break chromosomes randomly as do ionizing radiations. Others have a more localized action: 8-ethoxy coffeine for instance, breaks chromosomes preferentially at the centromeres and secondary constrictions. Maleic hydrazide-induced aberrations also occur nonrandomly, usually around the nucleolus, probably at least partly due to accumulation in the nucleolus. Part of the localization effect may not be due to higher sensitivity per se but to differences in repair or to differences in opportunity for interaction. The de-

tails of the action of radiomimetic substances are largely unknown. Some are strongly mutagenic and carcinogenic, others much less so. See further Rieger and Michaelis (1967).

Numerous viruses, among which measles virus, induce chromosomal aberrations as well as many other disturbances of the mitotic chromosomes and their behaviour.

1.4.8. *Primary lesion. Types of aberrations*

The first effect of the action of an ionizing irradiation or of a radiomimetic substance is the primary lesion, the nature of which is not entirely clear. According to an elaboration by Evans (1966) of a hypothesis of Revell, a lesion does not directly lead to a break in a chromosome. Lesions remain unstable and reactive for some time (after irradiation for instance, approximately 1 hr) and are then stabilized. After another 6 hr they are repaired during which process damaged DNA is replaced, for which DNA and protein synthesis are required. This may occur at S-phase, but also during other stages of interphase. At the beginning of this repair process there is a period of about one hour of renewed instability. In both periods of instability different lesions can become associated, provided they occur within a certain physical distance (*rejoining distance*). They can then be repaired together. The result is one of two situations: (1) The original situation is restored (fig.1.12B);

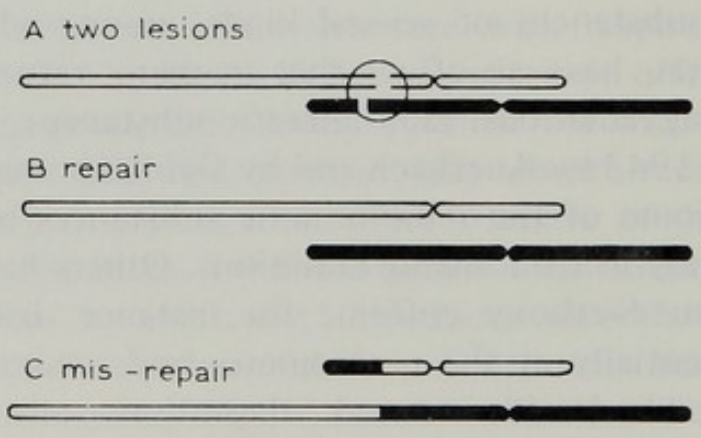


Fig.1.12. The interaction between two lesions (diagrammatic), one in each of two chromosomes. (A) Both lesions still open but close enough to interact. (B) Repair has led to restitution of the old structure, although DNA may have been altered. (C) Mis-repair has resulted in exchange.

(2) As a consequence of "mis-repair" (fig.1.12C) chromosome parts are exchanged. According to the older theory of Sax, open breaks would be formed first, that subsequently could undergo illegitimate associations resulting in exchange (breakage first hypothesis). Permanent open breaks are found in relatively low frequencies after ionizing irradiation and probably result in some way from incorrectly repaired lesions. They cause terminal deficiencies (figs.1.13A, 1.14A). The fate of chemically induced lesions is probably somewhat different. There, realization of exchange is frequently possible only during the proper S-phase, when the units involved in exchange are chromatids and not whole chromosomes. With ionizing radiations chromosomes are the unit of interaction at G_1, chromatids during S and G_2.

Irradiation in G_2 results in *Vicia faba* and a number of other organisms in a large number of uncondensed, non-stainable, apparent interruptions (*gaps*) in the chromatids at metaphase and anaphase immediately following irradiation. They were formerly considered to be true breaks, but probably are temporarily stabilized lesions of some kind that are repaired in the following S-phase. Irradiation in G_1 does not result in gaps; if they are induced, they are repaired in the S-phase immediately following G_1. Since the size of the gaps is variable and their actual significance unknown, they are unfavorable for quantitatively assaying radiation effects. In Tradescantia microspores, often used in radiobiological experiments, gaps are not observed.

Depending on the stage at which lesions are induced and repaired, different types of exchange configurations will arise. After ionizing radiation a chromosome acts as a unit in G_1: *chromosome-aberrations* are formed (fig.1.13). According to Evans and to Wolff, just before (10-15 min) DNA synthesis the chromosome changes into a double structure in respect to exchange, and it remains so during the rest of S and G_2. The two chromatids behave independently and *chromatid-aberrations* (fig.1.14) are formed.

A chromosome-lesion induced in G_1 and not associated with another lesion, can undergo mis-repair in S: of the (now double) chromosome the two sister chromatids can be exchanged (*sister-reunion*). Two orientations are possible here: (1) The exchange is

Interphase(G1)
Prophase
Metaphase
Anaphase
One lesion
No attachment
Sister chromatid reunion
Terminal deficiency
Dicentr. chrom. + acentr. fragm.
A
B
Two lesions
Intra-chromosome
Intra-arm
Symmetry
undecided
Interstitial deficiency
Paracentric inversion
Pericentric inversion
Ring chrom. + acentr. fragm.
C
D
E
F
Inter-chromosome
Asymmetric
Symmetric
Reciprocal translocation
Dicentr. chrom. + acentr. fragm.
G
H

symmetric: the orientation in respect to the centromere remains the same, and a visible aberration is not induced. (2) The exchange is *asymmetric*: the orientation is reversed in respect to the centromere. The two chromatid fragments with the centromeres unite and form a dicentric chromatid. The fragments without centromeres unite also and form an acentric fragment. One centromere of the dicentric chromatid always goes to one pole at anaphase and the other to the other pole: a bridge is formed, accompanied by an unoriented fragment. With all chromosomal aberrations where exchange has taken place, the distinction between symmetric and asymmetric reunion (fig.1.15) is of great importance. The terms symmetric and asymmetric should not be taken literally in this context: they merely indicate orientation in respect to the centromeres: With symmetric exchange the orientation of the chromosome segment involved is unaltered. With asymmetric exchange the orientation is reversed. This distinction is not always easily made (see figs.1.13 and 1.14). Although in some cases there is correspondence with symmetry and asymmetry in shape, this is not always so. The terms symmetric and asymmetric are not consistenly used to indicate the orientation of the rearranged chromosome segments in respect to the centromere. Often symmetric merely indicates "end result mechanically functional", asymmetric: "end result mechanically non-functional" (Rieger and Michaelis, 1967). Often the meaning coincides with that of the first definition, but when consulting the literature it is good to decide first in which sense the terms are used. It is sometimes assumed that symmetric and asymmetric reunions are equal in frequency but there apparently are exceptions.

The chromosomal segments involved in exchange may be part of the same chromosome (leading to intra-chromosome aberrations) or of different chromosomes (leading to inter-chromosome aberrations).

Fig.1.13. The origin of aberrations involving entire chromosomes, from the interaction of lesions in G_1 before the split of the chromosomes into chromatids is effected (compare fig.1.14). The dicentric anaphase chromatids of H, of which both centromeres go to the same pole (lower figure) can interlock, forming a bridge.

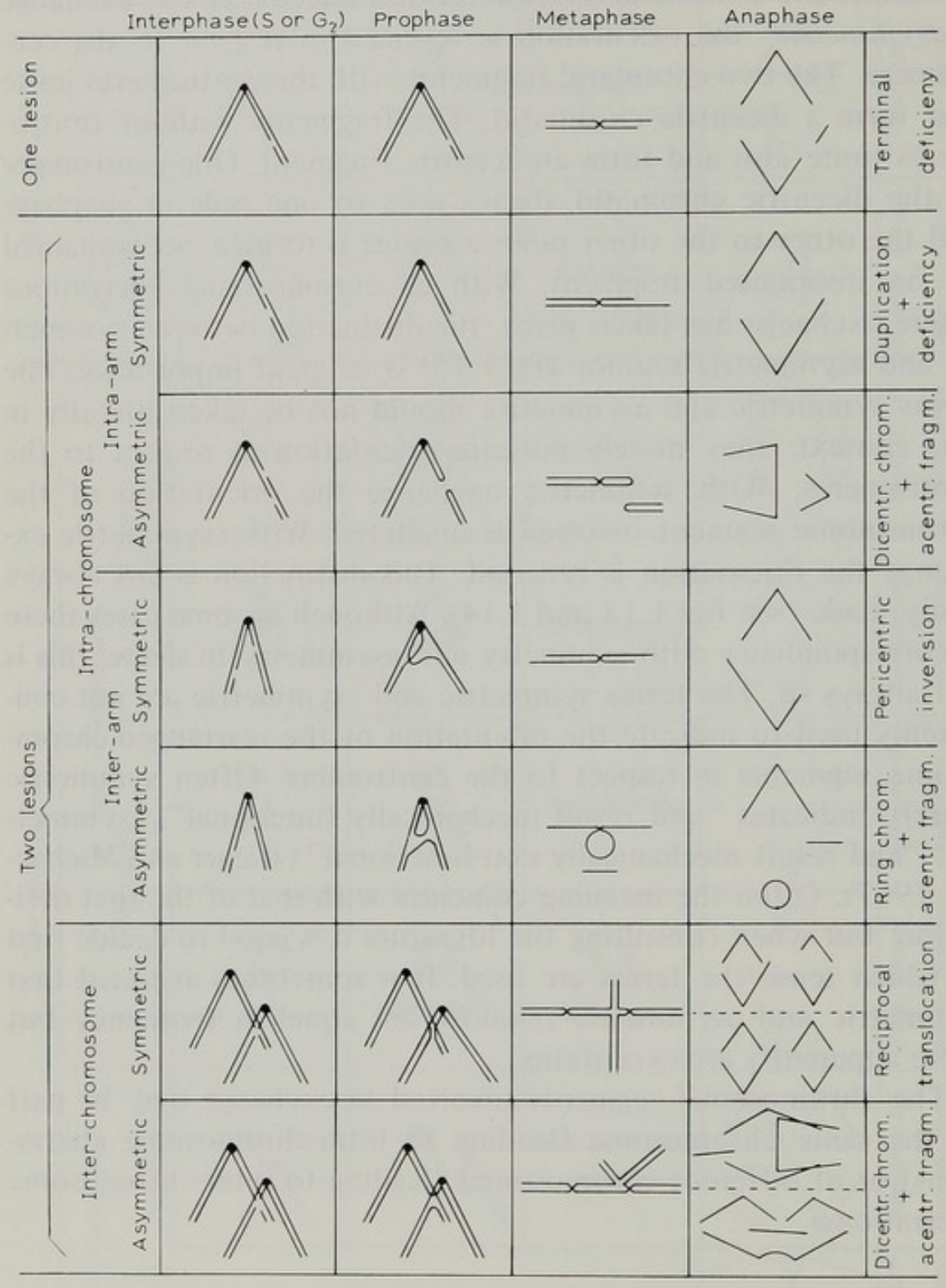


Fig.1.14. A selection of the most important chromatid aberrations, arising from interaction of lesions after split of the chromosomes into chromatids (G_2). The number of possible types is much larger than with chromosome aberrations (compare fig. 1.13).

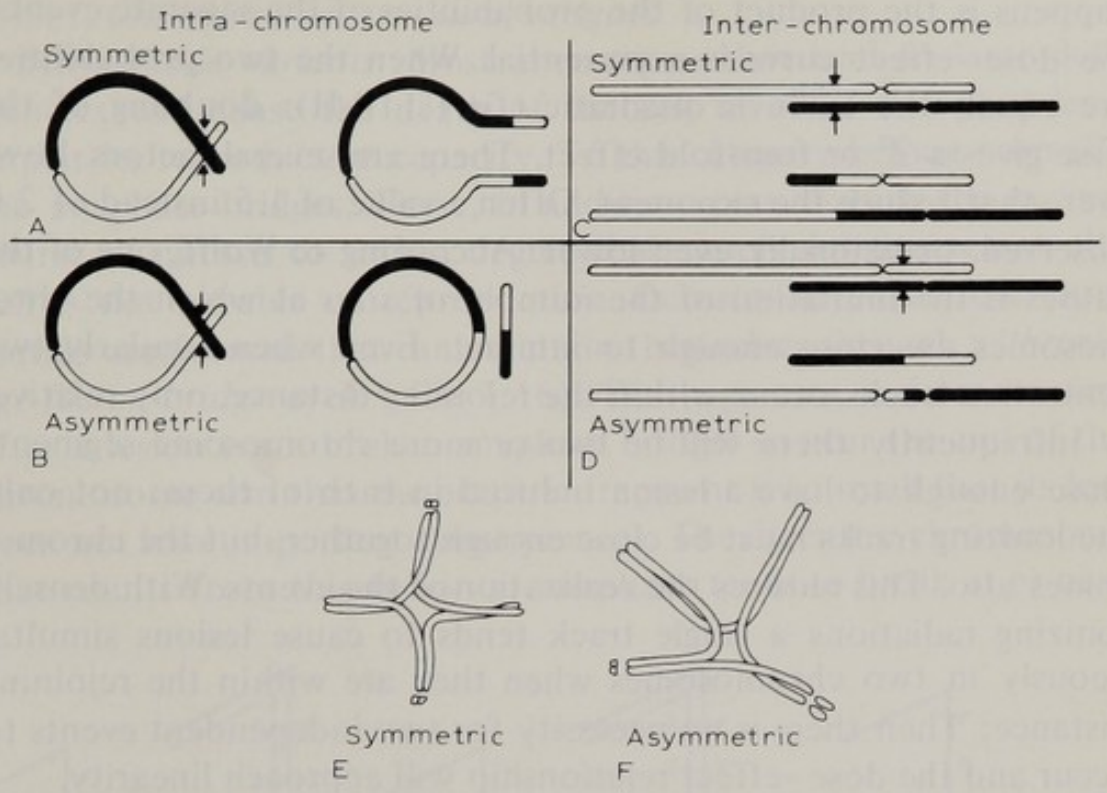


Fig.1.15. Examples of symmetric (A, C, E) and asymmetric (B, D, F) exchanges. In A, symmetric intra-chromosome (inter-arm) exchange leads to a pericentric inversion, a similar asymmetric exchange (B) leads to a ring chromosome with the centromere, and an acentric fragment. The symmetric interchromosome exchange of C leads to a reciprocal translocation (interchange), the asymmetric interchromosome exchange of D results in a dicentric chromosome and an acentric fragment. These four diagrammatic examples were chromosome aberrations, E and F are chromatid aberrations, where only one of the two chromatids is involved. Both are interchromosome exchanges. In E the exchange is symmetric, in F it is asymmetric. These are drawings of colchicine metaphase chromosomes of *Vicia faba* with subterminal centromeres (compare Rieger and Michaelis, 1967).

Aneuploidy may occasionally result from a damaging effect of ionizing radiations on the spindle.

1.4.9. *Dose–effect curves*

The frequency of chromosome- and chromatid aberrations resulting from a single lesion caused by a single ionization track, will increase linearly with radiation dose (fig.1.11B-I). This is the case, for instance, with chromatid bridges resulting from sister reunion in single chromosomes. It is not the case with aberrations based on more than one lesion. Here, two (or more) independent events must take place within a certain volume. The probability that this

happens is the product of the probabilities of the separate events: the dose–effect curve is exponential. When the two probabilities are equal, the curve is quadratic (fig.1.11B-II): doubling of the dose gives a 2^2 or four-fold effect. There are several factors, however, that reduce the exponent. Often a value of 1.5 instead of 2 is observed, occasionally even lower. According to Wolff, one of the causes is the limitation of the number of *sites* at which the chromosomes are close enough to interact. Even when regularly two ionization tracks occur within the rejoining distance, only relatively infrequently there will be two or more chromosome segments close enough to have a lesion induced in each of them: not only the ionizing tracks must be close enough together, but the chromosomes also. This reduces the realization of the events. With densely ionizing radiations a single track tends to cause lesions simultaneously in two chromosomes when they are within the rejoining distance: Then there is no necessity for two independent events to occur and the dose–effect relationship will approach linearity.

1.4.10. *Duration of treatment. Dose fractionation*

The period during which lesions are unstable enough to interact, is limited (about 1 hr). When an irradiation lasts longer than this, lesions induced during the first period of irradiation will have been stabilized before those of the last period are ready for interaction. A long drawn out irradiation, therefore, has less effect than one in which the same dose is applied in a shorter time, as far as effects are involved that result from interaction of different, independent lesions. When an irradiation is given in two or more fractions, the normal exponential dose–effect curve is found only when all fractions fall within the unstable period. Less effect (only about the sum of the composing fractions) is produced when the interval between the fractions is larger than the unstable period. When, however, part of the irradiation falls in the first and part in the second unstable period, the pre-repair period, then interaction is again possible and a larger-than-additive (exponential) effect is found.

1.4.11. *Chromosome-structural variants*

Semi-stabile variants. Most aberrations that lead to large deficiencies will cause the death of the cell involved or will at least impair its reproductive capacity. To this category belong normally the anaphase bridges. These, in addition, may disturb the normal course of mitosis to such a degree that simply for this reason the cell is not capable of reproduction. Occasionally, dicentric chromatids are formed that can maintain themselves through a "*breakage-fusion-bridge* cycle" (fig.1.16). The beginning is sister reunion after a lesion (or a broken anaphase bridge) so near the end of the chromosome arm that the acentric fragment is so small that its loss does not have important consequences. The dicentric chromosome that results from this fusion at anaphase tends to break at or near

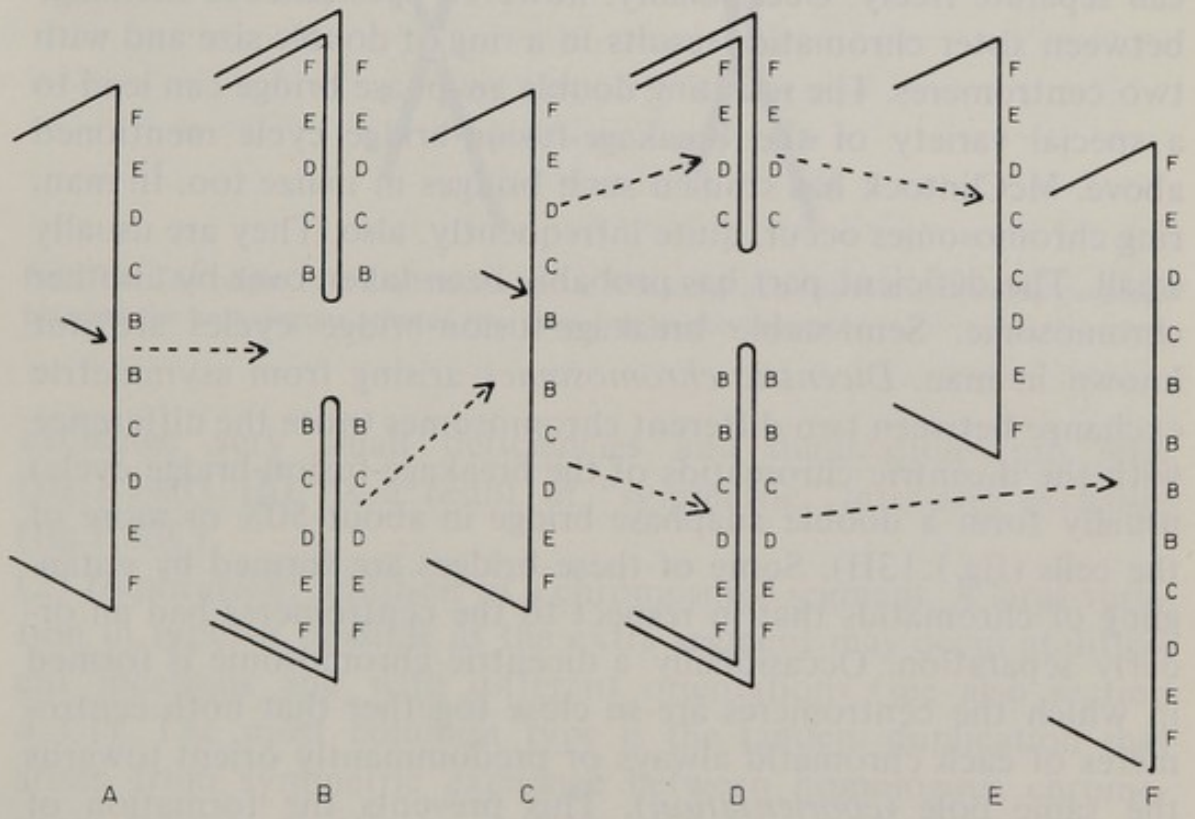


Fig.1.16. Breakage-fusion-bridge cycle of a dicentric originated from sister-chromatid reunion. The sister centromeres always go to opposite poles and the connected chromatids form a bridge at anaphase. A small fragment with locus A is lost. At anaphase the bridge breaks and the open ends after replication again by sister reunion lead to dicentrics. As long as the breaks occur between the two B loci, genetic consequences are not expected. Breakage between B and C, however, results in deficiency for B in one cell and its progeny and duplication in the other cell.

the old point of reunion, before nuclear division can be endangered by the bridge. In both daughter nuclei sister reunion occurs again and the cycle proceeds: break-fusion-bridge-break, etc. When the bridge breaks at another point than the location of the fusion, a duplication (extra segment of chromosome) results in one of the daughter chromosomes and a deficiency (a lacking segment) in the other. Then it may happen that with this deficiency a gene with clearly visible effect is lost, so that a mutant sector may grow out of the cell involved, provided the deficiency is not too large. Such a mutated sector may be recognized as a deviating area in the tissue. McClintock has described such cycles in maize endosperm. In other parts of the maize plant they are usually not observed. *Ring chromosomes* can behave normally as long as the two chromatids can separate freely. Occasionally, however, spontaneous exchange between sister chromatids results in a ring of double size and with two centromeres. The resulting double anaphase bridge can lead to a special variety of the breakage-fusion-bridge cycle mentioned above. McClintock has studied such bridges in maize too. In man, ring chromosomes occur, quite infrequently, also. They are usually small. The deficient part has probably been taken over by another chromosome. Semi-stable breakage-fusion-bridge cycles are not known in man. *Dicentric chromosomes* arising from asymmetric exchange between two different chromosomes (note the difference with the dicentric chromatids of the breakage-fusion-bridge cycle) usually form a double anaphase bridge in about 50% or more of the cells (fig.1.13H). Some of these bridges are formed by entangling of chromatids that in respect to the centromeres had an orderly separation. Occasionally a dicentric chromosome is formed in which the centromeres are so close together that both centromeres of each chromatid always or predominantly orient towards the same pole (*coorientation*). This prevents the formation of bridges and enables predominantly normal transfer of the dicentric chromosome. The proportion of anaphases with coorienting centromeres is inversely proportional to the distance between the centromeres.

Stabile variants. The most frequent types of stabile chromosome-structural variants have normal functioning chromosomes:

(1) *Deficiency* (deletion): loss of a chromosome segment, either interstitially or terminally. Large deficiencies generally lead to reduced vitality or even lethality, smaller ones are more easily tolerated. Terminal deficiencies result from "simple breaks" that become permanent. Interstitial or intercalary deficiencies arise in two ways: (a) Symmetric exchange between homologous chromosome arms from lesions at different places. One chromosome has a deficiency, the other a duplication (fig.1.17); with chromatid

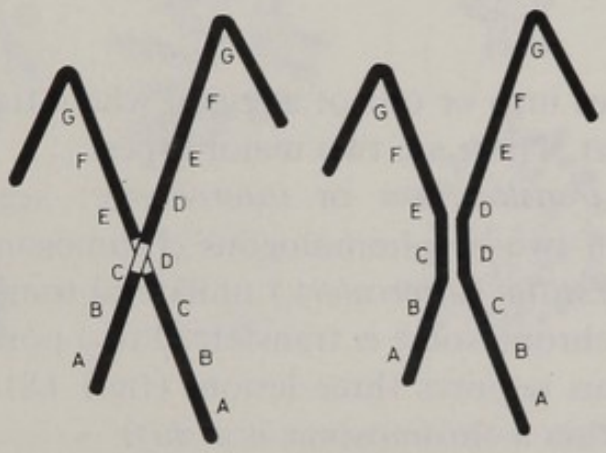


Fig.1.17. The origin of a duplication and a deficiency as a result of symmetric exchange between the homologous arms of two homologous chromosomes.

exchange very small deficiencies and duplications can arise (fig.1.14B). (b) As a result of "symmetric" inter-arm exchange (fig.1.13C).

(2) *Duplication*: addition of a chromosome segment. A large variation in types is possible as the extra segment may occur at different locations and with different orientations (see also section 4.3.1). The most common type is the tandem duplication that arises from symmetric exchange between homologous chromosome arms, as mentioned with the deficiencies (fig.1.17). The effect of duplications is less severe than that of deficiencies but often phenotypically observable.

(3) *Translocation*: transfer of a chromosome segment from one chromosome to another. There is no phenotypic effect unless the lesions have altered active genetic material, or the translocation

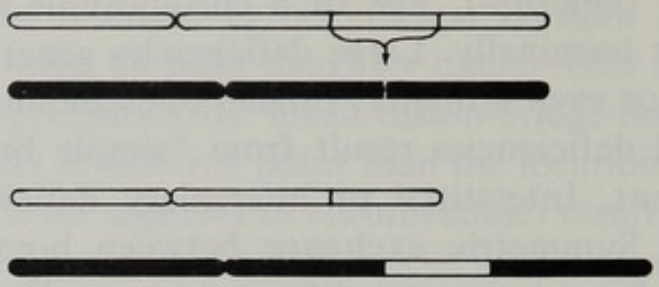


Fig.1.18. The origin of a simple interstitial translocation by the interaction of three lesions. The segment between the two lesions in one chromosome is inserted into the opening resulting from the single lesion in the other chromosome.

has brought genes into or out of a region where transcription cannot be carried out. There are two major types:
(a) *Reciprocal translocation* or *interchange*: segments are exchanged between two non-homologous chromosomes (figs.1.13G, 1.14F). (b) *Interstitial* (*intercalary*) unilateral translocation: a segment from one chromosome is transferred to a position in another chromosome. This requires three lesions (fig.1.18). An intercalary translocation within a chromosome is a *shift*.
(4) *Inversion*: a chromosome segment is inverted. A *pericentric* inversion contains the centromere; with a *paracentric* inversion the inverted segment is part of one arm. Pericentric inversions arise as a result of symmetric exchange between the two arms of the same chromosome (figs.1.13E, 1.14D). When in the case of chromatid exchange (fig.1.14D) two different chromatids are involved, of course a deficiency and a duplication result in both chromatids. A paracentric inversion results from asymmetric exchange within one chromosome arm (fig.1.13D).

Deficiencies, duplications, translocations and inversions will be treated in more detail in chapter 4.

1.5. The karyotype

1.5.1. *Karyotype. Idiogram. Karyogram*

Alterations in number (polyploidy, polysomy) and structure of the chromosomes occur in nature with a low frequency, but regu-

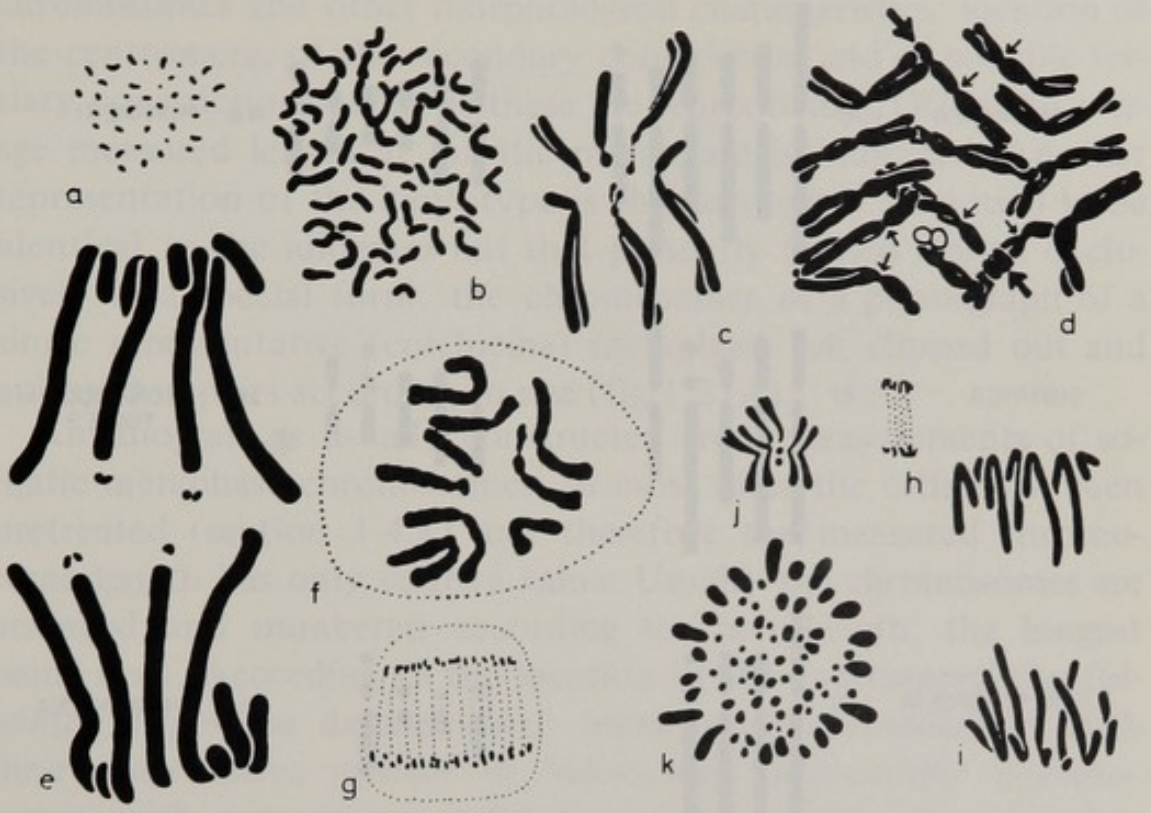


Fig.1.19. The karyotypes of eleven different plant and animal species. The magnification is the same, approximately 900 ×. (a-g) Angiosperms; (a) *Spirodella* (*Lemna*) *polyrhizza*; (b) *Mirabilis longiflora*; (c) *Crepis capillaris*; (d) *Secale cereale*; (e) *Aloe strigata*; (f) *Allium triquetrum*; (g) *Sparmannia africana*; all are root tip mitoses except (e) and (f) which are pollen mitoses. (h-i) Green algae; (h) *Sphaeroplea annulina*; (i) *Pithophora kewensis*; (j) *Drosophila melanogaster* (insect); (k) *Oceanodroma* (bird). According to Prakken, 1964.

larly. The same alterations are found as under experimental conditions. They are understood to be the basis of the great variation in chromosome form and number between species and genera. The expectation that spontaneous chromosome alterations should lead to a similar variation within species is not realized: actually a great stability is observed within species. A type that has proven to be functional is apparently maintained almost indefinitely. Stebbins and coworkers have shown that the chromosomes of the plant species *Plantago insularis* from America and *Plantago ovata* from Europe have a practically identical set of chromosomes. Yet the two species must have been separated from each other or from a possible common ancestor for over 20 million years. Although such an extreme stability does not necessarily have to be universal, it is cer-

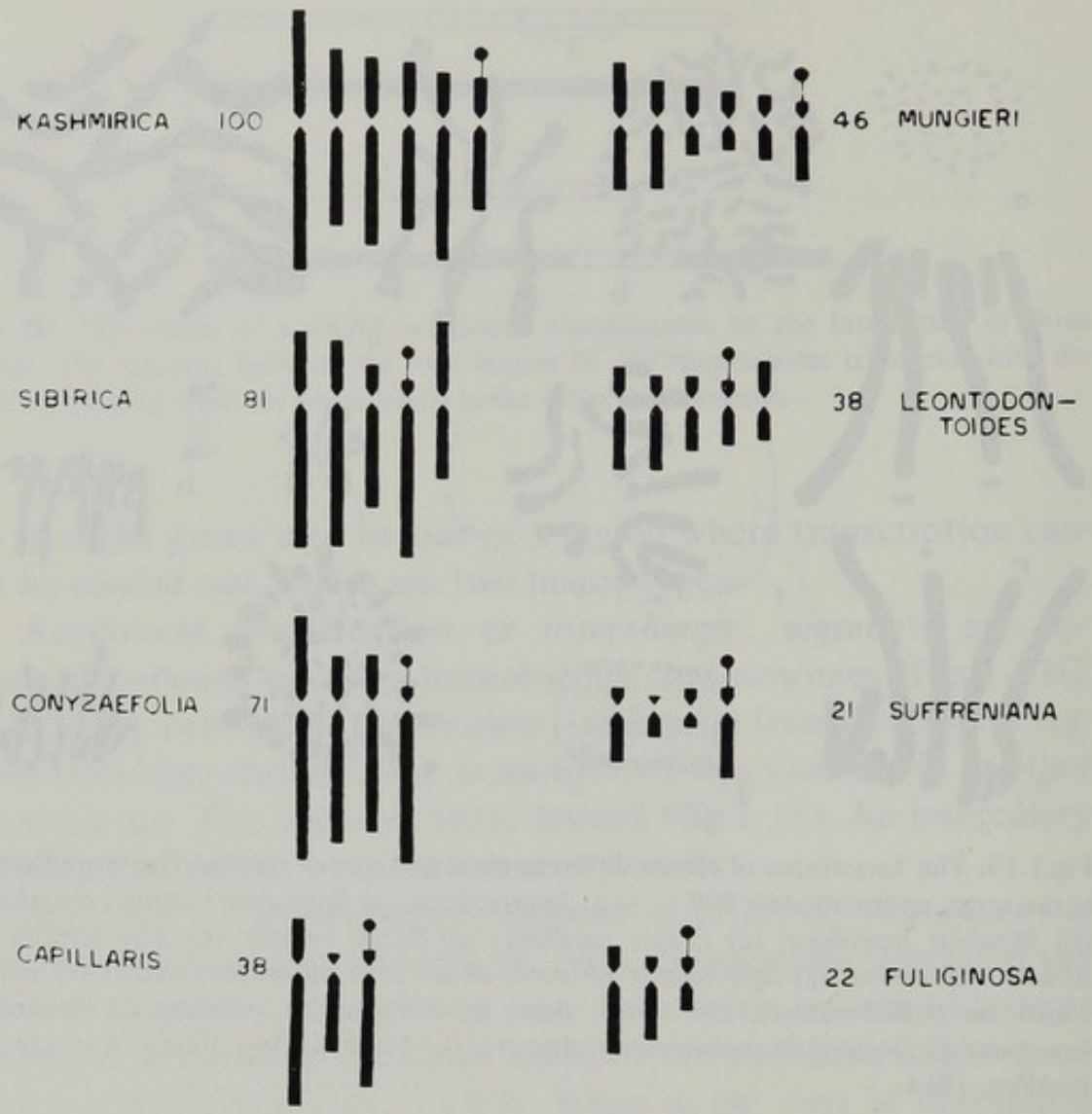


Fig.1.20. Simplified idiograms of eight species of Crepis. The relative genome length (*C. kashmirica* = 100) is shown. Evolutionary development is accompanied by reduction in chromosome number (top to bottom) and size (left to right). According to Babcock et al., 1942.

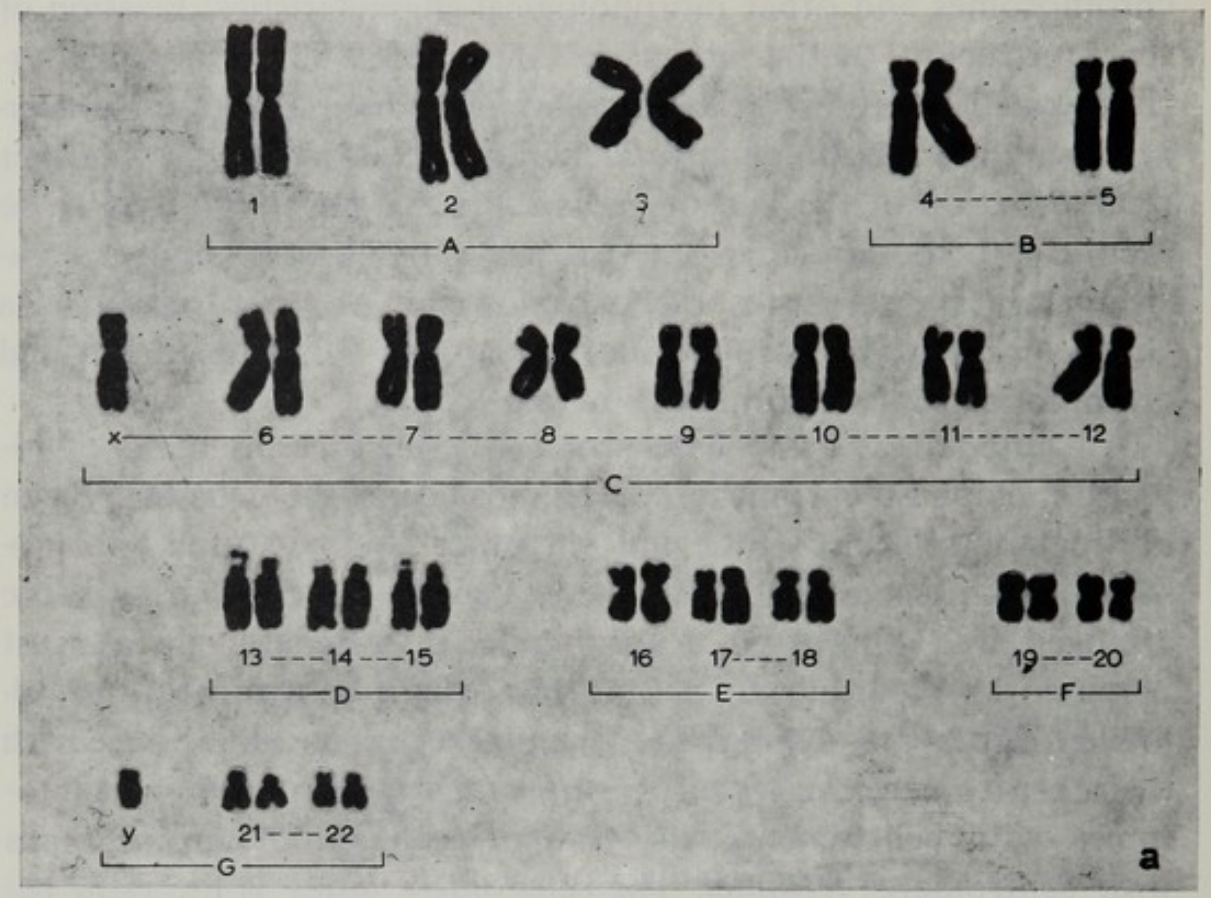
tain that deviations from the established type only very infrequently are an improvement, and consequently will gradually or rapidly be eliminated. Occasionally, within a species *polymorphism* in respect to number and chromosome structure is found. This means that more than one type can be satisfactory. The constant, characteristic haploid set of chromosomes of a species is the *karyotype*. The graphical representation of the karyotype is the *idiogram* (fig.1.20). It contains information on the length of the

chromosomes and other morphological characteristics: location of the centromere, of the secondary constriction and of possible tertiary constrictions as far as these are reproducible. Often the average measured length at mitotic metaphase is indicated. Another representation of the karyotype is the *karyogram*, that used to be identical to the idiogram but that presently is used almost exclusively in a special form: the chromosomes of a photograph of a single representative (colchicine) metaphase cell clipped out and arranged in pairs according to size (fig.1.21A).

An idiogram is usually constructed from measurements of somatic metaphase chromosomes. In most cases the cells have been pretreated (section 1.4.1) and therefore the measured chromosome length has only relative value. Usually the chromosomes are arranged and numbered according to their length, the longest being no.1. According to the location of the centromere, the following types are distinguished: *metacentric* chromosomes with their centromeres *median* or *sub-median*; *acrocentric* chromosomes with centromeres *sub-terminal*; *telocentric* chromosomes with centromeres *terminal*. The *centromere index* is the ratio between the length of the short arm and that of the long arm (s/l) of a chromosome, and therefore is smaller than unity. The ratio between long and short arm lengths (l/s) is larger than unity, and is usually called *arm (length) ratio*. In *holokinetic* chromosomes the kinetic centromere activity is diffusely spread about the entire chromosome ("diffuse centromere", a contradictio in terminis) or the chromosome is *polycentric*. Then, of course, no primary constriction can be observed.

Chromosomes vary in length from one cell to the other, due to differences in contraction, differences in reaction to treatments, preparation, etc. Consequently, two genetically identical homologous chromosomes in the same cell can show considerable differences. It is necessary therefore to measure the chromosomes in several cells and to use the averages for the idiogram. For the same reason it is useful not to use the absolute but the relative length of the chromosomes (or arms): the percentage of each chromosome (or arm) in the total length of the karyotype.

A number of methods of measurement are available. It is possi-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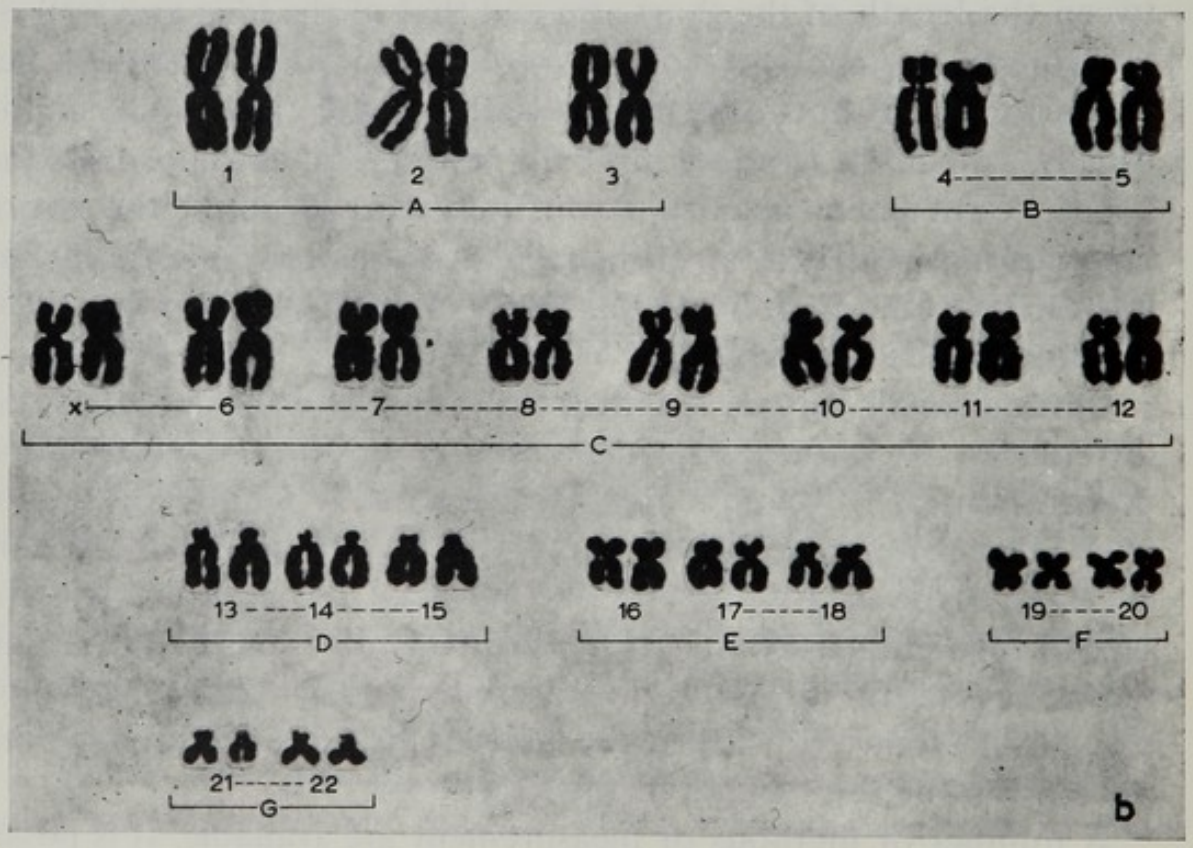
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ble for instance, to make measurements in enlarged prints of photomicrographs. The higher the resolution of the optical system used in photography, the more acurate the measurements. A requirement is that all chromosomes are in one horizontal plane, which is rather frequently realized in leucocyte preparations. In several other types of preparation it may be difficult to have all chromosomes in one plane and then several photographs are required. The photographic method is accurate but cumbersome. Direct accurate measurement in the preparation can be carried out with an ocular screw micrometer. Then it is not necessary that all chromosomes are in one plane: separate adjustment is possible for each chromosome. The requirement of horizontal alignment remains and only slight deviations are tolerated, as larger deviations affect the results. The chromosome arms must be straight, which is often well realized with the proper pretreatment. Similar or better results with less trouble and greater speed are obtained with a binocular drawing tube (camera lucida) that projects a special measuring scale over the microscopic image. Measurements along a simple ruler are less accurate and measurements in camera lucida drawings of the object are not satisfactory.

Fig.1.21. (A) Karyograms of (a) the normal human male and (b) the normal human female (Hamerton, 1969). The karyograms are composed of the clipped-out chromosomes of a photograph. Classification according to the Denver system (numerals) and the London system (letters). Group A contains the three largest, submetacentric chromosomes (1, 2, 3). Group B the two following in size, with subterminal centromeres (4, 5). Group C contains seven autosomes (6, 7, 8, 9, 10, 11, 12) forming a continuous series and not individually distinct, although the smallest and largest of the group are clearly different. The X-chromosome cannot normally be distinguished from this group, but with autoradiography it can sometimes be shown to be late labelling and thereby distinct from the others. Group D consists of three acrocentric chromosomes, some of which have a small satellite on the short arm, but this is not always readily recognized. Two small, almost metacentric chromosomes form group F (19, 20), while group G contains two small acrocentric chromosomes again with small satellites on the short arm (21, 22). The Y-chromosome is very similar to 21 and 22 but can sometimes be recognized as it tends to have its two chromatids less widely spread apart than the two smallest autosomes. The centromere is also more terminal, and there are no satellites. After treatment with quinacrine hydrochloride or quinacrine mustard the Y-chromosome shows a brilliant fluorescence in the UV-microscope, which makes it clearly recognizable, even in interphase.

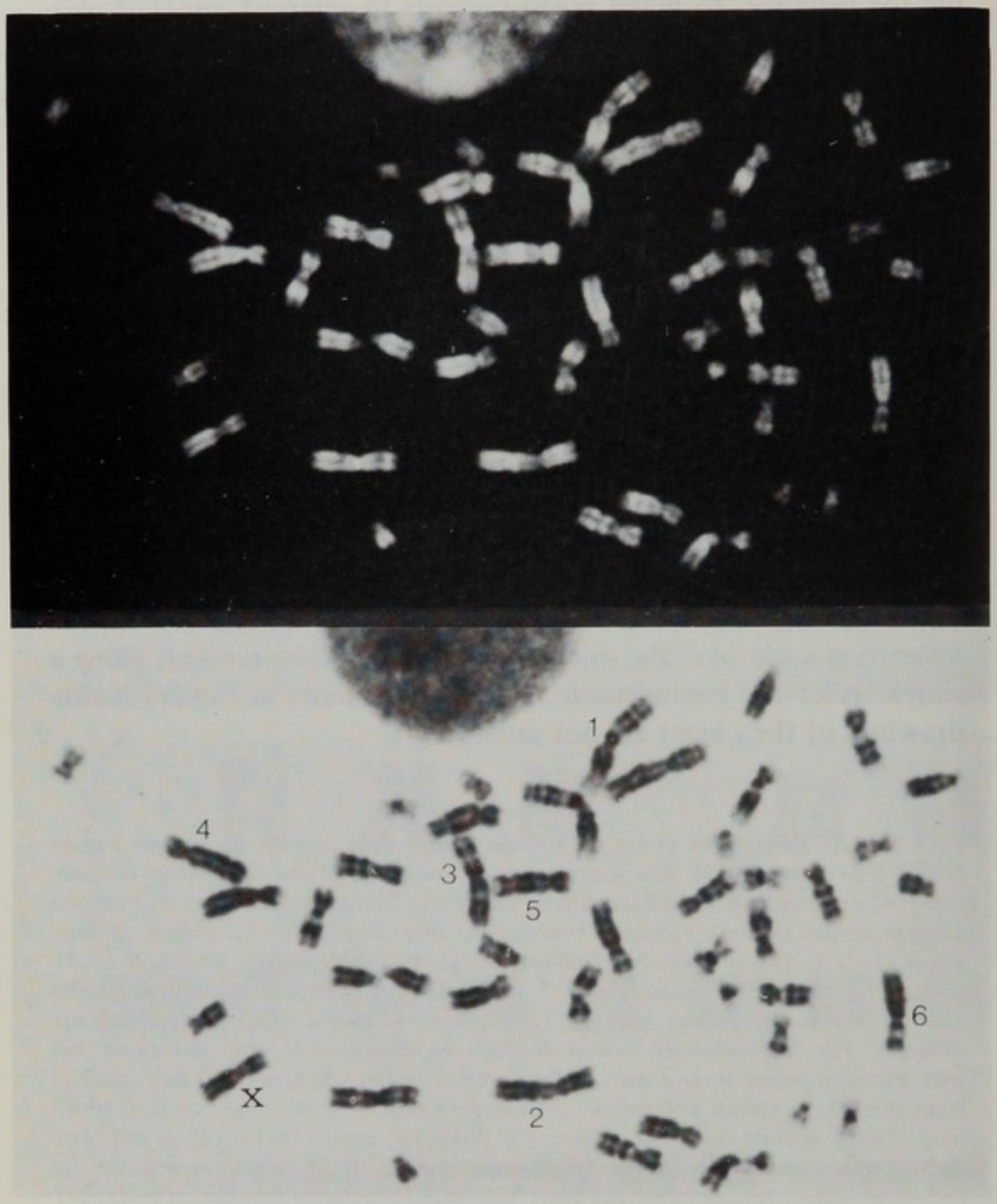


Fig. 1.21. (B) Metaphase cell of a normal male, stained firstly with ASG (acetic-saline-Giemsa) (b) and then with quinaerine (a), which makes specific chromosome parts fluorescent in UV (Evans et al., 1971). Bands appear that make recognition of individual chromosomes and even chromosome segments possible. The majority of the fluorescent and Giemsa bands are identical, apparently because of special chemical properties, perhaps a large content of repetitive DNA.

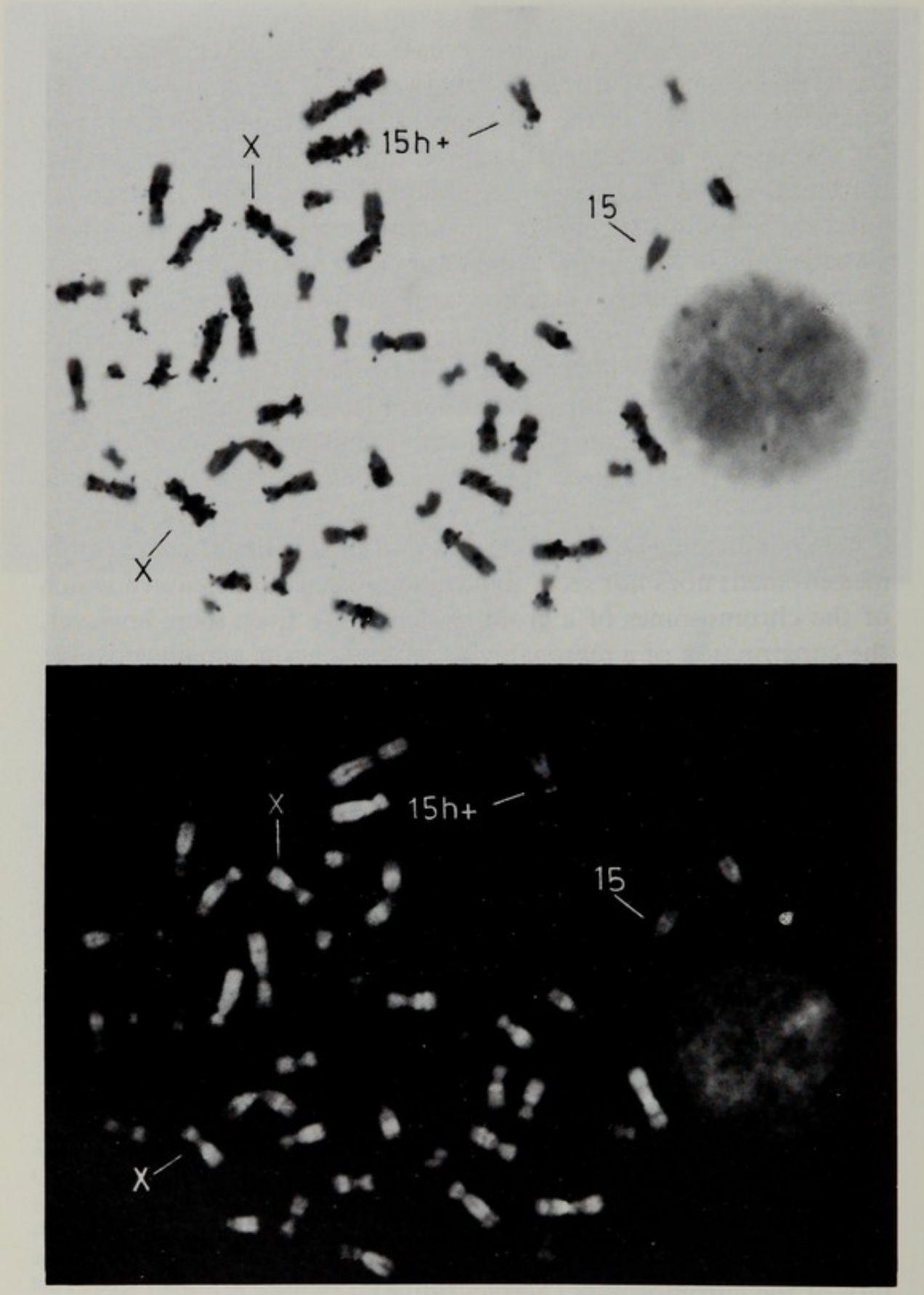
For the construction of an idiogram the chromosomes must be individually recognized, as is the case with the Crepis species of fig.1.20. Often, the difference between the chromosomes is so small that it is lost in the variation in the measurements. In man, for instance, it is impossible to differentiate between all individual chromosomes on the basis of size and of location of the centromere only: only groups of similar chromosomes can be distinguished. For the human karyotype a standard classification into groups has been designed (Denver classification, after a conference held for this purpose at Denver in 1961, compare fig.1.21A). Later at a conference at London a number of refinements permitting further sub-divisions of the groups, have been introduced.

Fluorescent staining, pulse labelling and special staining techniques can often be used for distinguishing specific chromosome segments.

When individual chromosomes cannot be distinguished, accurate measurement does not seem to have sense as it is not known which of the chromosomes of a group is measured. Even then, however, the construction of a reasonably exact karyogram remains possible when proper use is made of statistical analysis.

A simple method useful when the differences in length are small and mainly the location of the centromere can be used to characterize the chromosomes, makes use of a histogram of the arm length ratios. At certain classes of arm length ratio peaks show up that represent specific chromosomes. Even with considerable overlap the different chromosomes can often be well characterized. The occurrence of deviations from the normal karyotype (such as trisomy, translocations and inversions) can be recognized as changes in the histogram (fig.1.22).

There are several methods of distinguishing specific chromosome segments, and these can be important for refining the idiogram. One is the pattern of *tertiary constrictions*, or other forms of heterocycly that result in differentiation of chromosomal segments at mitotic metaphase. Sometimes this differentiation is the normal situation at metaphase. Sometimes it is inducible by cold, and sometimes by chemical agents that alter the condensation pattern of the chromosomes (5-azacytidine).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In several organisms, including man, heterochromatin or other chromosome segments with special chemical properties can selectively absorb quinacrine derivatives, especially quinacrine mustards, that make these segments light up in the UV *fluorescence* microscope (fig. 1.21B,C). An even more widely applicable technique for the identification of specific chromosome segments is the *acetic-saline-Giemsa* (ASG) technique, or modifications based on the same principles (fig. 1.21B). Especially in animal (including human) cytogenetics these techniques have been very successful.

A more laborious but often quite useful method is analysis of the *DNA replication pattern*, using incorporation of radioactive tritiated thymidine. Some chromosome parts, usually the heterochromatic segments, synthesize DNA later than the bulk of the chromosome and can be recognized when tritiated thymidine is applied during the later part of the S period only. With careful experimentation other areas, that synthesize during specific other periods of the S period, can be recognized by applying short pulses of tritiated thymidine at the corresponding periods. Compared with the measurement of chromosomes or with treatments that make specific segments directly visible microscopically, the labelling technique is rather cumbersome and inaccurate. The replication pattern is included only infrequently with the idiogram or karyogram (fig. 1.21C).

The *chromomere pattern*, usually not very distinct in mitosis but clear in the polytene chromosomes of many Diptera (section 3.1), and in pachytene bivalents in several species (section 2.4.1; fig.3.7), can greatly contribute to the detail of the idiogram, which then, however, takes on a somewhat different appearance than the normal metaphase idiogram.

Fig. 1.21. (C) Metaphase cell from a female heterozygous for an enlarged secondary constriction, and satellite region on chromosome 15. The cell was exposed to ^{3}H-thymidine in late S and the upper figure shows the autoradiographic pattern and the lower figure the fluorescence pattern for the cell. The autoradiograph shows that one of the X-chromosomes is more heavily labelled than its partner but both show the same fluorescence profile. Note the label associated with the brightly fluorescing enlarged satellites on the 15h+ (Ganner and Evans, 1971).

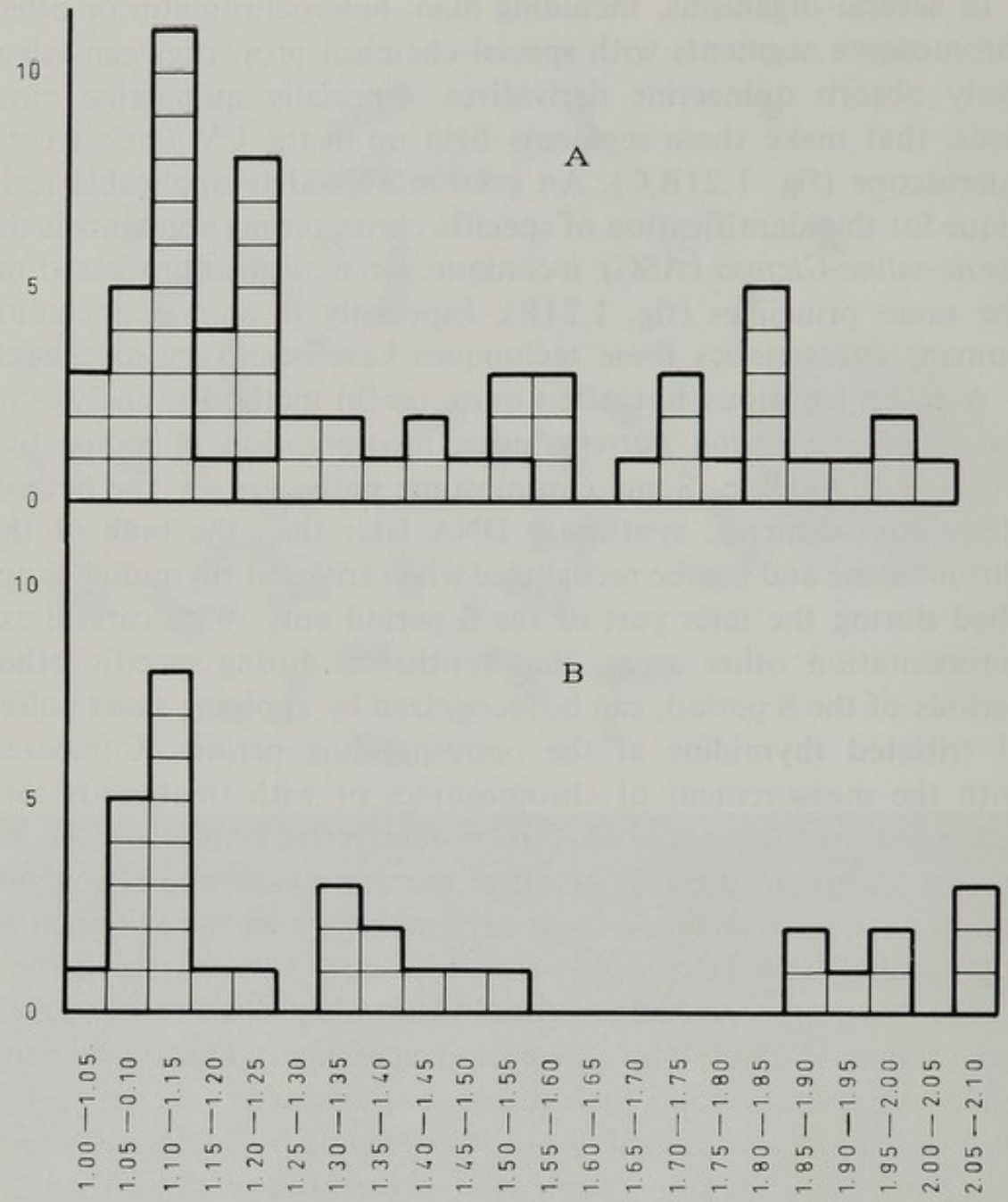


Fig.1.22. Histogram of the arm-length ratios of the chromosomes of rye (*Secale cereale*). Five diploid cells were measured, so each chromosome is represented ten times. As the recognizable satellite chromosome is not included, six different chromosomes remain. In A they overlap, but by separating them into six groups of ten, the approximate boundaries can be recognized and the corresponding arm length ratios deduced. If an extra chromosome is present (in a trisomic) it may be rather easily determined which chromosome it is. In B four cells were measured. Two more chromosome pairs were made recognizable by means of a translocation and only the remaining four were included in the histogram. Two of these are now free in the histogram (IV and VI) and it is clear that I and II have practically identical arm length ratios. The translocation must have been between III and V. Compare Sybenga and Wolters, 1972.

The differences between the karyotypes of different species can be very large (fig.1.19). Often within one karyotype the chromosomes have comparable sizes, but occasionally along with rather large chromosomes a number of small (as in Aloe) to very small (micro)chromosomes occur, as in some species of lizards and birds. Such karyotypes with striking size differences are called *asymmetric*. The same term, however, is used for karyotypes in which large differences in the shape of the chromosomes occur. In addition, the term is used for karyotypes in which asymmetric chromosomes occur, i.e. chromosomes with large differences in arm length. Whenever the term asymmetric is encountered in relation to the karyotype, it is necessary first to be sure about the meaning in which the author uses it. In general, animals more frequently have asymmetric chromosomes than plants.

Differences between species in respect to chromosome size are sometimes conditioned by simple genetic differences, that, for instance, affect the degree or type of spiralization, or the protein content. In a few plant species mutants have been described with unusually large chromosomes, that appear to function normally, and that contain the normal amount of DNA. The mutants are recessive; in hybrids all chromosomes have the same, normal size. Often the size differences between corresponding chromosomes of different species are due to properties inherent to the chromosomes: duplications, heterochromatin, perhaps polyteny and differences in repetititve DNA. Sometimes in hybrids between species with chromosomes of widely different size, as have been studied, for instance, in Crepis and Gossypium, the chromosomes can be of the same length during meiotic pairing.

1.5.2. *Constancy of the karyotype*

The constancy of the karyotype is only relative: there are variations that, however, do not detract from the principle of constancy.

(1) *Systematic variations.* (a) Different individuals of the same species can differ in respect to the presence or absence of certain uncommon types of chromosomes (B-chromosomes, sex-chromosomes, sections 3.2.7 and 3.2.1). In certain tissues the degree of

ploidy can deviate systematically from normal (compare section 1.3.1). (b) Polymorphism without known genetic consequences: not infrequently, homologous chromosomal segments in different individuals of the same species can exhibit small heritable differences in length, without any observable effect on the phenotype of the organism. According to Bose, the length of satellite and short arm of the nucleolar chromosome of rye can vary considerably from one inbred line of rye to the other. The same is true for most other rye chromosomes. The human Y-chromosome shows a similar variation (see section 3.2.1), as do other human chromosomes (fig. 1.21C). Heteromorphy of some chromosomes of Chorthippus involving considerable length differences have been observed in English populations. (c) Polymorphism with genetic consequences: in certain populations of plants and animals chromosomal rearrangements "float" in the populations and are maintained because of their favourable effects on the maintenance of heterozygosity with slight inbreeding, or the preservation of specific gene combinations (see sections 4.4.6 and 4.5.9).

(2) *Incidental variations.* Abnormalities as a result of "accidents", that are usually readily eliminated.

(3) *Fluctuations.* Variations between cells of the same organism in respect to size and shape of the mitotic metaphase chromosomes, sometimes depending on the tissue involved, sometimes even within an apparently homogeneous group of cells. In the latter case the cause may be random variation in chemical content or physiological conditions of the cells. Of course, the handling of the chromosomes during preparation can influence their appearance and especially in squash preparations considerable variation may be entirely artificial.

1.5.3. *Development of the karyotype. Cytotaxonomy*

In spite of the considerable stability of the karyotype, radical changes have occurred during evolution. Taxonomical divergence is often accompanied, therefore, by karyotypic divergence, in which number and shape of the chromosomes are involved. Related species often have related karyotypes. But the relation between taxonomical affinity and correspondence in karyotype is

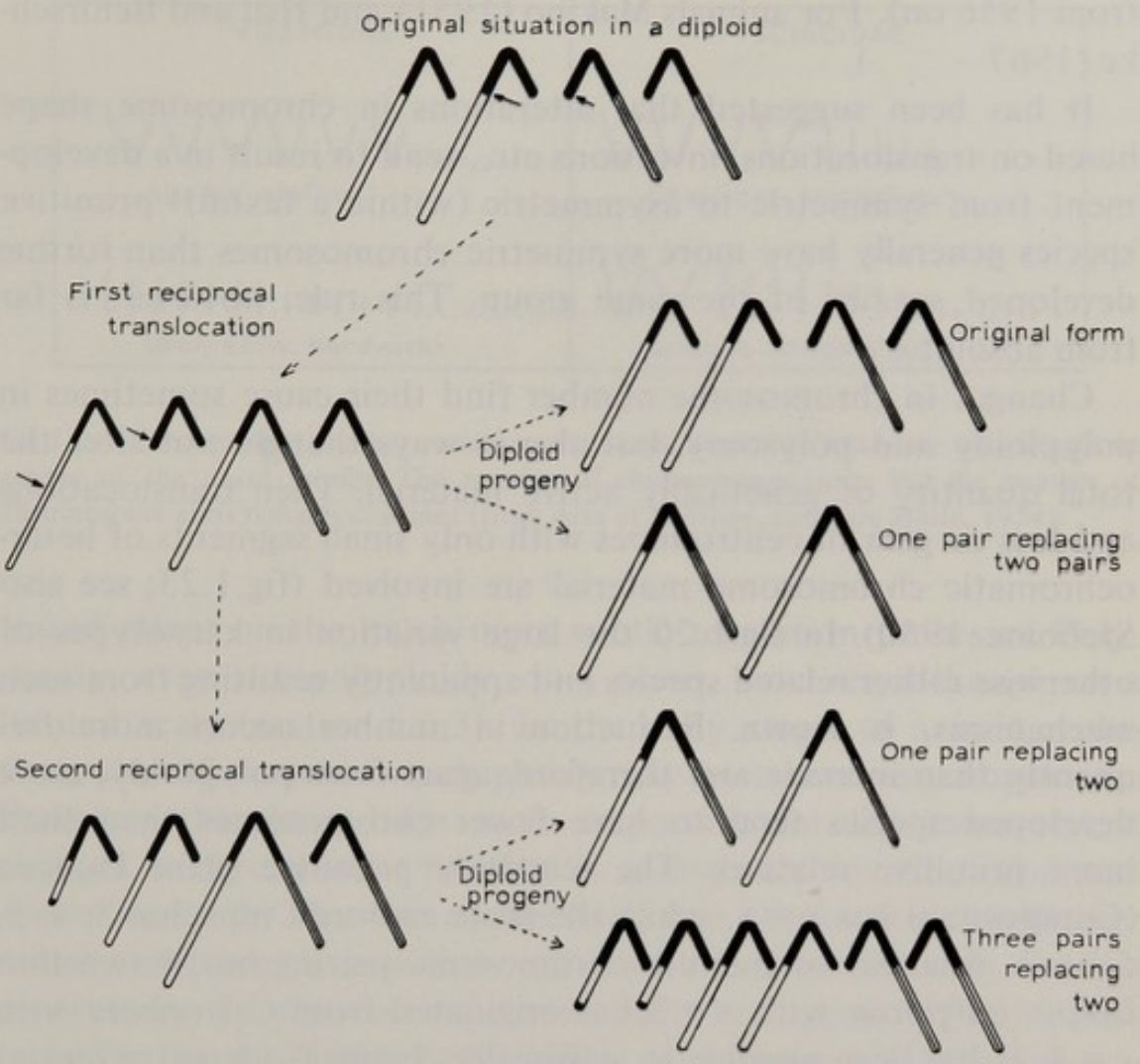


Fig.1.23. Alterations in chromosome number without gain or loss of genetically active material. Constitutive (permanent) heterochromatin around the centromeres (black) does not contain active genes. Translocations involving such segments are here involved. Reduction is more probable than increase as the gain of a centromere requires both a previous translocation and non-disjunction.

complex. Although chromosomal differentiation tends to accompany speciation rather than to induce it, the study of karyotype relationships can be very useful for taxonomy. The branch of science that analyses this relationship and that uses the description of the karyotype as a taxonomical characteristic, is *cytotaxonomy*. A simple but taxonomically often very useful characteristic of the karyotype is the chromosome number. These numbers have been collected for numerous species. For plants: Darlington and Wylie (1955); *Index to Plant Chromosome Numbers* (annualy

from 1956 on). For animals Makino (1951) and Hsu and Benirschke (1967–).

It has been suggested that alterations in chromosome shape based on translocations, inversions etc., tend to result in a development from symmetric to asymmetric (within a taxon): primitive species generally have more symmetric chromosomes than further developed species of the same group. This rule, however, is far from absolute.

Changes in chromosome number find their cause sometimes in polyploidy and polysomy, but also in ways that do not alter the total quantity of genetically active material. Then translocations and loss or gain of centromeres with only small segments of heterochromatic chromosome material are involved (fig.1.23; see also Stebbins, 1950). In fig.1.20 the large variation in karyotypes of otherwise rather related species and apparently resulting from such mechanisms, is shown. Reduction in number occurs more frequently than increase and therefore, apart from polyploidy, more developed species tend to have fewer chromosomes than their more primitive relatives. The relatively primitive plant Dubyea (Compositae) has $x = 8$, while the more evolved Crepis has 3, 4, 5, 6 or 7. Analysis of meiotic chromosome pairing has shown that *Crepis fuliginosa* with $x = 3$ has originated from *C. neglecta* with $x = 4$. It has been possible to artificially change *Godetia* (=*Clarkia*) *whitneyi* with $x = 7$ to a form with $x = 6$ by using two translocations. The new form, however, was not quite normal. The combination of reduction in number and polyploidy has given rise to a large variety of chromosome numbers, especially when hybridization of species with different numbers is followed by doubling of the chromosome number. Thus *amphidiploids* or *allopolyploids* arise. Crepis species with $x = 5$ have developed from species with $x = 6$ which together with the ancestral species have later given amphidiploids with $n = 11$. In nature as well as in the experiment species of Brassica and their relatives with $x = 10$, 9 and 8 have been combined to forms with $n = 17$, 18, 19, 22, 27, 29 etc.

While in plants polyploidy has played a great role in the recent evolutionary variation in chromosome number, this is much less so in animals. The cause may be that in animals gene dose has a pro-

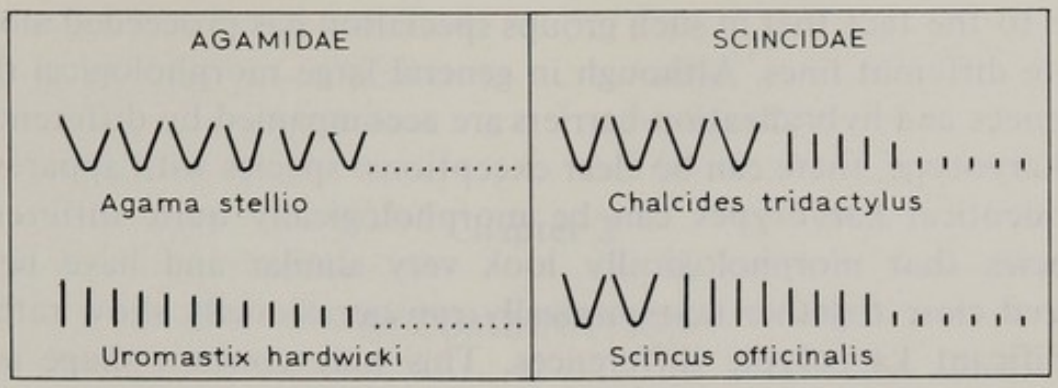


Fig.1.24. Chromosome number variation as a result of centric fusion or centric split in genera of the lizard family. The number of chromosomes varies but the number of chromosome arms remains constant (from data of Matthey, compare White, 1954).

found effect on the much more subtle regulation processes. Polyploidy, even autopolyploidy, does occur, however, although on a limited scale (section 5.6.3). In the early stages of animal evolution, also of mammals, polyploidy may have been very important. Processes that have been more important for the more recent changes in chromosome number in animals are *centric fusion* and *centric split*. In the case of the first, two chromosomes with terminal centromeres combine into one chromosome with median centromere. In the case of centric split the opposite occurs. Prerequisite for the latter is that half a centromere is as efficient as a complete centromere. Probably processes take place that are related to those of fig.1.23, with exchange predominantly at or near the centromere. Occasionally, however, different, as yet unexplained, mechanisms may be involved both in fusion and in split. The result of such alterations is that related species with widely different chromosome numbers still have the same number of chromosome arms. The importance of this system of change was first recognized by Robertson in 1916. Extensive studies on this aspect of karyotype development in lizards and small mammals have been conducted by Matthey (fig.1.24). For the role of chromosomal rearrangements in animal speciation see White (1954, 1969).

It is remarkable that karyotype development has taken a totally different course in some taxonomic groups than in others. This is

due to the fact that in such groups speciation has proceeded along quite different lines. Although in general large morphological differences and hybridization-barriers are accompanied by differences in karyotype, there can be clear exceptions: species with apparently identical karyotypes can be morphologically quite different. Species that morphologically look very similar and have been placed close together taxonomically can occasionally show rather significant karyotypic differences. This may concern shape and number of the chromosomes.

In respect to the development of chromosome number, according to Tischler three types may be distinguished in plants: (1) All species of one genus have the same chromosome number: Antirrhinum has $x = 8$ in all species, the related Nemesia has $x = 9$. Several related genera may share one number. (2) The species of one genus form a polyploid series with the same basic number: In Chrysanthemum the basic number is $x = 9$ and numbers with $2n = 18$, (27), 36, 54, 72, 90 occur. (3) A wide variation in basic numbers occurs: in Crepis 3, 4, 5, 6 and higher (cf. fig.1.20) are found. Carex with $x = 6, 7, 8, 9, 10, 13$ is a special case: it has holokinetic chromosomes (see section 3.2.7) and simple fragmentation is sufficient to give rise to a higher number: all fragments have centromere activity and can maintain themselves. Here, asymmetric fusions with chromosome structure alterations do not result in unmanageable dicentrics. This too will contribute to the greater variation of karyotypes. This system is not found in all species (plant and animal) with holokinetic chromosomes, probably because the chromosome ends (*telomeres*) play a role in the proper separation of the chromosomes at meiosis (section 3.2.7) and the fragments do not have telomeres. Of course, the systems of centric fusion and centric split, relatively common in animals, lead to a similar system of numerical variation.

Chapter 2

RECOMBINATION

2.1. The exchange of DNA

2.1.1. *Why recombination?*

Mutations are the basis of the adaptive improvement of the genetic make-up of all living organisms, but only a small minority has any direct positive effect. Random combinations of favourable mutations are extremely infrequent. When the process of adaptive improvement would depend on the chance occurrence of different favourable mutations in a single individual or in its line of descendants, it would be extremely slow. Moreover, many mutations are favourable only in combination with specific other mutations, lacking any selective advantage of themselves. Alone, they may even be deleterious. The probability of chance combination of such mutations is negligible. As stated in section 1.1.5, raising the mutation frequency to a significantly higher level would have quite negative results, such that only incidentally it can be considered desirable from an evolutionary point of view.

Apparently, very soon after the origin of life (later than, but probably in relation to the development of systems of DNA repair) systems of recombination have developed that serve to bring together into one individual mutations originated independently in different individuals of the same mating group. Recombination has been so successful that at present no forms of life are known to exist without some system of recombination.

Organisms with a single chromosome, such as viruses or bacteria, exchange corresponding segments between two copies of this chromosome. When the original chromosomes carried allelic differ-

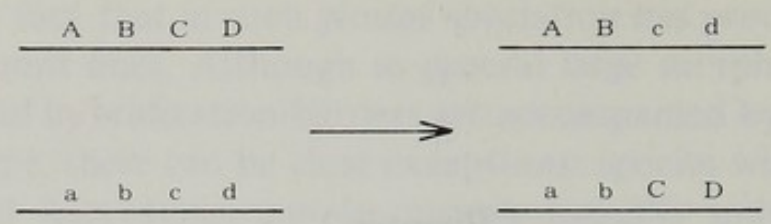


Fig.2.1. Recombination by exchange between two simplified hypothetical homologous chromosomes with different alleles for the four genes *A*, *B*, *C* and *D*. There is one point of exchange, situated between *B* and *C*.

ences, the new, recombinant chromosomes can have new allelic combinations (fig.2.1). Diploid higher organisms with several different chromosomes per cell have the additional possibility of recombining entire chromosomes (see sections 2.3.1, 2.4.3).

2.1.2. *The mechanism of DNA exchange. Crossing over*

All living organisms presumably have the same basic mechanism of exchanging DNA segments. Since exchange must involve equivalent sections of DNA, the break points in the two double-strands must be at exactly corresponding sites. This is realized by exact allignment (pairing) of the two chromosomes prior to exchange. The forces involved are uncertain. Single strands of DNA are known to associate quite readily with complementary strands, but here association is between double strands. After pairing breaks are formed. The suggestion that instead of both strands only one of each would break, the other remaining temporarily intact, was first made by Holliday in 1962. Since the endonucleases normally present break both strands, a special endonuclease must be available for single strand breakage. The broken strands wind loose perhaps over the length of an entire gene and in some way or another forms a pair (according to Whitehouse, 1965) with a complementary single strand, newly synthesized on one of the strands of the pairing partner (fig.2.2). The resulting double strand consists of two strands of different origin: it is *hybrid* DNA. When allelic differences occur within such hybrid DNA sections, *heterozygous* DNA arises, in which the nucleotides do not match. Non-matching of nucleotides calls for "repair", i.e. one of the two strands is excised and replaced by new DNA synthesized on the other strand as

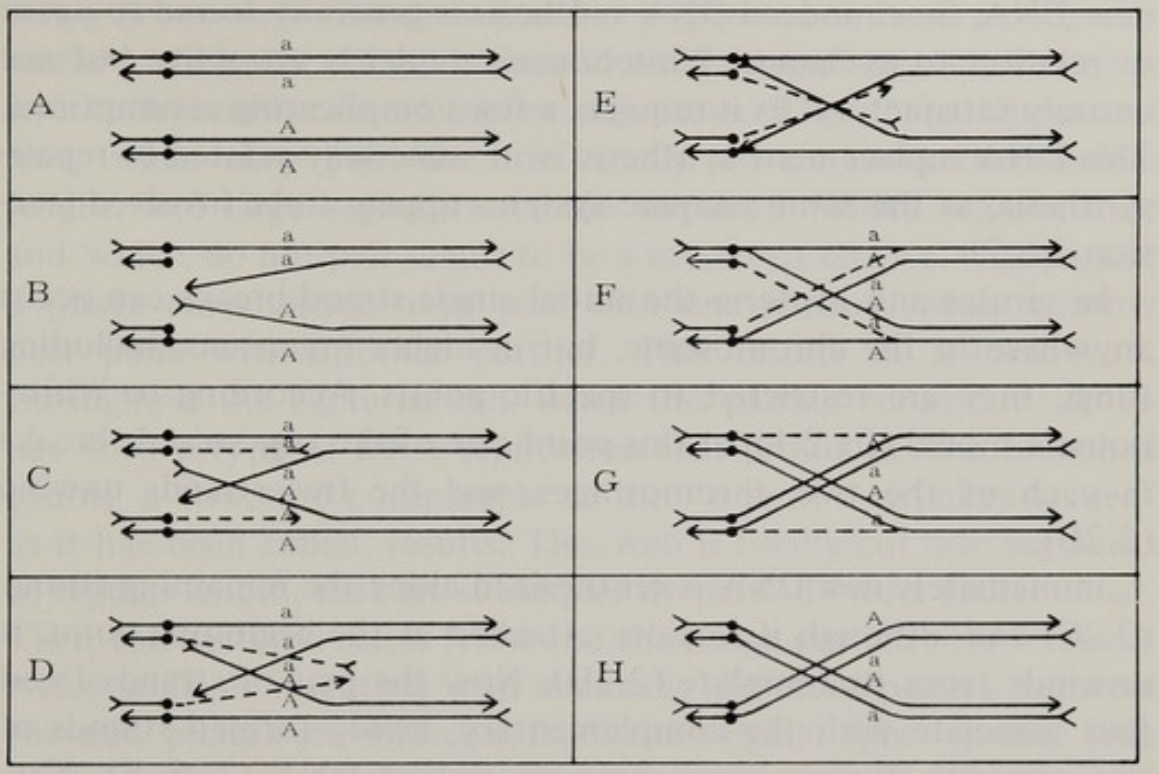


Fig. 2.2. The polaron-hybrid DNA exchange model according to Whitehouse (simplified). Two double DNA helices are shown between which exchange occurs. The black dot represents the limit of the polaron, which is the initiation point of DNA synthesis, and where single DNA strands start separating and unwinding from their sister strands as a preparation for exchange (B). Along the remaining strand DNA is synthesized (C) but this new DNA also unwinds from its template, now starting at the other end (D). These two new strands associate with the first released strand of the *other* DNA double helix to form a stretch of double-stranded DNA (E, F). These two pairing strands have a different origin and may even be slightly different in base composition: it is hybrid DNA. After new end attachments have been made and the now redundant stretch of old connecting DNA has been removed (G, H) the exchange has been completed.

a template. As a consequence, one of the alleles is converted into the other: *gene conversion*, which may involve either of the two alleles. It is observed quite regularly when exchange occurs inside a heterozygous gene. Actually this observation was one of the reasons for the adoption of the concept of hybrid DNA in exchange (cf. section 2.6.3).

Several models have been suggested for the course of events in exchange at the DNA level. An interesting one is that of Whitehouse. In addition to the presence of hybrid DNA, it assumes that half of the DNA involved in the exchange process is replaced by

new DNA. Since indeed DNA synthesis is generally found to occur in relation to exchange, Whitehouse's model is attractive but not entirely satisfactory, as it requires a few complicating assumptions. This DNA replacement synthesis is in some way related to repair-synthesis, as the same enzyme systems appear to be involved (section 2.6.2).

In viruses and bacteria the initial single strand breaks can occur anywhere in the chromosome, but in higher organisms, including fungi, they are restricted to specific points. According to Whitehouse's model (fig.2.1), at this point one of the two strands breaks in each of the two chromosomes, and the free strands unwind (2.2B).

Immediately new DNA is synthesized along the remaining strand (2.2C) and although it remains attached at the beginning point, it unwinds from its template (2.2D). Now the pair of strands freed first associate with the complementary, newly formed strands of the *opposite* chromosome, forming hybrid DNA (2.2E,F). New end-attachements are made and the redundant DNA of the original unbroken strand is removed (2.2G,H). The amount of DNA removed equals the amount newly synthesized: there is no net synthesis. At the end of this process, the two double strands have experienced a complete exchange. Neither of the two chromosomes has lost or gained material, but the exchange results in recombination of the genes in the two chromosomes.

2.2. Crossing-over in prokaryotes

2.2.1. *Viruses*

Viruses have their DNA (or RNA) molecules (chromosomes) free in the host cell. Recombination in bacterial viruses (bacteriophages) especially the T-phages of *Escherichia coli* has been studied thoroughly, but mainly by indirect methods.

Direct observation of these simple chromosomes is difficult, and electron microscope photography has yielded only limited information on the process of exchange.

Multiple infection of one bacterial cell with phage particles dif-

fering from each other in a number of genes, combines genetically marked, different chromosomes in one cell, and as such is equivalent to hybridization of higher organisms. The phage chromosomes, when they pair, do not usually do so along their entire length, and large segments remain unpaired. Which segments do and which do not pair seems to be a matter of chance. One part of a phage chromosome may pair with one partner, while another part pairs with another partner. This results in a mesh-work throughout the bacterial cell. When this pairing behaviour combines with a type of DNA replication that produces long branched chains, a structure comparable to the "web of a drunken spider" as it has been called, results. This web is finally cut into segments of regular length, that are subsequently packed into a protein coat. Then the bacterial cell is lysed and the phage particles are released. After multiple infection with allelically different phages, some of the new phages are different from the original types in that they contain new combinations of genes as a result of exchange (fig.2.3).

Since with phages several individuals can exchange with each other and a replication cycle can alternate with an exchange cycle, the recombination pattern can be rather complicated.

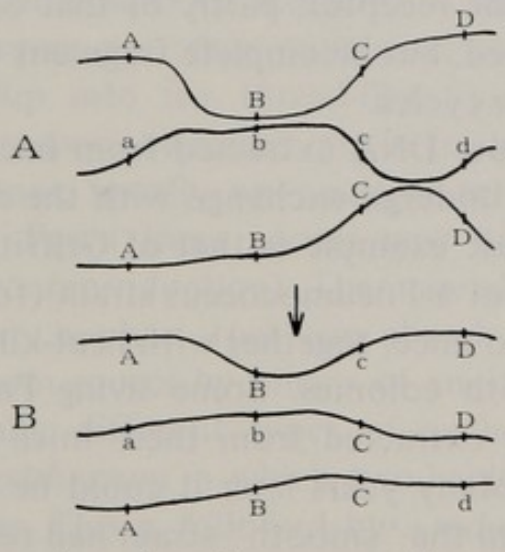


Fig.2.3. Exchange between three phage chromosomes (diagrammatic) in a bacterial cell. In some segments the DNA has paired and there exchange takes place. The top chromosome receives a segment of the middle one, which had also exchanged with the bottom chromosome. The new top chromosome therefore consists of parts of three different original chromosomes.

2.2.2. *Bacteria*

Bacteria have several recombination systems at their disposal, but they have the limitation of having only one chromosome per cell. This requires that, in order to come to exchange a chromosome or part thereof must be brought from the outside into an intact cell and in contact with the nucleus. For this purpose, bacteria have specialized sites on their cell-wall connected with channels in their plasma, for transport of DNA molecules. Compared to those in higher organisms these processes are only incomplete sexual processes: *parasexual* processes. The most important are:

Conjugation: At the time of replication one of the two daughter chromosomes is led through a hole in the wall and through a connecting tube into a cell of another bacterial cell. This second cell is the *receptor*, the first is the *donor*. Practically all strains of the thoroughly analysed bacterium *Escherichia coli* K12 can function as receptors, but only special genotypes can be donor: there is some, but not a complete sexual differentiation. It takes so much time for the chromosome to be brought over, that usually not more than a fragment will reach the receptor (see also section 6.1.5). This fragment takes part in exchange: the final result cannot be more than one complete chromosome that is composed partly of that of the receptor, partly of that of the donor, and a similarly recombined, but incomplete fragment that will get lost in subsequent division cycles.

Transformation: pure DNA extracted from bacteria can penetrate bacterial cells and undergo exchange with the chromosome of the receptor. The oldest example is that of Griffith who in 1928 injected living cells of a Pneumococcus strain (forming rough colonies in culture) into mice, together with heat-killed cells of a strain that formed smooth colonies. Some living Pneumococcus isolations shortly later extracted from these mice appeared to form smooth colonies. Many years later it could be demonstrated that DNA liberated from the "smooth" strain had recombined with the DNA of the "rough" strain. In many bacteria, isolated pure DNA fragments can similarly be taken up into a receptor cell and can then exchange with its chromosome.

Transduction: Bacteriophages, by exchange of a special kind, can

take up segments of bacterial chromosomes into their own chromosome and can carry this over into a new bacterial host. When this new host does not become lysed (= broken down to free a new generation of phage particles) the segment of bacterial chromosome in the phage chromosome can be taken up by exchange into the host chromosome. When the original and new host differ in a genetic factor that is located in the DNA involved in the transfer, the latter gets the allele of the former and looses what it had before.

2.3. Recombination in eukaryotes

2.3.1. *Fungi, Yeasts*

The step from single-chromosome *prokaryotes* (bacteria and viruses not having a true nucleus in which their chromosomes are packed) to multi-chromosome *eukaryotes* (organisms that have their chromosomes packed in a nucleus that is well separated from the cytoplasm) is large. Fungi and yeasts are relatively primitive eukaryotes. In the greater part of their life cycle they are haploid: each nucleus contains only one set of chromosomes. The cells of yeasts are usually uni-nucleate, and tend to remain separate. The cells of fungi, however, are often multi-nucleate and series of connected cells develop into the thread-like *hyphae* of the fungal *mycelium*. In Neurospora at certain places in the mycelium simple structures form loose, usually uni-nucleate cells (conidial spores) that can disperse. From these spores new haploid mycelia can grow out (vegetative reproduction). There are also small female organs with receptive hyphae, that can absorb nuclei from other hyphae or even from spores by means of anastomosis. Cells arise with two genetically different types of nuclei. The little organ develops into a *perithecium* in which two haploid nuclei fuse into one diploid nucleus. This is followed by a *reduction division*. The cells in which this takes place are called asci (singular *ascus*). The reduction division has a complex course, probably often resembling that in higher organisms (section 2.4.1), and involving a single cycle of chromosome doubling during two cycles of nuclear di-

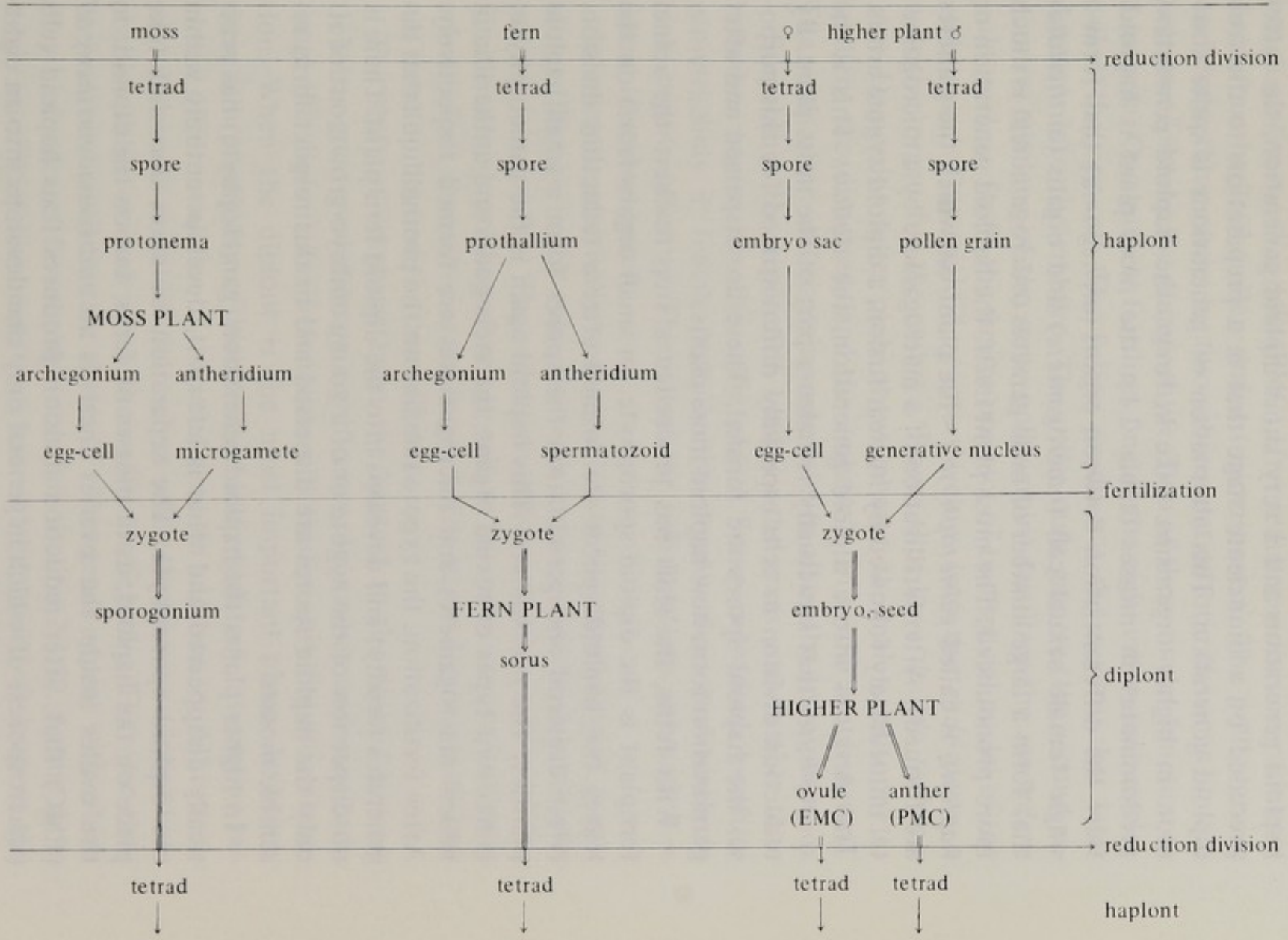
vision, resulting in four haploid nuclei. In the ascomycete Neurospora each of the four haploid nuclei goes through another division cycle which results in a total of eight haploid nuclei per ascus. The asci are oblong with the nuclei in a row (figs.2.7, 2.8). During the reduction division recombination takes place in two ways (compare sections 2.1.1, 2.4.3): (1) Chromosome recombination: the haploid nuclei have received some of their chromosomes from one parent, others from the other parent. The more chromosomes there are, the smaller the probability that all chromosomes of one parent are combined in the haploid daughter nucleus: the extent of chromosome recombination is proportional to the number of chromosomes. (2) Exchange between homologous chromosomes (one of one parent, the other of the other parent), comparable in effect but realized in a more complex way than in bacteria and phages. Both processes together result in an effective recombination, although, of course, large stretches of chromosome remain unaffected.

In the fungus Aspergillus, in addition to a sexual cycle that is comparable to that described above, another process of recombination is found. Apparently, non-sexual hyphae of different mycelia may anastomose and nuclei may pass from one to the other. When genetically different nuclei are thus brought together in one mycelium this is called a *heterokaryon*. Sporadically two such haploid nuclei fuse into a diploid nucleus. Then, as the result of successive loss of all chromosomes of one complete genome, *haploidization* occurs. Which chromosomes are lost is a matter of chance: the remaining haploid set is a random combination of the two parental nuclei. This is a form of chromosome recombination. There is also exchange of homologous chromosome segments after pairing. This somatic exchange is infrequent and, of course, takes place before haploidization. The result of these two processes is comparable to that of the reduction division but the course of the process is different. Heterokaryons occur in Neurospora too, but somatic recombination and haploidization have not been observed.

2.3.2. *Alternation of generations*

In the sexual cycle of fungi two generations alternate: a long

Table 2.1
Alternation of generations



haploid generation and a very short diploid generation, the latter preceded by a binucleate stage that is a preparation to the true diploid generation. This alternation of generations is quite common in higher organisms. Like in fungi, the haploid generation predominates in mosses (table 2.1): the moss plant is haploid. Here the differentiation between organs (*archegonia*) that form a single female sexual cell (*macrogamete*) and organs (*antheridia*) that form a large number of male gametes (*microgametes*) is much more pronounced. The moss plant itself, the haploid generation or *haplont*, is called *gametophyte* (= the plant on which the gametes are formed). After fertilization of a macrogamete by a microgamete, immediately followed by nuclear fusion, a diploid zygote arises. This develops into a diploid generation, the *diplont*. This is the *sporophyte*, that structually remains a part of the moss plant. By reduction division in cells especially differentiated for this purpose, the haploid spores are formed. These are dispersed and after germination form new haploid moss plants.

With ferns, the shift has proceeded a step further: the actual fernplant is the diploid sporophyte. In small organs (*sori*) on the leaves the haploid spores are formed after a reduction division. After dispersal and germination the spores form a small haploid plantlet: the *prothallium*. This haploid again is the gametophyte: it has two types of sexual organs, the archegonia and antheridia in which macrogametes and microgametes are formed respectively. After fertilization, the zygote remains on the prothallium (that degenerates rapidly) and develops into the diploid fern plant. There is no dispersion of the zygote or of a young embryo grown out of it: only the haploid spores are dispersed and in this respect ferns resemble mosses.

In higher plants the haploid generation (gametophyte) has practically disappeared and what is left of it, develops entirely within the diploid sporophyte. The separation of sexes does not take place on the haploid, as in mosses or ferns, but on the diploid: in the ovules inside the ovaries there is an *embryosac-mother-cell* (EMC) that, after reduction division produces four haploid cells (macrospores) of which in general only one develops into an eight-nuclear *embryosac* (the gametophyte). In the anthers a large num-

ber of *pollen-mother-cells* (PMC) after reduction division each produce four haploid pollen grains (*microspores*). The pollen grains each form three nuclei by two subsequent mitotic divisions, in fact together forming the gametophyte. Two of these nuclei function as a gamete. One gamete fertilizes one of the eight embryosac nuclei, called the *egg cell*. The fusion product is the zygote, that later develops into the embryo. The other male gamete (the second generative nucleus of the same pollengrain) fertilizes the *secondary pole nucleus*, itself the fusion product of two special nuclei of the embryosac. This triploid zygote develops into the *endosperm*, a nutritive organ for the embryo. Plants thus have *double fertilization*. Dispersion takes place at two phases: the male gametophytes (pollen) are dispersed, and the embryo's (in seeds) are dispersed. The seeds often still contain some or even all of the endosperm.

Sometimes the separation between the sexes goes further than between organs on the same plant: Some individuals produce gametes only in female reproductive organs others exclusively in male organs (*dioecy*). This is not the rule in plants although it is found in several genera.

In animals, practically nothing is left of the alternation of generations. Dioecy is the rule, although *monoecy* (*hermaphroditism*) occurs occasionally. The reduction division in the *oocytes* of the female animals produces one egg cell, in the *spermatocytes* of male animals four *spermatozoa* are formed. The haploid generation does not develop beyond the one-cell stage.

The shift of emphasis from the haploid to the diploid generation has great evolutionary significance. In mosses and especially fungi the diplont has a function practically only for recombination. When the diplont is the most important phase, however, there is a possibility of biochemical and regulatory interaction between different alleles of the same gene. In addition, mutant recessive alleles can maintain themselves when they have no immediate positive value or even when their effect is negative. They can thus be preserved until mutant alleles of other genes appear with which they can interact favourably (compare section 2.1.1). At the same time, since recessive mutations are not expressed as long as the companion dominant allele is present, the diploid organism has a much greater tolerance for mutations than the haploid.

Vegetative (non-sexual) reproduction regularly takes place in bacteria, in fungi and in plants (runners, bulbils, etc.). In some plants and animals, although the external phenomena and processes of the sexual cycle have been retained, the original function of recombination has been lost: embryos are then formed not from a zygote after fertilization, but from an unreduced cell in or near the sexual organs of the mother. Genetically, the embryo is identical to the mother. The phenomenon is called *apomixis*. There are several types of apomixis, especially in plants (Gustafsson, 1947; Rutishauser, 1965). It will be briefly considered in section 7.1.

2.4. Reduction division. Meiosis

2.4.1. *Function and description*

During reduction division (= meiosis) one of the two essential events in the alternation of generations takes place: the reduction of the chromosome number from diploid to haploid. The other essential event is fertilization. During the process of reduction, chromosome recombination and exchange of chromosome segments take place. Since the alternation of generations has evolved as the means to realize recombination, the *primary function* of meiosis should be considered to be recombination.

Meiosis is usually studied in pollen mother cells (PMC) or in spermatocytes as these are readily available in large numbers. It is often tacitly assumed that meiosis in the embryosac mother cells and in the oocytes is essentially equivalent to that in the male meiotic cells. This is not always so: especially quantitative differences between the sexes are often observed and occasionally the process may be qualitatively different.

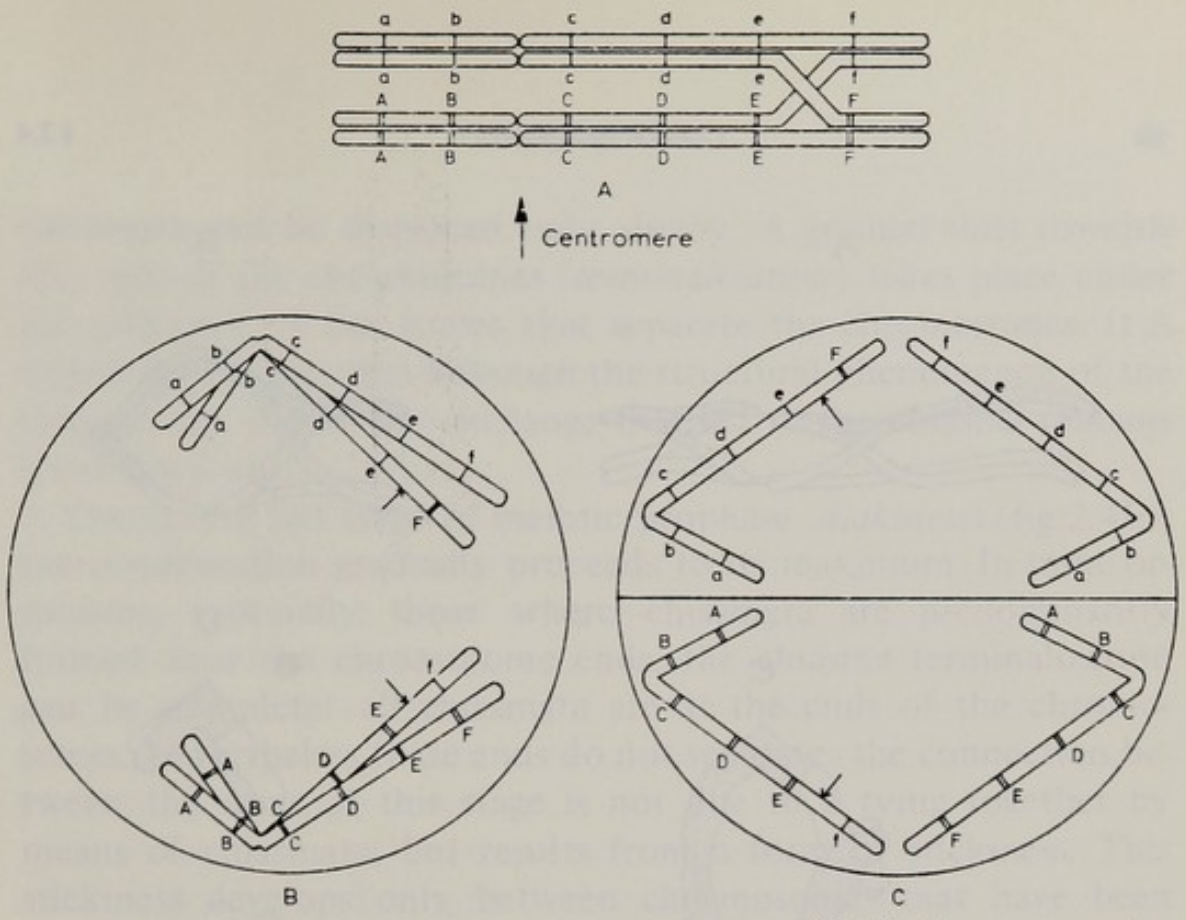
Meiosis is characterized by having a single chromosome division during two nuclear divisions. Consequently, the resulting four nuclei together contain only twice the original number of chromosomes which per nucleus is only half the original number. The requirements of meiosis, reduction combined with recombination, are complex and as a result the process necessarily is complicated.

Fig. 2.4. Meiosis, diagrammatic. See text, section 2.4.1.

The crucial steps are taken during the initial stages: the prophase of the first of the two divisions is long and usually divided into five sub-stages. During the first stage, *leptone* (fig.2.4A), the chromosomes becomes visible as long, thin threads. They have a finer structure than in mitotic prophase. In the second stage, *zygotene* (fig.2.4B), the chromosomes have slightly contracted and in favourable preparations of some material it can be seen that in some segments they have paired, in others not. The fact that this is not seen at leptotene and that it is complete at the following stage, *pachytene* (fig.2.4C), has suggested that during zygotene pairing of the chromosomes takes place into *bivalents*. Bivalents, of course, occur in the haploid number. The process of chromosome pairing is discussed in somewhat more detail in section 2.6.2.

At pachytene, contraction has proceeded further, but the bivalents are still quite long. In organisms with relatively short chromosomes in not too large numbers the individual bivalents can be distinguished. The double structure is recognizable, but it is not possible to see that each chromosome consists of two chromatids. Pachytene is considered to be the stage where the complex process of exchange is completed, but the points of exchange cannot yet be recognized microscopically. More details on exchange in the chromosome of higher organisms are given in section 2.6.2. The morphology of the pachytene bivalents is not homogeneous: when heterochromatic segments occur they can usually be distinguished at this stage. In addition, thicker and thinner parts succeed each other along the bivalents, sometimes giving them the appearance of strings of beads, the beads are the *chromomeres* (compare sections 1.3.1 and 3.1.1, fig.3.7). The distribution along the chromosomes is somewhat irregular but the pattern is constant and typical for the chromosome. In pachytene this pattern is usually clearer than in mitosis. In maize, tomato and several more organisms the pachytene chromomere pattern can be used to identify specific chromosomes and sometimes even segments of chromosome.

At the next stage, *diplotene* (fig.2.4D), the contraction has proceeded further again and probably because of the tendency of the chromosomes to stiffen with contraction and as a result of the torsion forces involved, the chromosomes separate. The two chro-



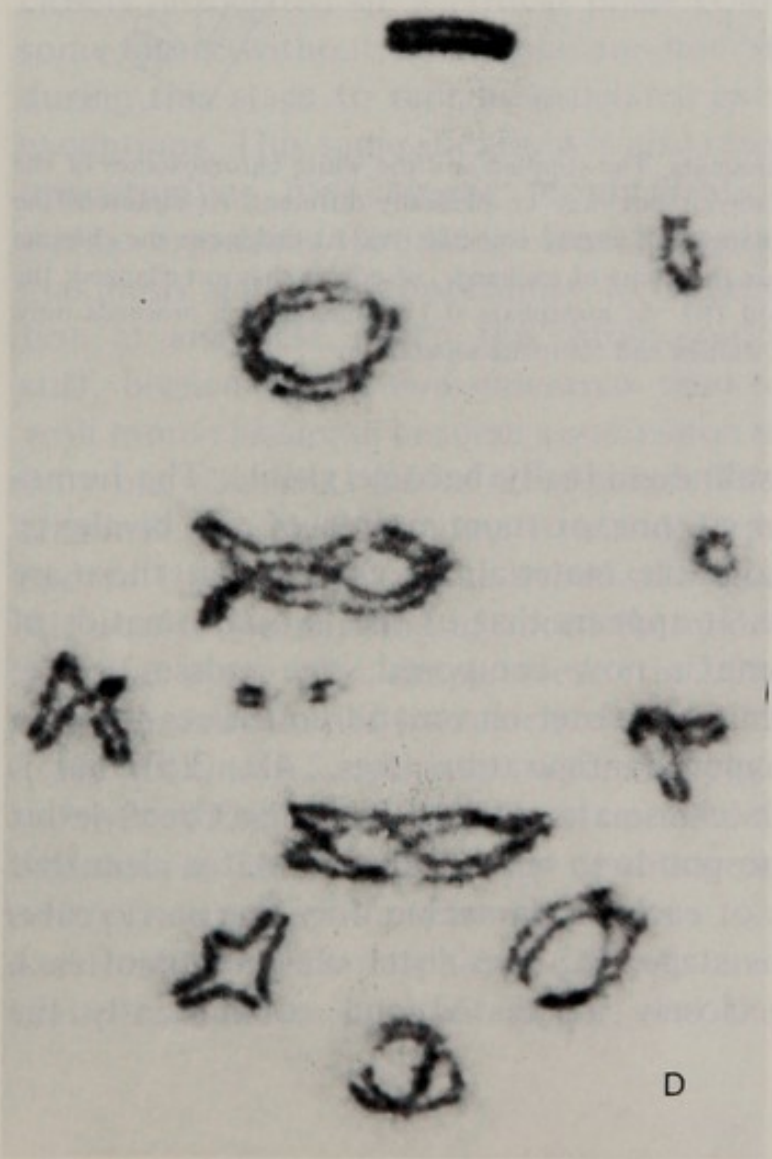


Fig.2.5. The structure of a chiasma and its genetic consequences. (A) Diagram of a diplotene bivalent with one chiasma, not yet terminalized. Only two chromatids are involved. (B) The same two chromosomes of the bivalent after anaphase I separation. Both chromosomes still have two chromatids, but distal from the point of exchange these are different, provided the original chromosomes had allelic differences. In this segment the two chromosomes now are identical: both being *Ff*. (C) After anaphase II separation each of the four groups has received one of the four chromatids of the original bivalent. All four chromatids are different as a result of the chiasma: two parental and two recombination types. (D) In the diplotene bivalents of *Schistocerca gregaria* the structure of the chiasmata, at least when they are not terminally located, is clearly visible (D from Henderson, 1969).

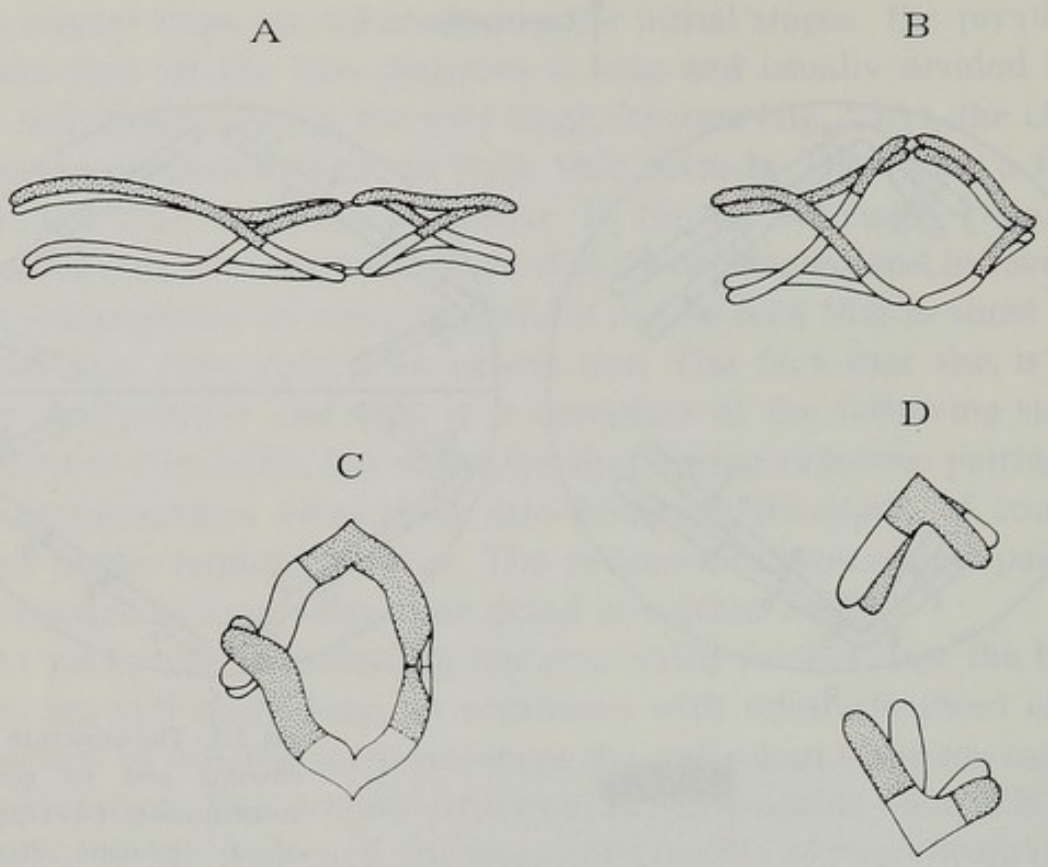


Fig.2.6. Terminalization of chiasmata. The stippled and the white chromosomes of the bivalent are completely homologous but may be allelically different. At diplotene the chiasma and the point of exchange still largely coincide (A). At diakinesis the chiasma has shifted towards the end but the point of exchange, of course, has not changed: the two points have been separated (B). At metaphase (C) terminalization proceeds only slowly but at anaphase there is sudden and complete separation.

matids in each chromosome gradually become visible. The homologues remain together at one or more points of the bivalents. Careful analysis in favourable material has shown that these are the points of exchange. It appears that of the two chromatids of which each chromosome is now composed, one crosses to the other chromosome, while the sister chromatid continues its way. This leads to an X-shaped configuration (figs.2.4D, 2.5) that is named *chiasma* (plural: chiasmata, derived from the Greek letter chi, χ). A chiasma corresponds to an exchange, and it is clear that of the two chromatids of each chromosome only one participates in the chiasma. At this stage, the two sister chromatids of each chromosome are still closely associated and consequently the

chiasmata can be displaced only slowly. A gradual shift towards the ends of the chromosomes (*terminalization*) takes place under the influence of the forces that separate the chromosomes. It is important to note that although the structural phenomenon of the chiasma can move, the exchange that led to the chiasma remains fixed (fig.2.6).

During the last stage of meiotic prophase, *diakinesis* (fig.2.4E), the condensation gradually proceeds to its maximum. In some organisms, especially those where chiasmata are predominantly formed near the chromosome ends, the chiasma terminalization can be complete: all chiasmata are at the ends of the chromosomes. Nevertheless these ends do not separate: the connection between the arms at this stage is not due to a tying together by means of chiasmata, but results from a form of stickiness. This stickiness develops only between chromosomes that have been closely associated at a critical phase. Chromosomes or chromosome arms without chiasmata are not associated close enough during this stage to remain associated except in a few important exceptions. This same stickiness is also responsible for the reduced opportunities for chiasma terminalization from diakinesis onwards: organisms that do not have their chiasmata terminalized by diakinesis, get little opportunity to do so before complete separation at anaphase. When the chromosomes at diakinesis become stiff, bivalents with two chiasmata tend to become circles, those with more chiasmata become a succession of circles and those with only one chiasma take on a rod-shape. The free chromosome ends terminal of a chiasma come to stand perpendicular to each other and to the bivalent at the other side of the chiasma. A bivalent with only one chiasma at about the middle of the chromosomes gets the shape of a simple cross (fig.2.5). More flaccid chromosomes usually do not have such extreme shapes.

At diakinesis, especially with complete terminialization, the number of chiasmata usually can not be determined exactly. When diplotene is not favourable for counting chiasma frequencies, it is only possible to make approximations.

After diakinesis prophase comes to an end, the nuclear membrane and the nucleoli disappear, and the spindle begins to de-

velop. The important difference with mitosis is that at meiosis the centromeres of the chromosomes behave as single units in respect to the spindle. Even when in favourable material the two chromatids of one chromosome can be seen to have their own centromere these always orient to the same pole. This orientation is stable only when it experiences a counterforce, and this is produced by the pairing partner in the bivalent which orients towards the other pole. The chromosomes of the bivalent appear to fumble about until finally a stable orientation is reached with one orienting to one pole and the other to the opposite pole: *coorientation.* A limited reorientation remains possible until anaphase. Coorientation is of great importance since it attends to the proper separation of the bivalent into its two constituent chromosomes. If no chiasmata or other connections are available the *univalent* chromosomes do not coorient and cannot select a pole to go to. In analogy to the situation in mitosis, the stage between the initiation of spindle activity and the equilibrium of the bivalents between the poles is (first) *prometaphase.* The stage of equilibrium is (first) *metaphase* (fig.2.4F).

In contrast to what is seen at mitotic metaphase, the chiasmata come to be situated in the equator, and the centromeres are already on their way to the poles.

Biochemical changes in the cell determine the end of metaphase by releasing the sticky connections between the chromosomes of the bivalents: the chromosomes separate and move each to its own pole: (first) *anaphase* (fig.2.4G). So many factors are involved in the selection of the pole towards which a chromosome moves, that it may be considered a matter of chance rather than of actual selection. Normally the chromosomes of different bivalents are independent in this respect.

The anaphase initiation is not entirely instantaneous: during, and especially towards the end of metaphase, several chromosome ends loose their connections. At anaphase the two chromatids are usually clearly visible in each chromosome: this is one way of distinguishing meiotic from mitotic anaphase.

After anaphase, there is (first) *telophase,* followed by an interphase stage, here usually called *interkinesis* (fig.2.4H). There are

two haploid cells, the *dyad* (note: the term dyad is occasionally used for bivalent). Interkinesis is short as replication of chromosomal material is not required; often little despiralization of the chromosomes is observed and sometimes the second prophase follows almost immediately after first telophase. The second division involves division of the chromosomes into chromatids and is very similar to a mitotic division, with the centromeres in the equator: (second) metaphase (MII, fig.2.4I). The MII chromatids are not quite as orderly arranged as mitotic metaphase chromatids, and this often gives second metophase a somewhat irregular appearance. Only at (second) anaphase (AII) the chromatids that have been present since prophase of the first meiotic division are separated (fig.2.4J).

In oocytes, one of the two cells of the dyad generally degenerates to form the polar body. Then the second division takes place in only one cell. Of the two daughter cells of the second division one again degenerates to form the second polar body, so finally only one nucleus remains: the egg nucleus. In the spermatocyte divisions all four products usually develop into spermatozoa, cell division and separation taking place after the first and again after the second division, which makes it impossible to recognize the four spermatozoa that have derived from one spermatocyte. In plants, the four products (tetrad) usually remain together for some time after meiosis in the pollen mother cell, but they separate later to form the pollen grains, except in some taxa where they remain together. From the embryosac mother cell a linear tetrad develops. Only one of the two terminal cells actually develops into an embryosac, the female gametophyte. The other three degenerate.

In respect to chromosome number, the first of the two meiotic divisions effects the reduction: prereduction, the first division is *reductional,* the second *equational.*

These terms have been applied also in a genetic sense, in connection with the exchange of chromosomal segments. Although not very meaningful, this use of these terms has had some circulation. The first division is then always considered reductional for the centromere and the segment between centromere and first chiasma. Here sister chromatids remain together when the chromo-

somes go to the pole. Beyond the first chiasma, however, sister chromatids separate as in mitosis, because they are now in different chromosomes: for this segment the first division is equational. For these same segments the second division must be reductional.

The genetic consequences of the formation of a chiasma are demonstrated in fig.2.5.

2.4.2. *Duration of meiosis*

The duration of meiosis varies considerably for different organisms and also depends on environmental conditions, especially temperature. In some plants meiosis starts so early in spring or even in the winter that, because of the low temperature, the process can take several months. All metabolic processes are similarly slowed down and therefore a long non-metabolic state of the nucleus as occurs during meiosis, does not present a problem. In many animals with relatively large eggs, meiosis also takes quite long, while high temperature and the development of the egg require that metabolic activity continues. In human females, for instance, meiosis starts in the late prenatal stages and is completed only at the time of ovulation: a period that can be as long as 40 years or more. The solution is complete despiralization at the stage at which meiosis is blocked, usually around diplotene. Sometimes an interphase structure appears (*dictyotene*) as in humans, sometimes the bivalents stretch to enormous size (500 μ for instance instead of some 10 μ with normal contraction) and develop side loops (*lampbrush chromosomes*, section 3.1.2). In such extended chromosomes transcription can go on at a high rate. Especially in amphibia the phenomenon has been analysed and described in detail. Before meiosis proceeds, the chromosomes condense and loose their sideloops: at metaphase the proportions are normal again. In other organisms, including plants, "diffuse" stages, probably with a similar function, may occur for instance between pachytene and diplotene or between diplotene and diakinesis. In other cases interkinesis can be long with complete despiralization and transcription.

Meiosis in general is somewhat longer in duration than mitosis

but of the same order of magnitude. A few examples for plants are: (at room temperature) Lilium sp., Gasteria sp., Vicia sp. 3-4 days; Antirrhinum 1-2 days; Tradescantia 2 days.
The following relation between temperature and duration of meiosis in Endymion was reported by Wilson (1959):

Temp (°C)	0	5	10	15	20	25	30
Days	36	15	7	3.5	2	1.25	0.83

Since meiosis is a very complex process and since different parts react differently on different temperatures, not only duration but several more meiotic phenomena are affected by temperature.

2.4.3. *The two types of recombination*

With the description of meiosis it was emphasized that chromosome recombination and exchange of chromosome segments are two different types of recombination. Each spore (and the gamete derived from it) receives a complete haploid set of chromosomes that is derived on the basis of randomness from the two sets (the originally paternal and maternal set) of the individual on which the spores are formed (chromosome recombination). The exchange of chromosome segments supplements chromosome recombination. Males of Drosophila and most other Diptera, do not have chiasmata, but chromosome recombination does occur: recombination is restricted, not eliminated.

2.5. Segregation on the basis of chromosome recombination

2.5.1. *Monofactorial segregation*

Two alleles of a single factor, one on one chromosome of a bivalent the other on the other chromosome, do not show meiotic recombination in a sense comparable to that between the alleles of two or more different factors (fig.2.5). There is only separation and subsequent segregation. This is best demonstrated in the asci of the ascomycete Neurospora. The spores are linearly arranged in an order that is determined by meiosis (section 2.4.1; figs.2.7, 2.8). Since after meiosis, but before spore maturation, an extra

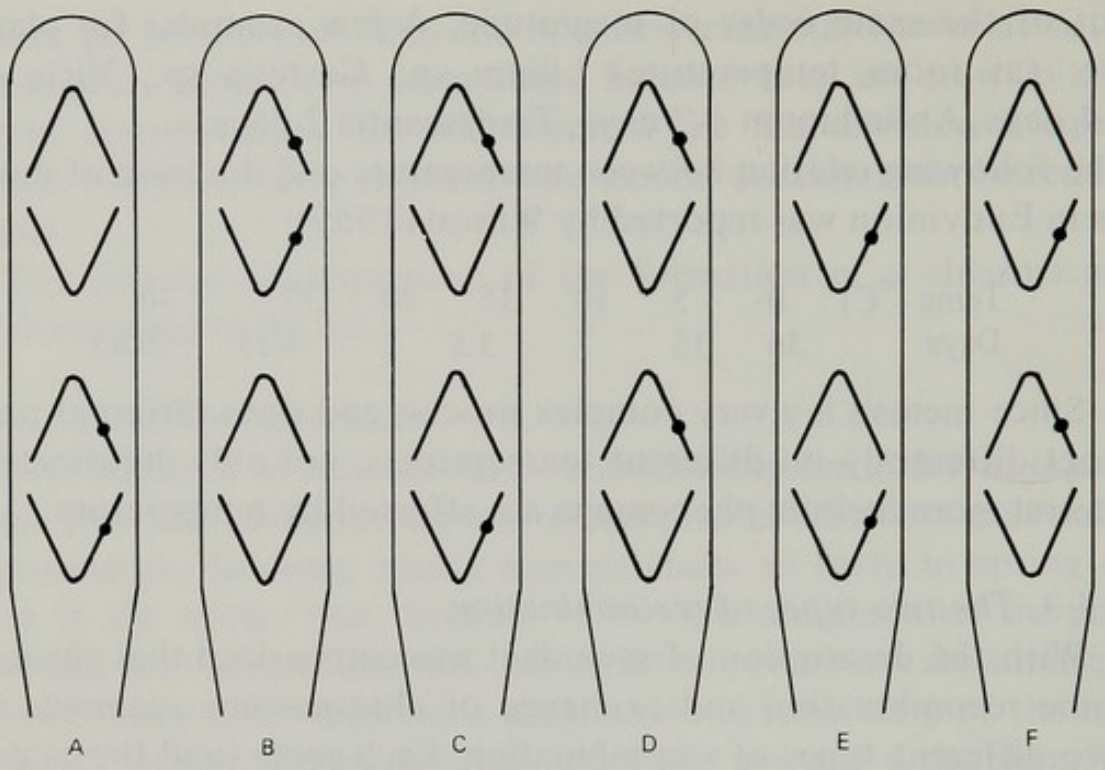


Fig.2.7. Linearly arranged second anaphase of meiosis in the ascus of Neurospora. The black dot symbolizes the allele for black of the spore-colour factor. In A there was no crossing-over, and at AI the chromosome with this allele in both its chromatids happened to move to the bottom pole. In B also, there was no crossing-over, but the chromosome went to the other pole. In C, D, E and F there was crossing-over between the locus of the spore-colour factor and the centromere (compare fig.2.5): both chromosomes had one chromatid with one allele and the other chromatid with the other allele. Depending on the orientation at second anaphase, one of the four types will be produced, but all have equal probability.

mitotic division occurs, there are eight instead of four spores. The top four are the sister-cells derived from the one anaphase I group, the bottom four are sister-cells of the other. In Neurospora there is a gene for spore colour of which one allele causes absence of colour, the other a brown-black colour. Dependent on the pole to which the chromosome with the colour allele passes at first anaphase, either the four top spores or the four bottom spores will be coloured (figs.2.7A,B, 2.8A,B). The chances are the same, so the two cases occur with equal frequency (see also fig.2.9). When other than spore-colour genes segregate, it is not possible to study their segregation in the asci. Such spores can be isolated by micromanipulation, and separate cultures can be bred from each spore.

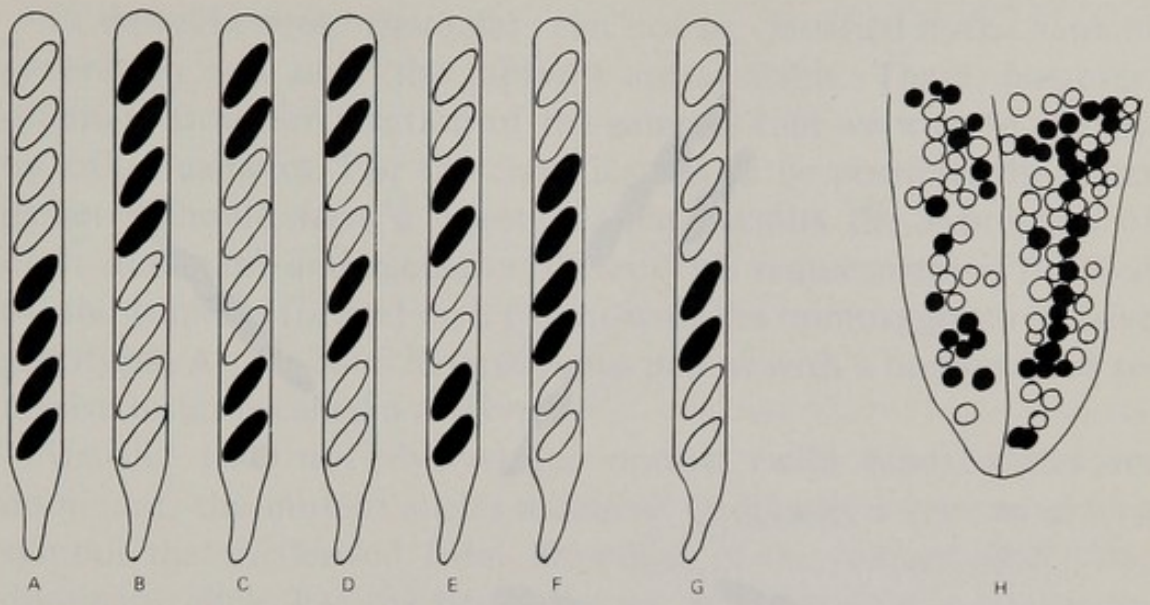


Fig.2.8. The eight linearly arranged ascospores of Neurospora, resulting from the six situations of fig.2.7. The four original products of meiosis have gone through another cycle of division, so that four pairs of identical spores are formed. In G conversion has occurred (see sections 2.1.2, 2.6.3): in one DNA strand during exchange a segment has been replaced by a copy of the pairing partner. It occurs only at or quite near the actual point of exchange and therefore is rather scarce for each individual locus. It can also result in 5:3 and other segregations. (H) Part of an anther of a rice plant heterozygous for the amylodextrin factor. Fifty percent of the pollen has starch, and stains black with iodine. The other fifty percent contains amylodextrin and stains only weakly. The gametic types can be classified, but tetrad analysis is not possible as the spores have separated (H according to Parnell, 1921).

There are numerous morphological, colour and biochemical characters that can thus be classified. The analysis is called *tetrad-analysis*; all four meiotic products can be analysed in their specific location in the ascus and this location is determined by the meiotic processes. The phenomenon that the two alleles of a gene end up in different gametes is called *segregation*. When only the alleles of one factor are considered, it is *monofactorial* segregation.

In plants higher than fungi, there is usually no possibility of tetrad analysis. In exceptional cases such as Salpiglossis, the tetrad remains intact until polliniation and with the proper techniques the genetic composition of the four meiotic products can occasionally be analysed, but not their order. Also, chromosomal forms can be constructed that permit a half-tetrad analysis (compare section 6.4.1).

Fig.2.9. A group of asci in a perithecium of *Neurospora crassa*, demonstrating segregation for spore colour. In young asci the spore colour has not yet developed: all spores are colourless (C.J.Bos, Dept. of Genetics, Wageningen). Compare figs.2.7, 2.8.

Normally, when haplonts can be classified, merely the frequencies of the different segregating types can be determined. In an old experiment by Parnell (1921) rice plants heterozygous for a starch factor were used: one allele determined the formation of normal starch, the other of amylodextrin (glutinous rice). Staining with iodine made the normal pollen grains purple-black, those with amylodextrin light brown (fig.2.8). The number of pollen grains with starch varied somewhat between plants (43.2-51.8%) with an average of 48.1%, close to the expected 50%. A few starch grains may have formed insufficient starch because of immaturity or may simply have lacked sufficient starch to take colour.

In diploids, most characters can not be classified in the haploid generation and only the diplonts are available. These, however, originate from fertilization of the gametes that we wish to classify by other gametes. For the classification to be possible, the latter gametes should have a genotype that permits the expression of both dominant and recessive alleles. This requirement is fulfilled by the gametes formed on a parent with the homozygous recessive genotype. A cross of a heterozygous parent with a homozygous recessive tester is called a *test cross.*

Usually (but not always) the normal (wild type) alleles are dominant, the mutant alleles recessive. Ordinarily a gene receives a symbol that is derived from the name of the mutant allele. The dominant allele has the first letter of the symbol in capital type, the recessive allele in small type, or the dominant allele is indicated by +. *Drosophila melanogaster*, normally with red eyes, has a series of eye-colour mutants, one of which is white eye, symbol *w* (*white*). Normal red can be indicated by *W*, but it is very common in Drosphila genetics to designate this allele by +, $+^w$ or $+_w$.

If the parents (P_1 and P_2) of a hybrid are *homozygous* for different alleles of a gene *A*, i.e. P_1 is *AA* and P_2 is *aa*, then the hybrid (F_1 of filius) is *heterozygous Aa* and forms gametes *A* and *a* in equal frequency (compare fig.2.5). A test cross of this F_1 with homozygous recessive *aa* gives progeny *Aa* and *aa* in the ratio 1:1, the gametic ratios.

Often it is difficult to carry out a test cross. Then two heterozygotes of the same genotype can be crossed or, in hermaphrodites and monoeceous plants, the F_1 can be self-fertilized. The generation thus obtained is the F_2. Since now two segregating populations of gametes are combined, an additional chance element is introduced. Table 2.2 shows the types of gametes that participate, and the segregation in the diploid progeny after selfing, i.e. after random combination of the male and female gametes. With complete dominance, *Aa* and *AA* can not be distinguished and a 3*A*.: 1 *aa* segregation results (*A*. indicates the total of progeny with at least one dominant A allele, i.e. *AA* and *Aa* together). A well-known example is that of Mendel (1865) who analysed the genetics of the characters "smooth" and "wrinkled" in peas. The par-

ents were *AA*, smooth (dominant) and *aa*, wrinkled (recessive). *Aa* was smooth. The peas on the F_1 plants are the diploid F_2 progeny and can be classified immediately. On 253 F_1 plants Mendel obtained 7324 seeds after selfing: 5474 were smooth and 1850 were wrinkled, a ratio of 2.96:1. Wrinkled is a property of the starch in the pea cotyledons and close observation reveals that heterozygotes have slightly abnormal starch, which makes it possible to recognize them. If the phenotype of the heterozygote is inbetween that of the homozygous dominant and the homozygous recessive type, inheritance is said to be *intermediate*. The segregation (table 2.2) now can be recognized as 1:2:1 (*AA* : *Aa* : *aa*). Another old and well-known example of intermediate inheritance in plants is that of Correns (one of the re-discoverers of Mendel's "laws"): flower colour of *Mirabilis jalapa*: *RR* is red, *Rr* is pink and *rr* is white. In such cases gene dose apparently is important for character expression.

Table 2.2
Monofactorial segregation.

Parents *AA* and *aa*. F_1 *Aa* with gametes *A* and *a* formed with equal frequency. Random combination in F_2:

♀ \ ♂	*A*	*a*
A	*AA*	*Aa*
a	*Aa*	*aa*

There are four combinations of two gametes. Of these three have at least one *A* allele, and when *A* is dominant, with random combination the segregation is 3*A*.: 1*aa*. With intermediate inheritance, *Aa* is distinct from *AA* and *aa*, and the segregation is 1:2:1.

With complete dominance, the heterozygotes can only be distinguished from the dominant homozygote by growing another generation, the F_3: upon selfing, *AA* F_2 individuals give exclusively *AA*, but *Aa* again gives a 3 : 1 segregation. In the same series of experiments by Parnell (1921, see above and fig.2.8H) on starch-amylodextrin segregations in rice, where a 1:1 ratio had been demonstrated in pollen grains, the starch in the seed on F_1 plants

(upon selfing) was analysed. Like in peas, this seed represents the F_2 generation. In 9 plants, 5292 seeds had starch and 1587 amylodextrin. With 3:1 segregation, 5159 and 1720 respectively were expected. The seeds had to be sacrificed for the test, but from another 81 F_1 plants, 17961 F_2 plants were grown, that were analysed in respect to *their* seeds i.e. the F_3 was tested. It appeared that 9211 F_2 plants had only starchy seeds. These apparently were homozygous for the (dominant) starch-allele; 13729 F_2 plants had seeds of both types (were heterozygous) and 5021 had only seeds with amylodextrin. With a 1 : 2 : 1 segregation this should have been 6990, 13980 and 6990 respectively. Although the principle of an F_3 classification is demonstrated, the discrepancy between observed and expected frequencies is considerable. Yet the segregation in the anthers had been shown to be normal. This represents a rather common tendency in such segregations: there is a *shortage of recessives* in the progeny. Recessive mutations often have an unfavourable effect that makes the haploid gametes carrying them (or the diploid homozygotes) less competent. In plants where the haploid generation completes a certain development of its own, the effect can occasionally be rather severe. In animals the effect on the haploid is usually small; but here the diploid generation tends to be more sensitive to genetic unbalance, especially in the very early stages of development.

When only two alleles of one factor are considered, there is no recombination with respect to the formation of the gametes; only segregation of the two alleles occurs. With respect to the diplont, however, a certain form of recombination can be observed: from the single genotype *Aa*, different recombination types arise: *AA* and *aa* along with the original *Aa*. Recombinants: (*AA* and *aa*) and original types occur in the same frequency 2 : 2.

2.5.2. *Segregations involving more than one factor with chromosome recombination*

When two factors are considered, each in a different chromosome, and each with two alleles (a *bifactorial* segregation) there is a possibility of recombination of the two pairs of alleles in the haplont: a hybrid *AaBb* of parents *AABB* (gametes *AB*) and *aabb*

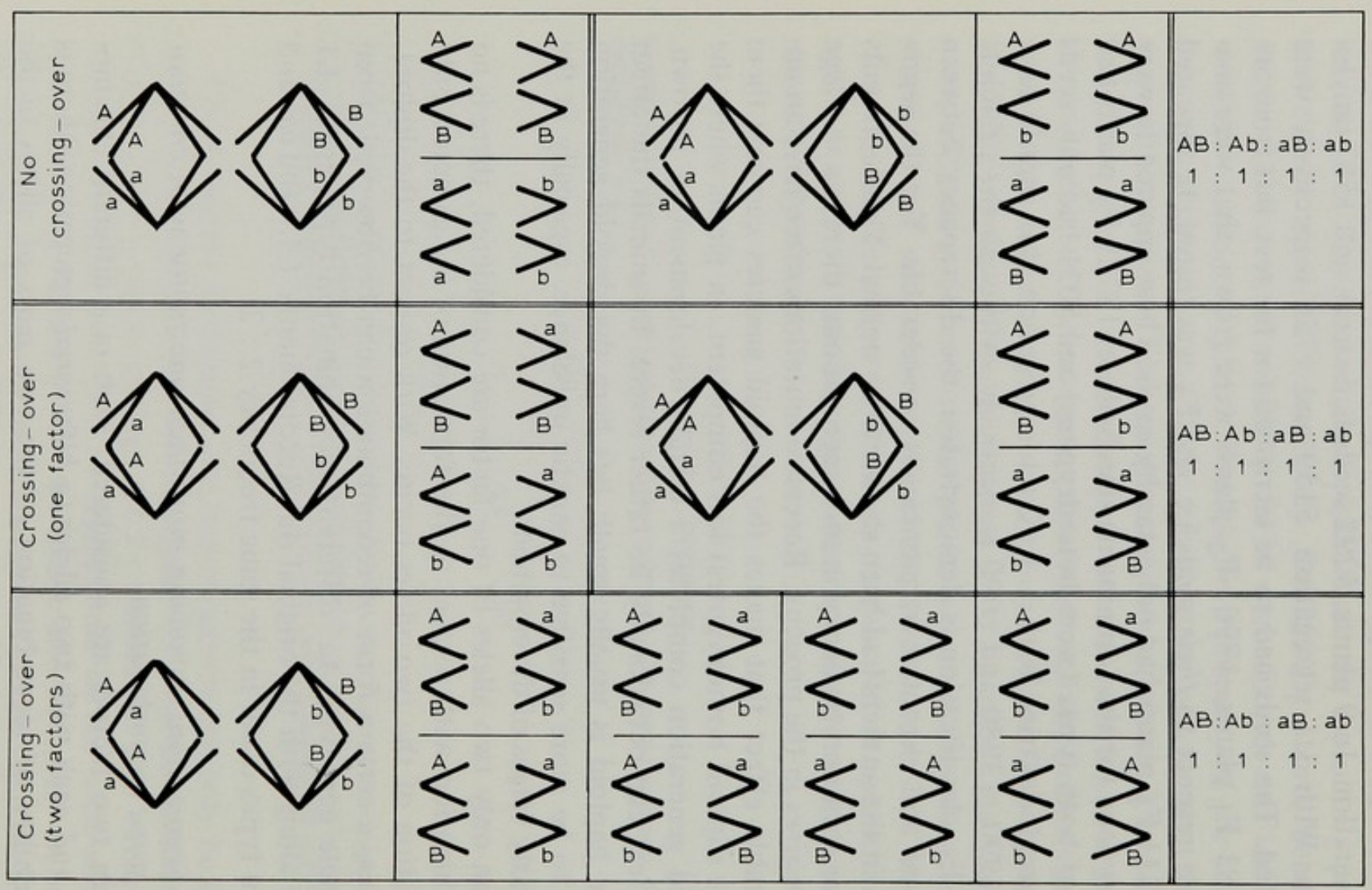


Fig. 2.10. Independent segregation of two genes in two chromosomes. Because of independent orientation of the two bivalents at MI and of the chromosomes in AII, the two types of tetrad occur with equal frequency, and this results in equal frequency of the four classes of gametes: *AB*, *Ab*, *aB*, *ab*.

(gametes *ab*) respectively, can have recombinant gametes with *Ab* and *aB* in addition to the parental gametes *AB* and *ab*. Direct observation is again possible with tetrad analysis in Neurospora. Sufficient spore-colour factors are usually not available, but with micro-manipulation the spores can be isolated and grown to mycelia that permit the analysis of a large number of morphological and biochemical mutants. In diploids a test cross is preferably applied since it has a better resolution than an F_2. Frequently, however, an F_2 is all that is available. The following example is again from Mendel, who formulated the foundations of classical genetics on the basis of the segregations he observed in these and similar experiments. The pair of alleles *A* and *a* for smooth and wrinkled peas, was combined with the pair *B* and *b* for green and yellow cotelydons. For the present it may be assumed that the two genes are on different chromosomes (fig.2.10). The orientation of the bivalents is independent (for exceptions see chapter 7), and therefore *A* goes to the same pole with *B* as frequently as it goes to the same pole with *b*. For the same reason *a* is combined with *b* as often as it is with *B*. The second division does not affect this segregation. The two types of tetrad of fig.2.10 are formed with equal frequency. In the final population of gametes, *AB*, *aB*, *Ab* and *ab* occur with equal frequency. With parents of genotypes *AABB* and *aabb*, the gametes *Ab* and *ab* are the result of chromosome recombination. If the parents would have been *AAbb* and *aaBB*, which is equally well possible, the gametes *AB* and *ab* on the hybrid would be the recombinant types. In both cases parental and recombinant types occur with equal frequency: there is 50% recombination.

Recombination in higher organisms is generally expressed in *gamete frequencies.*

In a test cross of *AaBb* with *aabb* (gametes exclusively *ab*), the (diploid) type *AaBb* is formed as often as the types *aaBb*, *Aabb* and *aabb*. In the F_2 (formed by self fertilization or by crossing two genotypically equal F_1 individuals), the four different types of gametes are combined randomly, which results in $4 \times 4 = 16$ types (table 2.3), formed with equal frequency. Nine out of these 16 have both *A* and *B* and therefore are phenotypical-

Table 2.3
Bifactorial segregation.

Parents *AABB* and *aabb* or *AAbb* and *aaBB*. F_1 *AaBb* with gametes *AB*, *Ab*, *Ab* and *ab* formed with equal frequenc· when *A* and *B* are on different chromosomes. Random combination in F_2:

♀ \ ♂	*AB*	*Ab*	*aB*	*ab*
AB	*AABB*	*AABb*	*AaBB*	*AaBb*
Ab	*AABb*	*AAbb*	*AaBb*	*Aabb*
aB	*AaBB*	*AaBb*	*aaBB*	*aaBb*
ab	*AaBb*	*Aabb*	*aaBb*	*aabb*

There are sixteen combinations of which with dominance of *A* and *B* nine have at least one dominant allele of both factors (*A.B.*). Three have at least one dominant allele for one factor and two recessive alleles for the other factor (*A.bb*) and three have at least one dominant allele for the other factor and two recessive alleles for the first (*aaB.*). There is one with only recessive alleles for both factors. With random combination the segregation is 9:3:3:1. For interaction between factors see text section 2.5.2.

ly (double) dominants (superficially the dominance in Mendel's experiment was complete). One dominant and the other (double) recessive (*aaB.* and *A.bb* respectively) occurs three times out of 16. Only one out of 16 is a double recessive (*aabb*). The ratio is 9 : 3 : 3 : 1. Mendel observed 315 : 101 : 108 : 32, which is in very good agreement with expectation. *This ratio results from the chance combination of two 3 : 1 segregations.*

This was the simple case in which both alleles of both genes could be distinguished. Sometimes, however, the two genes interact and then the expression can be altered such that classification is complicated and that not all four classes can be distinguished. With intermediate gene action on the other hand, a larger number than four classes can be recognized, up to 16.

With *polymery* both genes have the same action. As soon as one of the four alleles is dominant the complete effect is realized. This is the case in 15 out of the 16 F_2 classes, and therefore a 15 : 1 F_2 segregation is expected, and a 3 : 1 segregation in a test cross. Gene action can also be intermediate with polymery and when the effects are cumulative, the expression depends on the number of + alleles, and there is a series of transitions between one extreme

(double homozygous + type) and the other extreme (double homozygous – type). In this case only infrequently the 16 classes can be recognized. When the expression is affected by environmental variation, as may be expected in quantitative characters such as length, weight etc., classification becomes impossible and the phenotypes blend. With such quantitative characters, usually more than two factors are involved (occasionally even quite many) and in addition dominance and epistasy (see below) occur in varying degrees. Then these characters are not accessible for simple genetic analysis. Statistical analysis of the pattern of variation, however, can give some insight in the genetic system (*quantitative genetics*).

Two or more *complementary* factors each regulate the formation of one enzyme, while both enzymes are required for a function to be carried out. In the simplest case, the dominant allele enables the enzyme to be formed, while the recessive allele is not functional. Only when of both factors at least one dominant allele is present, the complete function can be realized. It appears from table 2.3 that this is the case in 9 out of the 16 possible combinations: the segregation is 9 : 7.

With *dominant epistasy* the action of one factor is not expressed when the dominant allele of the other factor is present. An example is the feather colour of white leghorns. These have a colourfactor, here indicated by *K* and a suppressor of colour, *O*. The cross between *KKOO* (white, because colour is suppressed) with *kkoo* (also white because the colour gene is in its recessive state) gives a F_1 *KkOo* (white, because there is an *O* allele). Crosses among F_1 animals result in a F_2 progeny in which all those with *O* are white. These are 12. In addition, *kk* is white, whatever the condition of the suppressor: *o* or *O*. This gives one additional type (*kkoo*) and the segregation is 13 white: 3 coloured. When the recessive suppressor allele is not present in the population, all animals are white, irrespective of the colour factor *K*. The factor that prevents the expression of another gene is called *epistatic*. In the present example it is a *suppressor*, but this is not necessarily always so. It may, for instance be a factor for a dark colour against which a lighter colour is not visible. The gene that is not expressed is *hypostatic*.

Related to epistasy and complementary factors is *cryptomery*. Only the initial situation is different from that with complementary factors: now one factor is dominant and the other recessive in one parent. In the other parent, on the other hand, the first is recessive and the second is dominant: in neither parent complementary action is possible. In the F_1, one dominant allele of both factors is present, and the function is expressed. An example is *Lathyrus odoratus*, that has two genetic types of white flower colour: *CCrr* and *ccRR*. Crossed they give violet colour in F_1 because *C* and *R* together can make anthocyanin, which separately they can not. In the F_2, of course a 9:7 segregation can be expected. In both parents *R* and *C* respectively were cryptomerous. This situation is also called *reciprocal recessive epistasy*. Actually it is nothing but a pair of complementary genes. There are more 9:7 segregations, all to be explained along similar lines.

With other interactions between factors 9:3:4, 12:4 and other segregations are possible.

Three factors in three different chromosomes, each with two alleles (*Aa Bb Cc* is the triple heterozygote) give $2^3 = 8$ different types of gametes: *ABC*, *aBC*, *AbC*, *ABc*, *Abc*, *abC*, *aBc* and *abc*. In F_2 this results in 64 zygote genotypes, one of which is the triple (homozygous) recessive. Dependent on dominance, complementation and epistasy, a large number of different segregations are possible.

Deviation from normal segregation ratios can have different causes. Reduced viability of gametes has been mentioned in section 2.5.1. Further causes are partial or complete lethality of homozygous recessive diploids, or of male or female gametes. Often gametes of a certain genetic make-up lag behind in fertilization in competition with gametes with a different constitution (*certation*). A special case are certain forms of incompatibility, that have been especially thoroughly analysed in plants. Pollen grains with an incompatibility factor do not germinate or do not develop normal pollen tubes on styles with the same allele of the incompatibility factor. Several systems have been described. Usually many different incompatibility alleles of the same factor occur in one population. Incompatibility systems are very effective in pre-

venting like genotypes to be combined and thereby in maintaining heterozygosity.

Again in plants, unfavourable interactions between embryo, endosperm and motherplant in hybrids of special genetic constitution can lead to embryo abortion, and thus also represent a form of incompatibility (*somatoplastic sterility*). These interactions sometimes have their origin in unusual combinations of ploidy levels. In most diploids the relation is: diploid embryo, triploid endosperm, diploid mother. In a number of species the cross between diploid and tetraploid is sterile since the ploidy levels triploid embryo, pentaploid endosperm and tetraploid mother appear to be incompatible. Comparable phenomena, usually not involving differences in ploidy level have been reported for the interaction between embryo and mother in animals. The causes usually can not easily be traced, but in the well-known case of the rhesus incompatibility in humans, there is a single allelic difference in a blood factor between mother and child.

2.6. Segregations involving crossing-over

2.6.1. *Chiasmata and crossing-over in eukaryotes*

Already in 1909, Janssens in his *chiasmatype theory* suggested that the chiasmata visible at diplotene would correspond with chromatid exchange. An alternative explanation of chiasmata was that they would result from a special mode of sorting-out the four

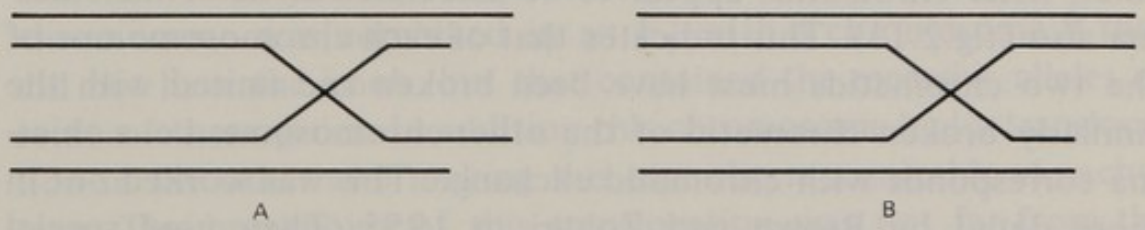


Fig.2.11. A chiasma in a heteromorphic bivalent: one chromosome has a terminal deficiency, the other is normal. If the chiasma would have resulted merely from wrongly sorting out the four chromatids, the shape of A would have been observed every time a chiasma was seen. This is never the case. Actually only the shape of B is found, and this can only be produced by breakage and "illegitimate" reconnecting of the four chromatids.

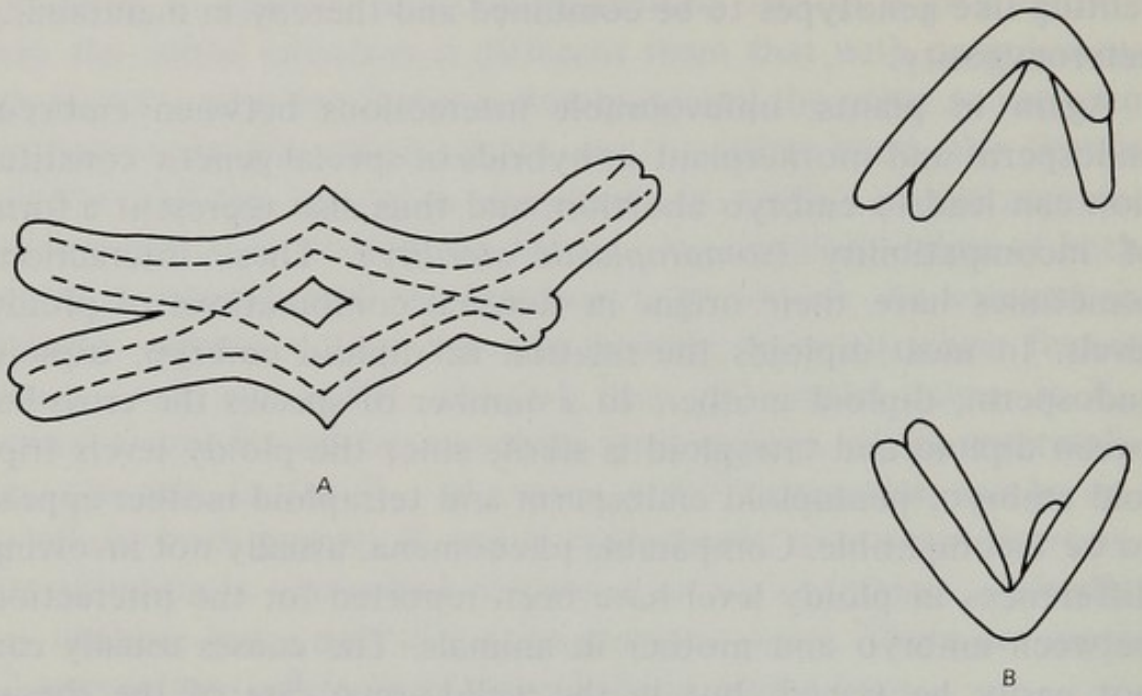


Fig.2.12. Illustration of the demonstration by Brown and Zohari that a chiasma observable at diakinesis and MI, and a chromatid exchange seen at AI are expressions of the same event: The frequency of heteromorphic bivalents (A) with one chiasma (more were not found) between the centromere and the end of the deficiency arm, agrees perfectly with that of chromatid exchange scored at anaphase I (B).

chromatids of the two pairing chromosomes. On the centromere-side of the chiasma (the proximal segment) the sister chromatids of each chromosome would have remained together, distal of the chiasma non-sister chromatids would be associated (*two-plane* theory). This hypothesis is not tenable since in *heteromorphic bivalents* in which the two chromosomes are morphologically distinct, sister chromatids appear to be associated distal of the chiasma also (fig.2.11). This indicates that of each chromosome one of the two chromatids must have been broken and united with the similarly broken chromatid of the other chromosome: i.e. a chiasma corresponds with chromatid exchange. This was worked out in more detail by Brown and Zohari in 1955. They used special stocks of *Lilium formosanum*, in which the *A* bivalent was heteromorphic. It had a large deficiency in one of the two homologous chromosomes. The presence or absence of a chiasma (there never appeared to be more than one) in the segment remaining between the centromere and the deficiency was scored in diakinesis and in

metaphase I in the pollen mother cells. Whenever a chromatid exchange had occurred, the two chromatids of an anaphase chromosome should have become different and recognizable as such. Brown and Zohari, therefore, also scored the frequency of cells at anaphase I with chromosomes that had different chromatids, i.e. the frequency of chromatid exchange (fig.2.12). In one plant 70% of the heteromorphic bivalents at metaphase I had a chiasma between the centromere and the deficiency, and at anaphase in sister cells in the same anther, 71% exchange was observed. In a progeny-plant, equivalent in respect to chromosomal constitution, there were chiasmata in 51% of the cells at metaphase and exchange was scored at anaphase in 55%. A comparable deficiency in another bivalent showed 90% chiasmata at diakinesis and 89% chromatid exchange at anaphase in the year 1951. In 1953 the percentages were 79 and 78% respectively. Such a striking correspondence between the frequencies of observed chiasmata and scored chromatid exchanges is convincing proof that chiasmata correspond with chromatid exchange. Several more arguments against the two-plane theory can be produced, but are not necessary.

That genetic crossing-over (recombination between genes in the same chromosome, as observed in segregating progenies of a heterozygote) results from exchange of chromosomal segments had already been shown by Stern for Drosphila and by Creighton and McClintock for maize, both in 1931. Creighton and McClintock used stocks of maize in which the short arm of chromosome 9 was marked by a terminal knob, recognizable at pachytene. The same arm contained the dominant alleles of several known genes. This chromosome was combined in an F_1 with a chromosome 9 that did not have the knob, but that contained the recessive alleles of some of these genes. In addition this chromosome had a translocation in the other arm making this arm also recognizable at pachytene. The breakpoint of this translocation was not far from the centromere. The F_1 was crossed to a normal stock homozygous for the recessive alleles. In the testcross generation knob and translocation could be classified microscopically and the presence of either the dominant or recessive alleles of the marker genes could be scored morphologically. All cases of exchange in the F_1 could

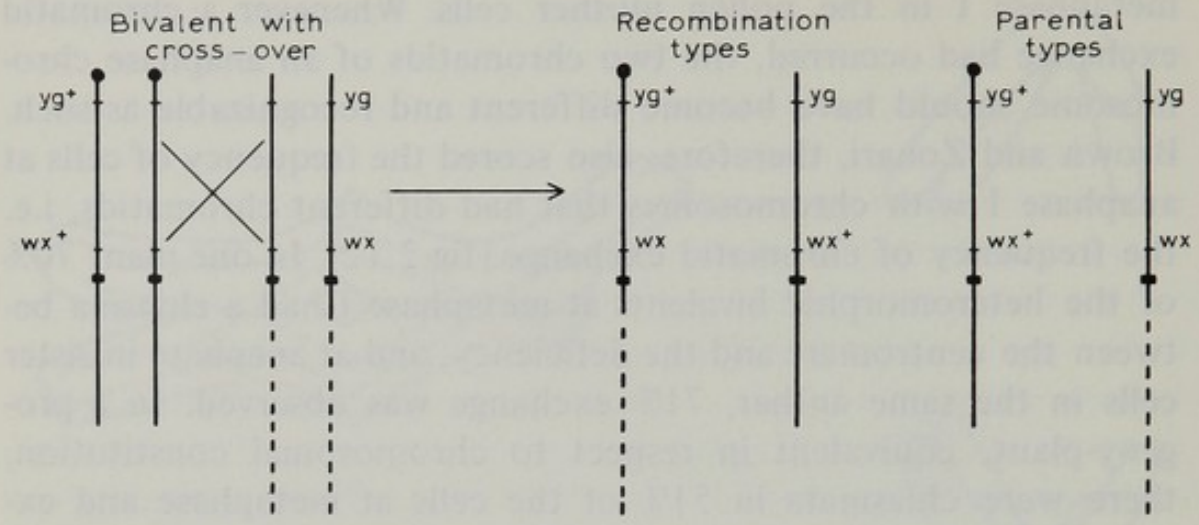


Fig.2.13. Diagram of the demonstration by Creighton and McClintock that exchange between chromosome segments corresponds with genetic recombination (crossing-over). Exchange between the genes *wx* and *yg* is accompanied by exchange between the morphological markers on the two homologous chromosomes 9, of which one had a heterochromatic knob at one end, and the other could be recognized by a reciprocal translocation (striped). The centromere is represented by a small square.

thus be traced (fig.2.13), and it appeared that whenever genetic crossing-over was observed between the two genes, there also had been exchange between the translocation and the knob (see also section 6.4.2). Since the genes were close to the chromosomal markers, exchange between the genes and these markers was negligible. When genes were studied that were further away from the knob and the translocation, recombination between these genes and the chromosomal markers could be demonstrated.

The effect of exchange between the centromere and a locus (= the place of a gene on the chromosome) in tetrad analysis is shown in figs.2.7, 2.8 and 2.9). In the example a spore colour factor in Neurospora is used and the position of the recessive and the dominant allele in the ascus is shown. Like in A and B of these figures, where exchange had not occurred, anaphase I orientation determines to which pole the respective (double) centromeres will go. With exchange, both chromosomes have one allele in one of the two chromatids, and the other allele in the other chromatid. As a consequence, the top spores contain both types as do the bottom spores. Anaphase II orientation determines the final arrangement and in this respect the top and the bottom are indepen-

dent. There are four possibilities, and these are realized with equal frequency. Since exchange types (C,D,E,F) can be distinguished from non-exchange types (A,B) the percentage of recombination between locus and centromere can be determined.

When the four tetrad cells are unordered or free from each other, or when only one cell develops, it is impossible to conclude whether or not crossing-over between locus and centromere has occurred. Then it is occasionally possible to construct special chromosome constitutions that permit the estimation of crossing-over frequencies between locus and centromere (section 6.4.1).

2.6.2. *The mechanism of exchange*

In eukaryotes and in prokaryotes, DNA exchange is preceded by chromosome pairing and followed by separation (segregation). In prokaryotes the process is elaborate, but in eukaryotes, with their long and complex chromosomes that often occur in large numbers per cell, an extravagant biochemical and structural machinery is required to realize a single chromatid exchange. In prokaryotes perhaps temporary and local strand separation is sufficient to cause attraction between homologous strands of different chromosomes. In eukaryotes, the concentration of homologous DNA available for association is so low and the mobility of the protein-complexed DNA is so restricted that chance association is improbable. The subtle genetic regulation of the chromosome pairing processes also indicates that other processes than mere chemical association are involved, especially in the initial attraction between homologous chromosomes.

In a limited number of species of animals (Drosophila is a well-known example) and even a few plants, the pairing of homologous chromosomes is not restricted to meiosis but occurs quite regularly in all somatic and meiotic cells. The association is most easily recognized during the condensed stages (metaphase, anaphase) but it is not clear whether pairing at these stages is autonomous or is merely a remnant of pairing at the diffuse stages. Incidental close somatic pairing has been observed repeatedly in organisms that normally do not show somatic pairing (Arabidopsis, sugar beets, Haplopappus, etc.). In other instances weak, hardly detectable as-

sociation is believed to occur regularly in special genotypes (wheat, according to Feldman et al., 1967). Occasionally, somatic association in heterozygotes can lead to crossing-over, that is detectable when it gives rise to sectors in which recessive alleles are expressed. Although the frequency of somatic crossing-over generally is not of much genetic importance, somatic pairing has often been suggested to be important for preparing meiotic pairing. Especially in late premeiotic stages, somatic chromosomes can often be seen to associate (Rhoeo, Haplopappus) and several authors, for instance Brown et al. (1969) and Maguire (1967) have suggested that premeiotic pairing of condensed somatic chromosomes is a general phenomenon. Initiation of pairing at condensed stages would indeed prevent the entangling of the chromosomes and interlocking of bivalents, which is surely expected when unassorted, extended chromosomes pair. Indeed in a number of fungi (Barry in Neurospora, and Lu and Raju in Coprinus) it can be seen that homologous chromosomes pair in a state of contraction just after nuclear fusion. They remain relatively unextended throughout the later stages of meiotic prophase, although during a great part of these stages they have a somewhat diffuse appearance. Apparently crossing-over also takes place in this condition, and DNA pairing may only involve short apposed segments.

Premeiotic pairing at contracted (not necessarily *compact*) stages are not universially observed. In *Lilium longiflorum* "Croft", in which a stage of relative contraction occurs between S-phase and leptotene, Walters (1970) could not see any indication of alignment of homologues. Whether nevertheless in cases without clear alignment at condensed stages pairing is initiated immediately upon decontraction when the chromosomes are diffuse but still relatively short and rod-shaped, is not easily decided.

Whatever the stage of pairing, the mechanism is still unknown. It has been suggested that pairing of relatively condensed chromosomes that cannot be a function of the DNA, is regulated by specific loci on the chromosomes, the *zygomeres*. In some species a few might occur in each chromosome, in others many (Sybenga, 1966).

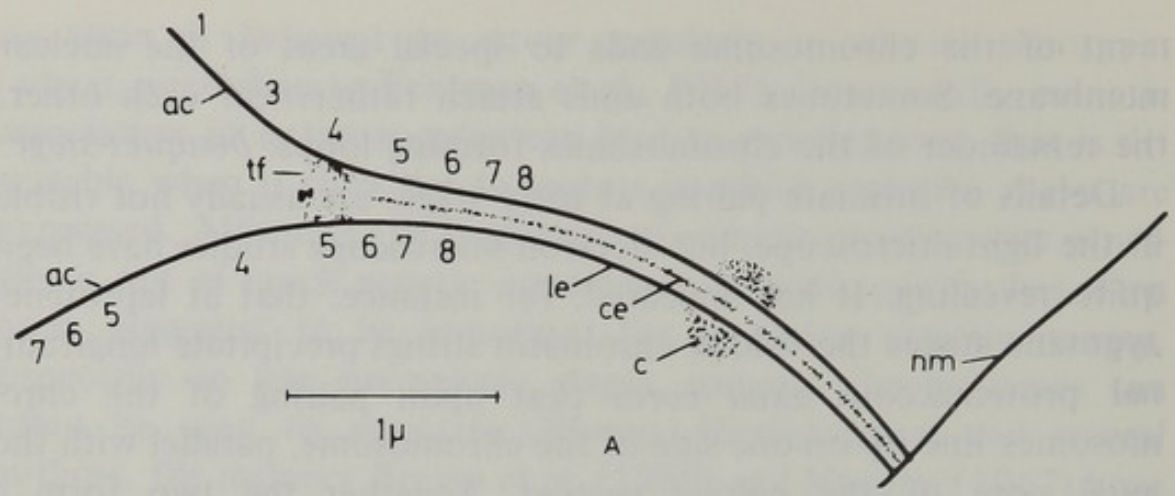
Alignment of chromosomes is often brought about by attach-

ment of the chromosome ends to special areas of the nuclear membrane. Sometimes both ends attach rather near each other, the remainder of the chromosomes forming loops: *bouquet-stage.*

Details of intimate pairing at early stages are usually not visible in the light microscope, but electron microscope studies have been quite revealing. It has appeared, for instance, that at leptotene-zygotene stages the folded chromatin strings precipitate longitudinal proteinaceous *axial cores* that upon pairing of the chromosomes line up on one side of the chromosome, parallel with the axial core of the pairing partner. Together the two form a third band, the *central element*, between them. It sometimes is double or even triple and may show striations (fig.2.14). The three bands together, two lateral and a central element form the *synaptonemal* or *synaptinemal complex.*

The formation of the central element depends on the association of the two homologues. It is not formed when the chromosomes do not pair intimately, even when the lateral elements are formed in the two chromosomes. Details of the synaptonemal complex in several insects have been described by Moses (1969a,b), Sotelo and Wettstein (1966) and others, while Moens (1968) made detailed studies of the synaptonemal complex in plants like lilly and tomato. Moens (1969) also demonstrated that in *Locusta migratoria* the formation of the central band is preceded by protein connections between the associating chromosomes (fig.2.14). Close association of the chromosomes was seen to start at the ends that attached to the nuclear membrane. From here on the formation of the synaptonemal complex proceeded. At these stages individual chromatids are not visible. At diplotene and diakinesis the synaptonemal complex is not immediately dissolved. It is released from the chromosomes and often appears to maintain a nonspecific form of attraction that results in a stacking-up of individual fragments of the lateral elements. At these stages the individual chromatids become distinguishable.

The synaptonemal complex probably has a role in the alignment of the chromosomes, but its function in crossing-over is not clear. Although there is a strong correlation between the occurrence of chiasmate meiosis and the occurrence of a synaptonemal



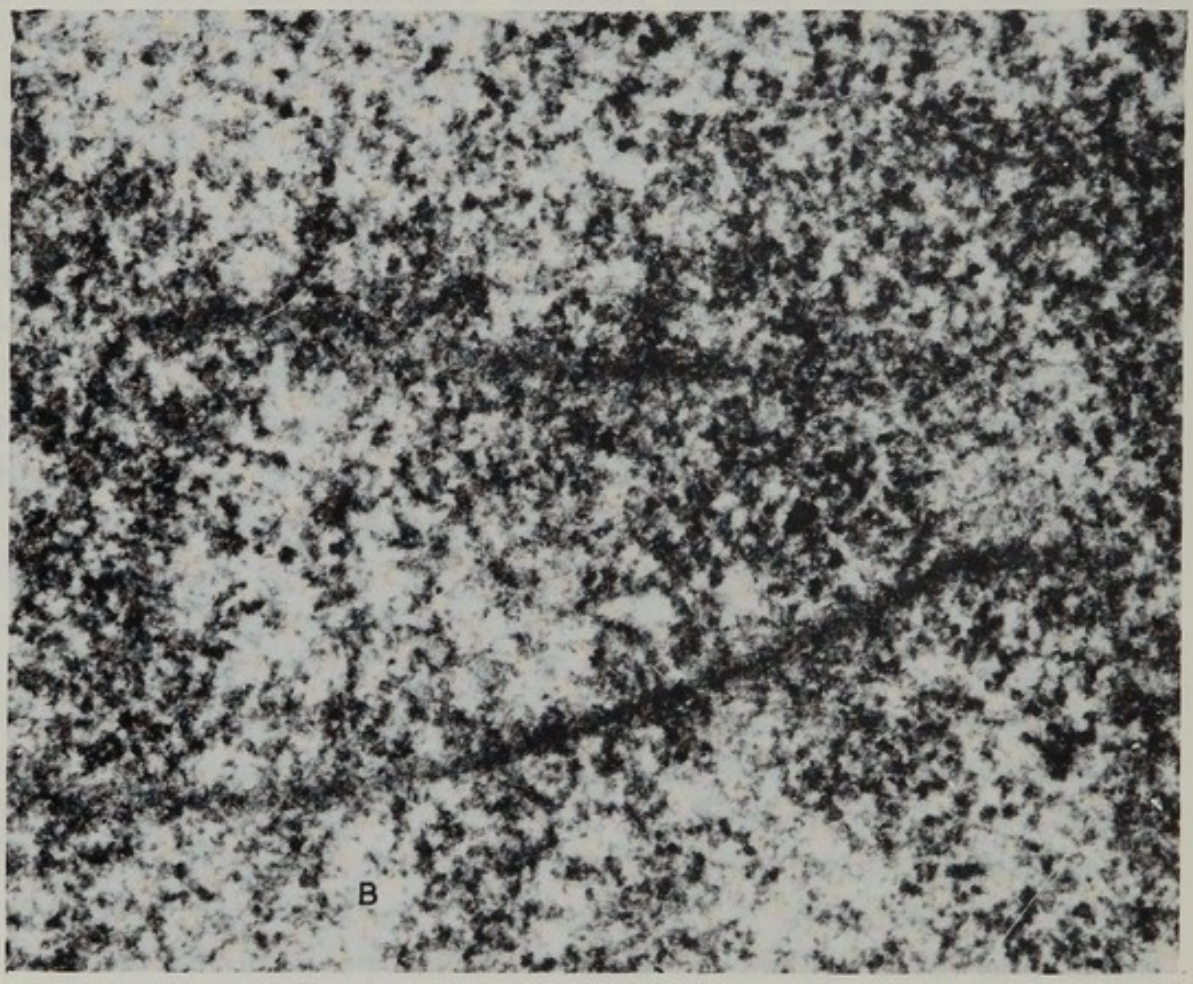
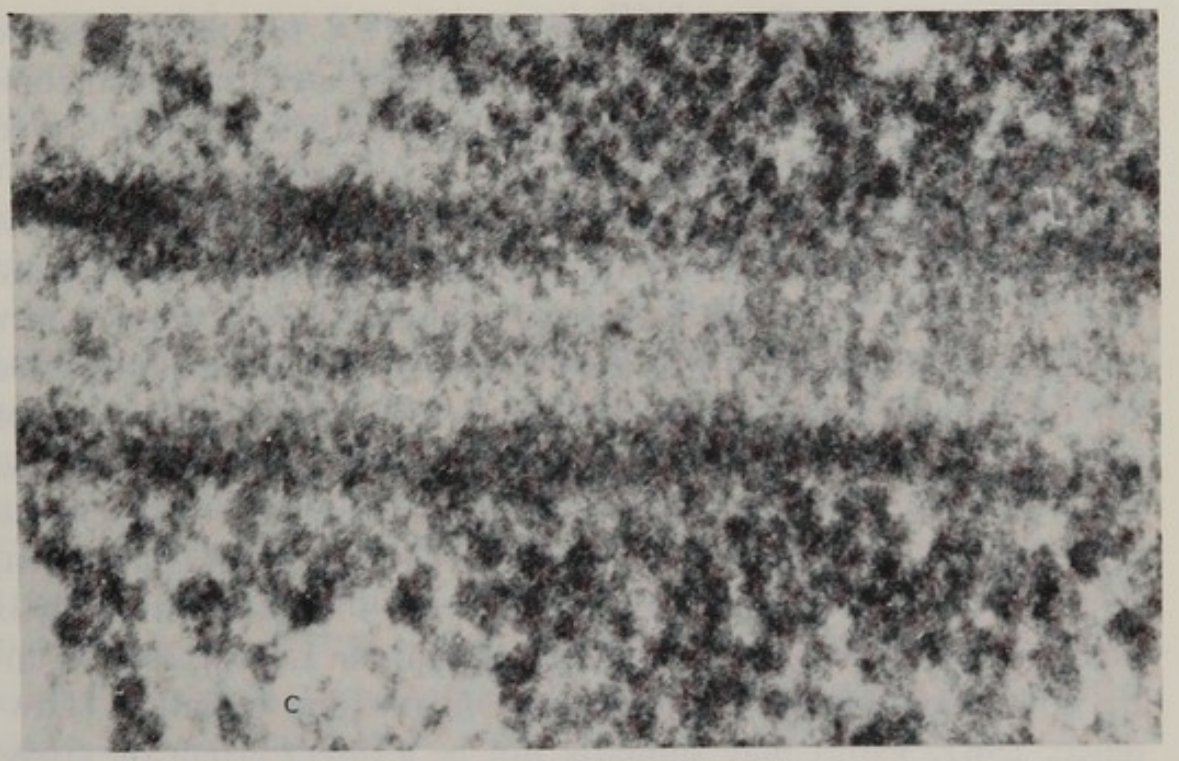
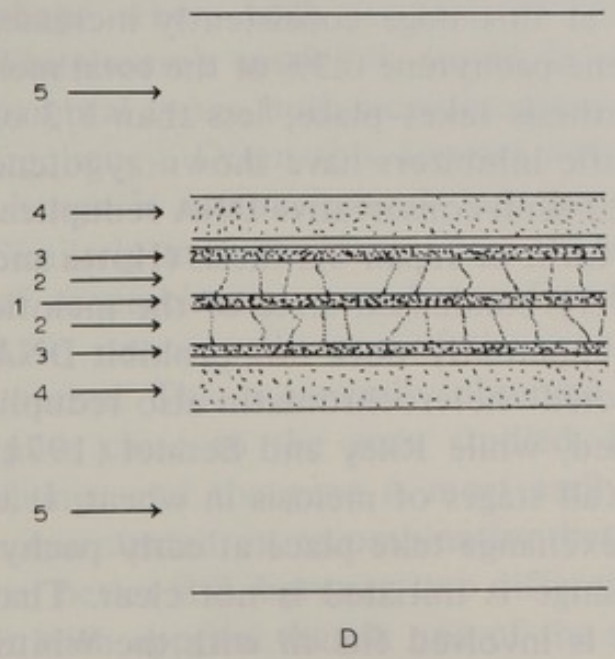


Fig.2.14. The synaptonemal complex. (A) The process of pairing in *Locusta migratoria*: pairing has started at the chromosome ends associated with the nuclear membrane. Here the complex is complete with the two axial cores of the paired chromosomes, now the lateral elements of the synaptonemal complex, and the central element. The axial cores are present also before pairing, but the central element is formed during pairing and this is preceded by the formation of protein strands (transverse filaments) connecting the pairing chromosomes. Diagrammatic composition from a series of electron micrographs (Moens, 1969). (B) One electron micrograph of the series of A, showing the two axial cores approaching each other and forming the transverse filaments.



(C) Completed synaptonemal complex at zygotene–pachytene of *Lilium tigrinum*. (Courtesy Dr. P.B. Moens.)



(D) The components of the synaptonemal complex. To make the different parts visible and their composition recognizable, different staining and digestion methods are required which can not be carried out in the same preparation. (Diagrammatic after Moses, 1968.)

(1) Central element composed of basic and acidic proteins and perhaps some DNA. (2) Central space traversed by protein filaments at least some of which contain DNA. (3) Inner segments of the axial cores with DNA, and basic and acidic proteins. (4) Outer segments of the axial cores with basic and acidic proteins, and some DNA. (5) Main microfibrilar mass of chromatin with DNA, and basic and acidic protein.

It should be noted that the chromatin surrounds the complex on all sides.

complex, this correlation is not absolute. A few instances are known of crossing-over without demonstrable synaptonemal complex, and occasionally a synaptonemal complex has been observed in meiosis without chiasmata.

Although neither the exact nature of exchange nor the exact structure and form of the DNA in which exchange takes place are known, experiments with externally applied modifying factors have given indications of the stages at which the processes decisive for exchange take place. In Lilium perturbance of the course of meiosis, for instance by transplantation or by heat shock, at the time of DNA synthesis greatly reduces crossing-over. Often the cells revert to mitosis (Stern and Hotta, 1969a,b; Hotta and Stern, 1971). Where only crossing-over is affected the effect probably is merely through pairing. A simular reduction in Lilium, Tradescantia and Chlamydomonas resulting from ionizing radiation (Lawrence, 1961, 1965) may similarly not be a direct effect on crossing-over. However, the observation that some DNA inhibitors increase and others decrease crossing-over when applied during premeiotic DNA synthesis makes the picture complicated (Davies and Lawrence, 1967).

A second stage that is sensitive to external influences is early pachytene. Here again transplantation and heat shock cause a reduction in chiasma formation in Lilium, but no reversion to mitosis. Ionizing irradiation, however, at this stage consistently increases crossing-over. In lilly at zygotene-pachytene 0.3% of the total meiotic and premeiotic DNA synthesis takes place, less than 1/3 of this at pachytene. While specific inhibitors have shown zygotene synthesis to be a completion of semi-conservative DNA reduplication, pachytene synthesis is a form of repair synthesis (Hotta and Stern, 1971). That the relatively condensed state of the meiotic chromosomes at these stages apparently does not prohibit DNA synthesis is not quite unexpected: heterochromatin also reduplicates when relatively condensed, while Riley and Bennet (1971) found some DNA synthesis at all stages of meiosis in wheat. It is clear that the final stages of exchange take place at early pachytene, but exactly when exchange is initiated is not clear. That some form of DNA synthesis is involved fits in with the White-

house model, and that it is repair DNA synthesis agrees with the many observations that mutants affecting radiation sensitivity also affect crossing-over. The first observations along these lines were made by Holliday (1965, 1967) who found that UV-sensitive mutants of Ustilago had drastically decreased recombination frequencies: the DNA repair enzymes were deficient and these same enzymes were necessary for DNA exchange. Later, others made similar observations on fungi and yeasts. Subsequently many recombination-reduced (*rec*$^-$) mutants in bacteria were found to be radiation sensitive (Howard Flanders and Theriot, 1966). This again was followed by many similar observations in other bacteria. Even desynaptic mutants (section 2.9.10) in higher organisms (barley: Riley and Miller, 1966; Drosophila: Watson, 1969; man: Pearson et al., 1970), having greatly reduced chiasmafrequencies, could be shown to be more radiation sensitive than their normal sibs.

2.6.3. *Reciprocity of exchange. Conversion*

Usually in bacteria exchange takes place between one complete chromosome and an incomplete one, often merely a fragment. Recombination still leaves one chromosome complete, the other incomplete. The incomplete chromosome is usually not recovered in the progeny and the exchange seems to be non-reciprocal. In phage, where both products of exchange are viable, on basis of Whitehouse's model it would be expected that besides the two parental types, both recombination types would appear with equal frequency. Often this appears not to be the case: here again exchange is non-reciprocal, but the mechanism must be different. Similarly in eukaryotes where the separate products of one event of recombination can be analysed, as in Ascomycetes (figs.2.7, 2.8), occasionally one of the recombination products is not recovered. This is generally observed when the point of exchange is quite close to the gene studied. Proximity of the point of exchange and the gene is most easily realized when the analysis is concentrated on recombination between very closely linked genes or, better still, between two different mutant sites of a single gene. It then appears that in one of the sites, occasionally in both, one

allele is converted into the other. This leads to a 3 : 1 segregation in the ascus, or 6 : 2 when there are 8 spores. While 3 : 1 segregations can be explained by occasional spontaneous trisomy or tetraploidy (compare sections 5.1.5, 5.6.4), 5 : 3 segregations and deviating arrangements occur also, for which only gene conversion seems to be the possible explanation. One explanation of conversion, based on the Whitehouse model (fig. 2.2) is that in the stretches of hybrid DNA after exchange the paired bases do not match when two different alleles are apposed. Such mismatching of bases resembles the situation after induced mutagenesis, and triggers the repair machinery (sections 1.4.8, 2.1.2): one of the two segments is excised and a new segment is synthesized complementary to the other strand. This results in removal of one of the alleles. It may happen at one or at both hybrid segments. Since it occurs at the single strand (half-chromatid) level, it is detected as a difference between the spore pairs that are formed after the post-meiotic division. Since many complications observed with exchange and conversion are hard to explain on basis of the Whitehouse model, Stahl (1969) has proposed an alternative, more complicated model of exchange that will not be discussed here.

If the system of exchange is fundamentally the same in all eukaryotes, one would expect conversion to occur in higher organisms as well as in Ascomycetes. Since the frequency of conversion is too low for any specific marker to result in significant changes in segregation ratios, it can only be observed when tetrad analysis is possible. There are indeed indications that conversion occurs in the plant *Salpiglossis variabilis*, where the pollen tetrads remain together and a crude form of tetrad analysis can be carried out. Large scale half-tetrad analysis as is possible with isochromosomes (attached-X) in Drosophila (see sections 4.3.1, 5.2.1) led Smith et al. (1970) to suspect non-reciprocality at the *Maroon-like* cistron. In the liverwort Sphaerocarpus, however, conversion was not detected in a large population of tetrads.

2.6.4. *Terminology: exchange, chiasma, crossing-over, recombination, linkage*

When in a double heterozygote (*AaBb*) the two factors are in

the same pair of chromosomes (*AB/ab*), the alleles remain together in the chromosome in which they occur, unless between them *exchange* takes place (fig.2.5). The characters that are conditioned by these alleles will therefore tend to remain associated in the progeny. Already in 1905, before it had been generally accepted that genes are located in chromosomes, this phenomenon of *linkage* of characters was known. Breakage of linkage was usually found to produce the reciprocal types, and was called *crossing-over* (the single event a cross-over). Later this was explained by exchange of homologous chromosome segments. Since a cross-over was detected by *recombination* of characters, crossing-over and recombination were not clearly distinguished. The physical basis is an *exchange* between two of the four chromatids. This leads to the microscopically visible *chiasma* that may move towards the chromosome end (terminalization). Consequently, at the time of observation of a chiasma at diakinesis and metaphase I, it is not necessarily located at the site of formation, the point of exchange (fig.2.6). Exchange at the same time leads to the genetic phenomenon of crossing-over, but only in 50% of the chromatids: there are twice as many chiasmata as there are cross-overs. Crossing-over is recorded as recombination only when it occurs between two factors that are both heterozygous for two alleles and that both can be classified in the progeny. When between these factors two exchanges, i.e. two chiasmata involving the same two chromatids are formed, these two cancel each other and recombination is not observed (see also section 2.7.3): there is crossing-over, but no recombination. Such double crossing-over can only be recognized when a third factor inbetween the other two is available. Thus the terms exchange, chiasma, crossing-over and recombination each have their own meaning. Crossing-over and recombination have sometimes been used interchangeably, but it is better to avoid this. However, as long as there is no certainty that double crossing-over occurs, i.e. when there is no means to distinguish between the two it is customary to use the term crossing-over also when actually merely recombination is meant.

2.7. Estimating recombination and crossing-over percentages

2.7.1. *Test-cross*

Direct estimation of a recombination percentage between two factors is possible in haplonts, for instance in a tetrad analysis in Neurospora, or on moss plants. In diplonts a test cross reveals the gametic genotypes from which the recombination frequency can be estimated. A simple example is Hutchison's experiment with maize where kernel colour and kernel shape can be observed directly on the cob. Shrunken kernel, *sh*, is recessive, *Sh* (normal, smooth kernel) is dominant; colourless aleurone, *c*, is recessive, coloured aleurone (either red or blue) *C*, is dominant. The F_1 *C Sh/c sh* was crossed with the double recessive that gave gametes only of type *c sh* (test cross). As the two genes are in the same chromosome, without crossing-over the F_1 forms the gametes *C Sh* and *c sh*; with crossing-over *C sh* and *c Sh*. There are four gametic types: two parental and two recombination types. The recombination frequency p is calculated as the frequency of the two recombination types. When all meiotic products are recovered, on the basis of the chiasma-type theory the reciprocal recombination classes are expected to be equal, each $\frac{1}{2}p$. In the experiment, however, both are a sample from a large population: p can only be estimated. With two factors in two different chromosomes, recombination can also be estimated, but p is expected to be 0.5 (= 50%). The four classes of Hutchison's experiment contained the following numbers:

coloured smooth	coloured shrunken	colourless smooth	colourless shrunken	total
$\frac{C \quad Sh}{c \quad sh}$	$\frac{C \quad sh}{c \quad sh}$	$\frac{c \quad Sh}{c \quad sh}$	$\frac{c \quad sh}{c \quad sh}$	
4032	149	152	4035	8368
$\frac{1}{2}(1-p)\times N$	$\frac{1}{2}p\times N$	$\frac{1}{2}p\times N$	$\frac{1}{2}(1-p)\times N$	N

There are three independent equations. There is only one unknown (p). The best estimate of p is obtained by dividing the sum

of the two recombination classes by the total number in the experiment, as if these two classes were not just a sample, but contained all crossing-over chromatids. The crossing-over percentage is thus estimated as: $[(149 + 152)/8368] \times 100\% = 3.6\%$. Usually the correspondence between the two non-cross-over categories (4032 and 4035) and between the cross-over categories (149 and 152) is not quite as good as it is here: chance fluctuations (sampling error) can result in considerable variations.

In this example the two dominant alleles (*C* and *Sh*) were located in the same parental chromosome, the two recessive alleles (*c* and *sh*) in the other. This is the *coupling phase*. When the recessive allele of one factor is in the same chromosome with the dominant allele of the other (*c* with *Sh* and *C* with *sh*), it is the *repulsion phase*. In the repulsion phase, the parental and crossing-over classes are interchanged compared to the coupling-phase. Of this situation too, Hutchison had an example:

coloured smooth	coloured shrunken	colourless smooth	colourless shrunken	total
638	21379	21096	672	43785
$\frac{1}{2}p \times N$	$\frac{1}{2}(1-p) \times N$	$\frac{1}{2}(1-p) \times N$	$\frac{1}{2}p \times N$	N

Crossing over percentage $[(638 + 672)/43785] \times 100\% = 3.0\%$. The slight difference between the two results is quite normal. Although actually only recombination percentages have been estimated, in view of the absence of any information to the contrary and the negligible probability that in such a short interval two exchanges would have taken place, the recombination percentage has been assumed to correspond to the crossing-over percentage.

2.7.2. F_2

Frequently, haploid or test cross generations are not available. Then one must resort to the F_2. Here the recombination percentages must be estimated from recombination classes that have originated in a more complex way: they form a random combination of the gametes of the father and the mother. With complete dominance, *Aa* and *AA* cannot be distinguished. Similarly *Bb* and *BB*.

There are again four classes, as with factors in different chromosomes (compare table 2.3): *A.B.* (composed of *AABB*, 2 × *AABb*, 4 × *AaBb*); *aaB.* (composed of *aaBB* and 2 × *aaBb*); *A.bb* (composed of *AAbb* and 2 × *Aabb*); *aabb*. With independent segregation the ratio is 9 : 3 : 3 : 1. With linkage in coupling phase the recombination classes *aaB.* and *A.bb* are less frequent. The crossing-over percentages in father and mother need not necessarily be identical. They are again (preliminarily) estimated as percentage recombination chromatids (sampled as gametes), but their frequency can only be derived indirectly. *Ab* and *aB* gametes *together* are formed in the mother with a frequency p_1, in the father with a frequency p_2; *AB* and *ab* gametes with frequencies $(1-p_1)$ and $(1-p_2)$ respectively. The *separate* frequencies for *AB* and *ab* gametes are $\frac{1}{2}(1-p_1)$ or $\frac{1}{2}(1-p_2)$ each. Therefore the double recessive has the frequency $\frac{1}{4}(1-p_1)(1-p_2)$. It can be similarly deduced that *aaB.* and *A.bb* occur each with a frequency $\frac{1}{4}[1-(1-p_1)(1-p_2)]$. The remainder is the class with at least one dominant allele of each of the factors: the frequency of *A.B.* equals $\frac{1}{4}[2+(1-p_1)(1-p_2)]$. The form $(1-p_1)(1-p_2)$ occurs in all equations and apparently is the parameter that can be estimated. It may represented by *P*. In 1931 Imai found in an F_2 of the bind weed Pharbitis for two flower colour factors in the same chromosome:

A.B.	*aaB.*	*A.bb*	*aabb*	total = *N*
187	37	35	31	290

This clearly deviates from a 9 : 3 : 3 :1 ratio. The expected ratio with crossing-over percentages p_1 (mother) and p_2 (father) or $(1-p_1)(1-p_2)=P$ can be formulated as shown above, summarizing:

A.B.	$\frac{1}{4}(2+P)$
aaB.	$\frac{1}{4}(1-P)$
A.bb	$\frac{1}{4}(1-P)$
aabb	$\frac{1}{4}P$

To obtain the actual expected numbers, these fractions must be multiplied by the total number *N*. Now four equations arise, from which the best approximation of the single unknown *P* must be

estimated. The best way to do this is by the use of the maximum likelihood method, on the condition that the two monofactorial segregations, that for *a* and that for *b*, are undisturbed, i.e. are essentially 3 : 1. The method involves the maximalization of a logarithmic function, the differential quotient of which is made equal to 0. For details see Mather (1951). Applied to the example of Imai's in Pharbitis, the following relation is obtained:

$$0 = \frac{187}{2+P} - \frac{37}{1-P} - \frac{38}{1-P} + \frac{31}{P}$$

from which the quadratic equation $0 = -290P^2 + 12P + 62$ is derived: $P = 0.4835$.

If one cares to assume that $p_1 = p_2$, $(1\text{-}p) = \sqrt{p} = 0.6953$ and $p = 30.47\%$.

Another approach used rather frequently, and acceptable when there is a slight deviation from the 3 : 1 segregation of the separate factors (for instance when a certain genotype has reduced viability, compare section 2.5.1), is the *product ratio* method. The four equations are here combined by dividing the product of the non-crossing-over classes by the product of the crossing-over classes. In coupling phase:

$$\frac{A.B. \times aabb}{aaB. \times A.bb} = \frac{2P+P^2}{1-2P+P^2}$$

This expression is too complicated to be worked out every time a crossing-over percentage has to be estimated, but it can easily be used in conjunction with the tables composed by Immer (1931). From $P = (1-p_1)(1-p_2)$ and assuming $p_1 = p_2$, the corresponding cross-over frequency can again be calculated.

On the assumption that the frequency of crossing-over is proportional to the distance between two genes on a chromosome, cross-over percentages have been used to determine (genetic) lengths in the construction of one-dimensional maps. Chromosome mapping will be considered in chapter 6.

2.7.3. *Double crossing-over. Maximum crossing-over percentage*

As seen before (section 2.6.4) the calculated crossing-over per-

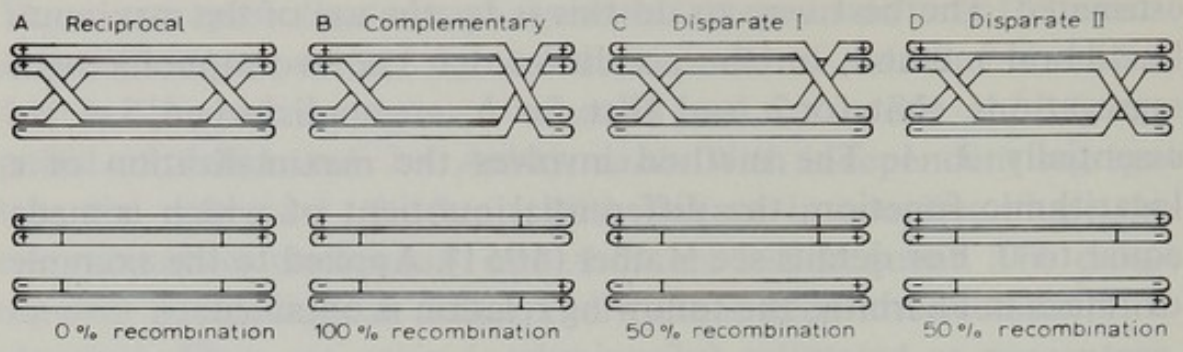


Fig.2.15. The four combinations of two chiasmata.

centage strictly speaking is not more than the recombination percentage observed in the gametes. When two chiasmata are formed between the factors studied, some will involve the same two chromatids. In these double crossing-over occurs which does not lead to recombination. In fig.2.15 two chiasmata are shown between two factors at the ends of the chromosome, both with two alleles (here simply represented by + and –). In the first case (A) the same two chromatids are involved in both chiasmata (two-strand double cross-over): there is no recombination. The chiasmata are *reciprocal.* If there had been a classifiable third factor inbetween, both cross-overs could have been recognized (see section 2.8.1). In the second case (B) different chromatids are involved in the two chiasmata and this results in 100% recombination chromatids: the chiasmata are *complementary* (four-strand-double). In the third (C) and fourth (D) case, one chromatid is involved in both (no recombination), one in neither (no recombination) and the other two either in one or in the other (recombination). These are *disparate* chiasmata (three-strand-double), giving 50% recombination. The difference between the last two cases is that the double cross-over, non-recombination chromatids are in one chromosome in C and in the other in D.

When the two chiasmata are independent in respect to the chromatids involved, the four cases occur with equal frequency. The average recombination percentage (= observed crossing-over percentage) will then be: (100% + 0% + 50% + 50%)/4 = 50%. In other words: the four cases together have 16 chromatids, eight of which

are recombination chromatids, eight are not. Thus with two chiasmata, as with one chiasma, there will not be more than 50% recombination.

With three chiasmata $2^3 = 8$ types can be distinguished, all with equal frequency. These involve 32 chromatids: eight are non-cross-over chromatids, eight are double-cross-over chromatids and 16 are single or triple cross-over, i.e. recombination chromatids. On the average again 50% recombination. This can be extended to four, five, etc. chiasmata. Apparently 50 is the maximum possible recombination percentage. This is equal to the recombination percentage between factors on different chromosomes: with large distance between two factors on the same chromosome (= many chiasmata) segregation is independent.

2.8. Interference

2.8.1. *Chromatid interference. Chiasma interference*

Although it is a generally valid rule that a chromatid that has participated in one chiasma is free to participate in a second chiasma, there are exceptions. Then an exchange chromatid has a reduced chance to be involved in a second chiasma: *chromatid interference*. This results in a reduction in number of the double- and non-cross-over chromatids, while the single cross-over chromatids (the recombination chromatids) increase. The four combinations of two chiasmata (fig.2.15) now do not occur with equal frequency and the recombination percentage of the gametes will exceed 50%. Recombination percentages in excess of 50 have been observed in yeasts, fungi and the liverwort Sphaerocarpus, and have been ascribed to chromatid interference. In higher organisms recombination significantly in excess of 50% is usually not observed. Generally, in the few cases where the course of the chromatids in the chiasmata can be observed microscopically (locusts, grasshoppers, some Amphibia) chromatid interference is not found. On the other hand, Darlington (1965) mentions a case of Stenobothrus where reciprocal chiasmata were relatively frequent,

and a case of Melanoplus with a shortage of disparate chiasmata: both the opposite of chromatid interference.

Another form of interference is quite a general phenomenon: *chiasma interference*: one chiasma reduces the probability of a second chiasma in its vicinity. The average distance between chiasmata (*intercept length*) is thus increased and the distribution of the chiasmata over the bivalent does not correspond to a Poisson distribution as would be expected with random formation. Interference between cytologically observable chiasmata was demonstrated for the first time by Haldane (1931) on basis of observations on the bean *Vicia faba*. One consequence is that bivalents with very high chiasma frequencies occur much less frequently than expected. Mather has suggested that chiasmata in a bivalent are probably not formed simultaneously but in succession, beginning at specific points, usually the chromosome ends, or the centromere, or both. The first chiasma is free in its choice of a location and will appear close to the initiation point on a distance *d* (*differential distance*). The second chiasma maintains a certain although variable distance from the first, on the average of length *i* (*interference distance*). The following chiasmata maintain this average distance. In this way chromosome segments of high average chiasma intensities alternate with segments of low chiasma intensities. Mather arrived at this conclusion on the basis of genetic analyses of *Drosophila melanogaster*. Later (1963), Henderson conducted very detailed analyses on diplotene bivalents of the locust *Schistocera gregaria*, where chiasmata can be readily distinguished (see Lewis and John, 1965). At this stage terminalization has proceeded only very little. Here too, a good agreement with expectation was found and the distances *d* and *i* could be estimated accurately. Chiasma formation appeared to start sometimes at the chromosome ends, sometimes at the centromere and sometimes at both simultaneously.

There are several indications that in general formation of chiasmata and their location can be related directly to the course of chromosome pairing, which also often starts at the chromosome ends or at the centromere, proceeding from thereon over the chromosome. In other cases the relation is less clear. Such pairing and

Table 2.4
Three point test in maize.

All three factors involve properties of the seed: the segregation can be read on the cob of a selfed F_1 plant.

Parents: P_1 *c* (colourless aleurone) – *sh* (shrunken seed) – *wx* (waxy endosperm)
P_2 *C* (coloured aleurone) – *Sh* (smooth seed) – *Wx* (normal endosperm)

F_1: $\frac{c - sh - wx}{C - Sh - Wx}$, test-crossed with $\frac{c - sh - wx}{c - sh - wx}$

Types in testcross with numbers of seeds found (representing gametic ratios):

C Sh Wx	*C Sh wx*	*C sh Wx*	*c Sh Wx*	*C sh wx*	*c Sh wx*	*c sh Wx*	*c sh wx*	Total
2238	672	19	98	107	39	662	2198	6033

Monofactorial segregations: *C* : *c* = 3036 : 2997
Sh : *sh* = 3047 : 2986
Wx : *wx* = 3017 : 3016
slight shortage of recessives

Crossing-over *C* – *Wx*
C wx = 672 + 107
c Wx = 98 + 662
1539 crossing-over $\frac{1539}{6033} \times 100 = 25.51\%$

Crossing-over *C* – *Sh*
C sh = 19 + 107
c Sh = 39 + 98
263 crossing-over $\frac{263}{6033} \times 100 = 4.36\%$

Crossing-over *Wx* – *Sh*
Wx sh = 19 + 662
wx Sh = 672 + 39
1392 crossing-over $\frac{1392}{6033} \times 100 = 23.07\%$

The greatest crossing-over percentage corresponds with the greatest distance, and this must be between the outer two loci. The order therefore, is *C-Sh-Wx*. The sum of *C-Sh* and *Sh-Wx* is 27.43 which is more than *C-Wx* estimated directly (25.51). The difference is a result of double crossing-over.

Double crossing-over:
C sh Wx = 19
c Sh wx = 39
58 percentage $\frac{58}{6033} \times 100 = 0.96\%$

The product of 23.07% and 4.36% = 1.01% is the expected double crossing-over frequency. The difference is due to interference. The coincidence value *c* can be calculated as $\frac{0.96}{1.01} = 0.95$ and the interference equals 1-0.95 = 0.05.

The distance *C-Wx* can be estimated directly when double crossing-over is taken into account, i.e. the double crossing-over frequencies count twice:

C wx = 672 + 107 + 2 × 19
c Wx = 98 + 662 + 2 × 39
1655 crossing-over $\frac{1655}{6033} \times 100 = 27.46\%$

(Results from a student course, Department of Genetics, Wageningen.)

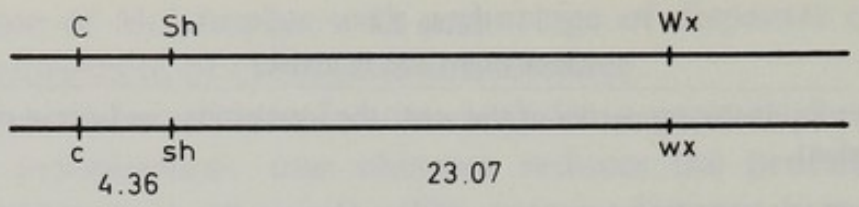


Fig.2.16. An example of a three-point-test in maize (compare section 2.8.1 and table 2.4). The order of the three factors *c*, *sh* and *wx* can be given, and the distance between them in percent crossing-over: this is the beginning of a genetic map.

interference patterns lead to a localization of chiasmata and crossing-over in certain segments of the chromosome. Such and other localization will be briefly considered in section 2.8.5.

Interference between points of exchange can be demonstrated genetically (in crossing-over). A prerequisite is that crossing-over is observed simultaneously in at least two preferably adjacent chromosome segments. The simplest case is that of three factors in one chromosome (fig.2.16, table 2.4). In such a three point test, say with the genes *a*, *b* and *c*, in this order, crossing-over can be observed between *a* and *b*, *a* and *c*, and *b* and *c*. There is interference when crossing-over in *a-b* and *b-c* simultaneously is found to occur less frequently than expected on the basis of the crossing-over frequences of *a-b* and *b-c* separately. The simultaneous occurrence of crossing-over in *a-b* and *b-c* is double crossing-over, discussed in section 2.7.3. Table 2.4 and fig.2.16 show a three-point test with seed-colour and seed-shape factors in maize. A three-point test is a favourable set-up for studying double crossing-over, but it is good to be aware of the fact that "double crossing-over" as observed in a three-point test does not imply that necessarily exactly two chiasmata have formed between the two outer marker genes. When one of the two segments is rather large (as in fig.2.4), this may occasionally have two chiasmata that cannot be recognized. Also, not observing double crossing-over in the three-point test does not exclude the possibility that both segments have had a chiasma. There may have been one in each, but involving different chromatids: the genetic observations considered concern merely recombination in chromatids, not chiasma formation in chromosomes.

2.8.2. *Coincidence*

The ratio between the double crossing-over frequency as observed in two segments and the frequency expected on the basis of independence from the frequency in each of the two segments separately is the coincidence, c. Interference can be expressed as $(1-c)$. In table 2.4 the observed double crossing-over is 0.96%. The crossing-over percentages in the two segments are 23.07% and 4.36%, their product 1.01%, which is the expected double-crossing-over percentage in the absence of interference. Coincidence, $c = 0.96/1.01 = 0.95$ and interference $1 - 0.95 = 0.05$. This relatively low value is due to the great (genetic) length of one of the two segments.

2.8.3. *Negative interference*

In general, the complex chromosomes of higher organisms appear to show only positive interference, i.e., points of exchange have a small probability of occurring in close proximity. In prokaryotes as phages and bacteria, but also in lower eukaryotes as fungi and yeasts, there is a second type of interference: crossing-over tends to take place in clusters in very close proximity: negative interference. Over larger distances positive interference can sometimes also be demonstrated, especially in fungi. A possible explanation of positive interference is the limitation of the number of chromosome segments in which the chromosomes can line up sufficiently intimately to undergo exchange at the DNA level. The cause of this may be torsion or a limitation in the tendency of the double DNA strands to separate. In the places where effective pairing occurs, however, there is little or no limitation to exchange and then it occurs in a high frequency, which is demonstrated as negative interference. Why negative interference is not observed in higher organisms, is not clear. It must have some relation with differences in chromosome structure.

2.8.4. *Interchromosome effects*

A fourth form of interference concerns exchanges in *different* chromosomes within a cell: when one bivalent happens to have very few chiasmata, other bivalents tend to have more, and vice

versa. This results in a negative correlation between bivalents in respect to chiasma frequencies. The phenomenon depends on a number of conditions and has frequently, although far from universally, been observed. In Drosophila interchromosome effects of a special nature have been intensively studied: heterozygous chromosomal rearrangements influence cross-over frequencies in other chromosomes. Especially inversion heterozygotes with reduced cross-over frequencies in the inversion segments appear to have increased crossing-over in rather specific segments of other chromosomes. The causes are as yet unknown. On the basis of interchromosome effects, Mather (1939) constructed the hypothesis that the total number of chiasmata in a cell has a specific maximum. When one bivalent has many, fewer are available for the remainder, and vice versa. The phenomenon, however, is too complex to be explained by a single simple mechanism. In chapter 4, it will be discussed again when the chromosome structural variants will be considered. For a review see Lucchesi and Suzuki (1968).

2.8.5. *Chiasma localization*

In section 2.8.1 concentrations of chiasmata in specific segments of the chromosomes were attributed to interference patterns. Frequently, localization of a different, unexplained nature is observed: chiasmata tend to be concentrated in specific areas of the chromosomes. Often these are the ends as in barley, rye, Tradescantia, Oenothera and many more; sometimes near the centromeres as in Fritillaria (Darlington, 1965). In maize, in many locusts and in Drosophila the distribution is more even and is mainly determined by interference (section 2.8.1). Strict localization is one of the means of maintaining recombination-free chromosome segments in which special gene-combinations can be kept intact. This type of localization is genetically determined, and is not predominantly an autonomous function of the chromosome segment involved. This was shown by Jones (1967) in his analysis of chiasma localization in the progeny of a hybrid between *Secale dighoricum* and the related species *Secale turkestanicum*, both species with terminal localization of chiasmata. In the progeny lines with the normal, localized chiasma distribution could be iso-

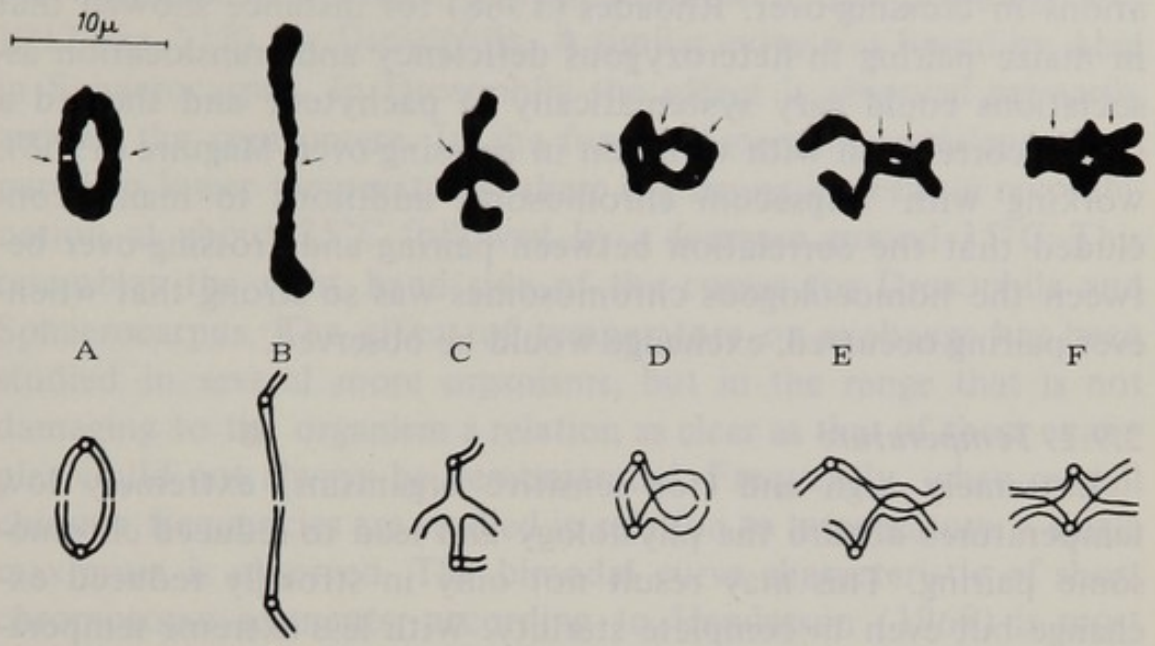


Fig.2.17. Genetically determined chiasma localization in rye. (A,B) The usual terminal localization of chiasmata at metaphase I in normal rye (*Secale cereale*), where only occasionally figures like C and D are seen, while the other types are quite infrequent. C, D, E, F are MI bivalents with terminal and proximal chiasmata as occur quite frequently in abnormal genotypes derived from intercrossing different species related to *S.cereale* (Jones, 1967).

lated along with lines that had lost this property and that had a random distribution (fig.2.17). Apparently genes regulating localization had segregated. To what extent this regulation acts through chromosome pairing is not known, but many instances have been found of an effect of pairing on chiasma formation (section 2.9.1).

2.9. Factors affecting chiasma formation and crossing-over

2.9.1. *Interference and chromosome pairing*

Interference (section 2.8.1), including interchromosome effects (section 2.8.4) are factors that affect genetic exchange. It is quite probable that several of the factors mentioned below affect chiasma formation and crossing-over by way of their effect on interference. The same is true for chromosome pairing: variations in the extent of pairing are common and are closely correlated with vari-

ations in crossing-over. Rhoades (1968) for instance showed that in maize pairing in heterozygous deficiency and translocation associations could vary systematically at pachytene and showed a strong correlation with variation in crossing-over. Maguire (1968), working with Tripsacum chromosome additions to maize concluded that the correlation between pairing and crossing-over between the homoeologous chromosomes was so strong that whenever pairing occurred, exchange would be observed.

2.9.2. *Temperature*

Extremely high and (for sensitive organisms) extremely low temperatures disturb the physiology and lead to reduced chromosome pairing. This may result not only in strongly reduced exchange but even in complete sterility. With less extreme temperatures maxima and minima are often found at temperatures not always close to the optimum temperature for the development of

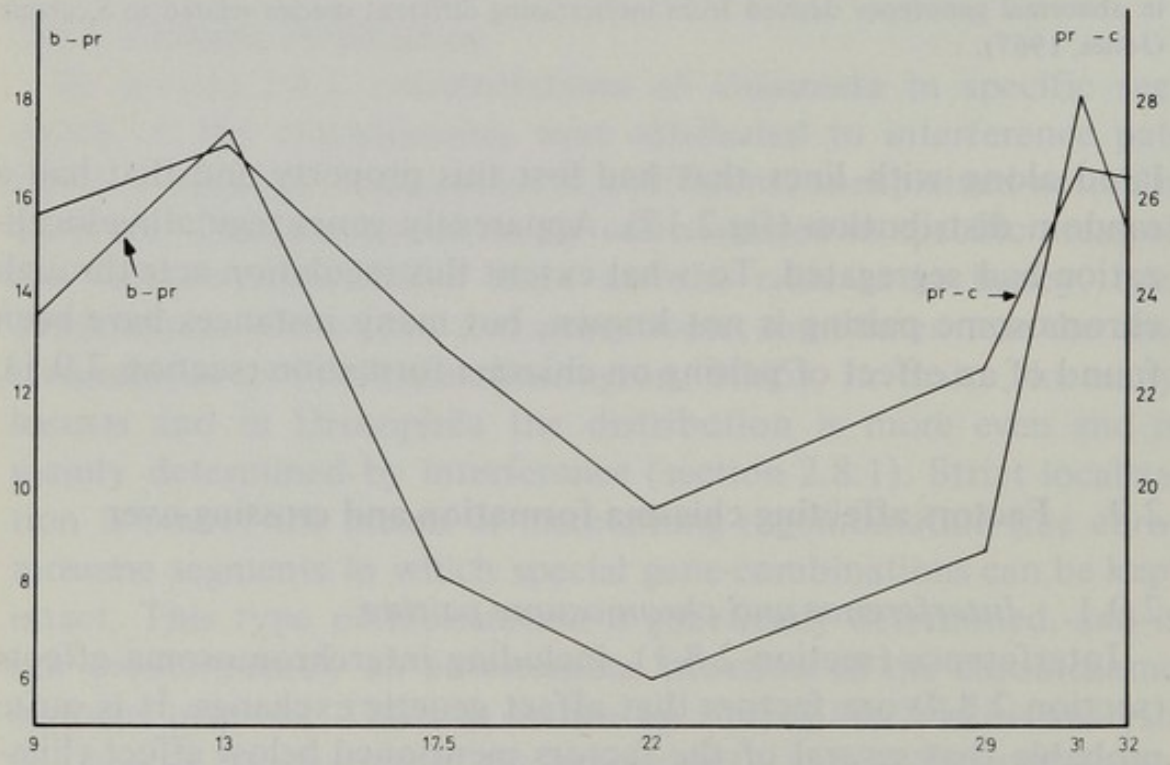


Fig.2.18. The effect of temperature on crossing-over in female *Drosophila melanogaster* according to Plough (1917). The segments *b-pr* and *pr-c* react similarly. Vertical: crossing-over percentages, at left for *b-pr*, at right for *pr-c*. Horizontal: temperature in centigrades.

the organism as a whole. An old example is that of Plough's of 1917 (fig.2.18) for Drosophila. A similar curve was found by Abel in Sphaerocarpus. In Drosophila the effect is observed primarily around the centromere. In the fungus *Aspergillus nidulans*, compared to lower temperatures, there is a strong increase in recombination at about 25°C followed by a decrease around 35°C. This resembles the right hand side of the curves for Drosophila and Sphaerocarpus. The effect of temperature on exchange has been studied in several more organisms, but in the range that is not damaging to the organism a relation as clear as that of these examples could not always be demonstrated. Frequently, when overall chiasma frequencies are studied in relation to temperature, a single maximum is observed. The bimodal curve characteristic of short chromosome segments, according to Henderson (1969) is most easily explained by a tendency for shifts in chiasma localization to accompany an overall reduction.

The role of heterochromatin (compare section 2.9.6) in the temperature effect was studied by Wolf (1963) in the dipterous species Phryne. With relatively low temperatures heterochromatinization was lower and crossing-over higher than with more normal temperatures. The greater sensitivity of centromeric segments to temperature variations in Drosophila may primarily be a heterochromatin effect. For a review of temperature effects see Wilson (1959).

2.9.3. *Age*

In general, with age crossing-over in female Drosophila decreases as a result of increasing intercept length. At a later age a rise can set in. Again the effect is strongest around the centromere. In the longer-lived males of the insect Phymateus, Oyidi (1968) found a similar effect at younger stages followed by another fall and a rise at older stages. In mice such W-shaped curves can also be observed. In most mammals, the sometimes observed decrease in chiasma frequency with maternal age cannot be an age effect, since exchange usually occurs at embryonic stages. It must reflect a gradient in the foetal ovary. Of course, when chiasmata are studied, the possibility of chiasma terminalization and subsequent loss

must be taken into account. Such a decrease, of course, does not result in a decrease in crossing-over (fig. 2.6).

2.9.4. *Water content*

Low water content can lead to reduced chiasma frequencies; in extreme cases it can result in complete failure of pairing and exchange. When the genotype tends to reduced chiasma frequencies, these are expressed more clearly under dry conditions, even when normal genotypes do not show a reaction yet: desynapsis in plants has been shown to be more pronounced in dry than in normal seasons.

2.9.5. *Sex*

When sex is determined by a chromosomal mechanism (see section 3.2.1), the homogametic sex has sometimes been found to have a higher crossing-over frequency than the heterogametic sex. In a sense, an extreme example are the Diptera, where most males (the heterogametic sex) are completely free of crossing-over. The rule formulated by Haldane in 1922, however, does not hold without exception. Rhoades in 1941 could show that in maize, where the organs of the two sexes are formed on different parts of the same plant, crossing-over in chromosome 5, close to a heterochromatic segment, was higher in the male organs (the tassel) than in the female cobs. In such cases slight variations in the conditions within the plant may be as important as sex.

2.9.6. *Heterochromatin*

The role of heterochromatin in the expression of the effect of temperature on crossing-over has been mentioned in section 2.9.2. In general crossing-over is reduced in heterochromatic regions as may be expected considering their compact structure, but it is not impossible, as has been suggested. Ramanna (1971), for instance, showed that, in contrast to earlier reports, in the entirely heterochromatic short arm of chromosome 2 of the tomato crossing-over occurred in a considerable proportion of the pollen mother cells. Perhaps because of the result of interference phenomena crossing-over is sometimes increased in regions adjacent to heterochroma-

tin. This may be the reason that in Drosophila, translocation of a chromosome segment from a euchromatic region towards a new location in the vicinity of heterochromatin usually results in an increase in crossing-over.

A slightly different effect is that of heterochromatic B-chromosomes (see section 3.2.7). In maize, for instance, where the B-chromosomes are almost entirely heterochromatic, crossing-over in the normal (A-) chromosomes is significantly increased when four or more B-chromosomes are present. In rye, with mainly euchromatic B-chromosomes this increase is only occasionally observed, while in truxaline grasshoppers, again with euchromatic B-chromosomes, the effect is absent.

2.9.7. *Ionizing radiation*

An occasionally observed increase of crossing-over after irradiation of Drosophila females may at least in part be due to induced non-meiotic exchange between somatically paired chromosomes. In male Drosophila too, crossing-over can be induced by ionizing irradiation. It is difficult in these cases to distinguish between isolocus breaks and induced meiosis-like phenomena accompanied by crossing-over.

More generally ionizing radiations, at least at higher doses and probably as a consequence of biochemical disturbances, induce a reduction of genetic exchange. Lawrence in 1965 reported that irradiation of Lilium, Tradescantia and Chlamydomonas at specific moments before and during prophase of meiosis strongly affected chiasma frequency (and crossing-over). Irradiation at premeiotic DNA synthesis had a reducing effect, irradiation at prophase (zygotene–pachytene) an enhancing effect, irradiation at prophase dies have been very valuable for analysing the mechanism of meiotic exchange (section 2.6.2). For a review of radiation effects see Westerman (1967).

2.9.8. *Chemicals*

The effect of chemicals can often best be studied in microorganisms where penetration problems are minimal. In yeast radiomimetic substances have been found to increase somatic (non-

meiotic) recombination. Non-radiomimetic strong mutagenic agents such as ethyl-methane-sulphonate (EMS) have a similar effect. Apparently this is not simply a matter of induced isolocus breaks but a phenomenon in some way related to meiosis. EDTA raises recombination frequencies in Drosophila, probably by changing chromosome structure by removing divalent cations. Colchicine prevents chromosome pairing and in this way reduces recombination. Selenium, when present in excessive quantities in the soil, is taken up in sufficient amounts in plants to have a reducing effect on crossing-over. Substances that influence DNA synthesis, influence crossing-over in different ways (section 2.6.2). When protein synthesis is blocked chemically at zygotene–pachytene, chiasma formation is prevented.

2.9.9. *Variation within the organism*

It has repeatedly been observed that in plants chiasma frequencies can vary from one flower to the other. Künzel in 1963 reported a gradient in the heads of barley in respect to number of chiasmata per cell. Such gradients may reflect gradients in heterochromatinization or other systems of regulation. Even within a single anther a gradient is sometimes demonstrable. In animals too, systematic and significant differences can occur within testes and ovaria (compare section 2.9.3). In the harlequin lobe of the testes of the axolotl, chiasmata are almost completely absent. Attempts to correlate such differences in chiasma frequency with differences in biochemical characteristics (histones, RNA content), although promising at first, have not yielded definite results. Apparently, many as yet unknown factors play a role.

2.9.10. *Genetic factors*

Chiasma frequency and crossing-over frequency are part of the phenotype: they are determined by environment–genotype interactions. Effects resulting from structural and numerical variations will not now be considered, but in chapters 4 and 5. The importance of genetic factors is observed with inbreeding, which in crossbreeders causes a prominent decrease in crossing-over. After 5 to 6 generations a plateau is reached, different inbred lines having

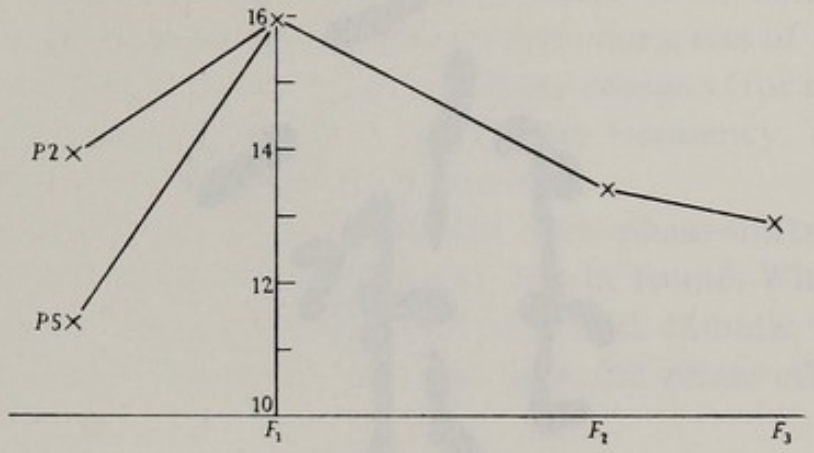


Fig.2.19. The two inbred lines P_2 and P_5 of rye, a natural crossbreeder, have relatively reduced chiasma frequencies. The hybrid (F_1) has a much higher frequency as a result of heterosis. In the subsequent inbreeding generations (F_2, F_3) a drop is observed (F_2: average of 30 plants, F_3 of 32 plants). Vertical: average number of chiasmata per cell with seven bivalents. Horizontal: generations. (From Rees, 1955.)

different plateaus. After crossing two inbred lines of rye, Rees (1955) found an appreciably higher chiasma-frequency in the F_1 than in the parents. In subsequent generations of selfing (F_2, F_3, etc.) the level fell back to about the average of the parents, but the parental levels themselves were not recovered. Apparently recombination had taken place in a polygenic system and the gene combinations of the parents were not realized (fig.2.19).

The relatively low chiasma frequency in some species-hybrids that morphologically may show hybrid vigour, can partly be explained by structural differences between the parents. Also important, however, is that gene unbalance inhibits the proper functioning of the complicated processes of pairing and exchange.

Monofactorial variations have been reported on many occasions. The effects are usually drastic, as otherwise classification would not have been possible and the factors would not have been recognized as monofactorial. Several instances of monofactorial *asynapsis* are known, where chromosome pairing completely or nearly completely fails. More frequent is *desynapsis* (formerly not distinguished from asynapsis) where pairing apparently is normal, but where exchange is restricted (fig.2.20). These mutations are most readily expressed under unfavourable environmental conditions

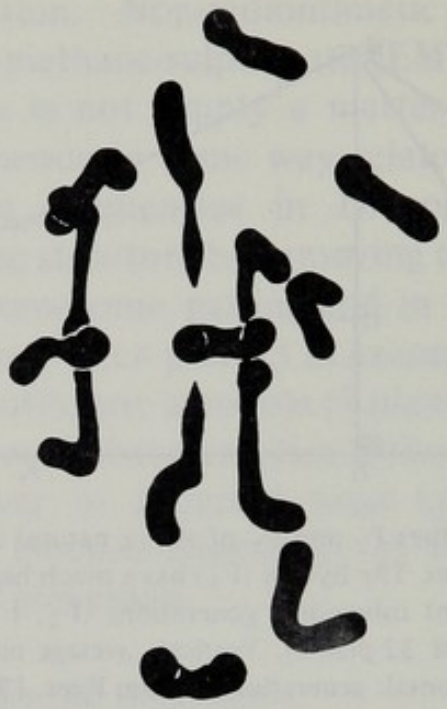


Fig. 2.20. First metaphase in the PMC of a desynaptic rye plant. There are relatively few chiasmata and consequently many univalents. (From Prakken, 1943.)

(compare section 2.9.4). As discussed earlier (section 2.6.2) desynaptic mutants generally appear to be deficient in DNA repair, in lower organisms as well as in higher organisms. Desynaptic mutants in Drosophila, wheat and tomato may be abnormal in the formation of their synaptonemal complexes. These may fail entirely, although the axial cores are usually formed. Nothing is yet known of a correlation between DNA repair and synaptonemal complex formation.

As suggested by Simchen and Stamberg (1969 and later), fine control of recombination at specific loci seems to be under the control of numerous independent genes, each with its effect on a special, not necessarily nearby segment. The segments affected may be small or occasionally somewhat larger and some genes may control more than one segment. The controlling genes do not react identically on variations in the environment.

2.9.11. *Shifts in populations*

In South African locusts, Nolte (1967) found an increase in chiasma frequency with the transition of the solitaria to the grega-

ria phase, which can take several generations to be completed. A pheromone is involved that affects several characters of the insect. Mutants that failed to show some of these changes (for instance in colour) still showed the effect on chiasma frequency. The effect can be induced by the isolated pheromone.

In organisms that do not undergo such phase-shifts, seasonal variations in chiasma frequencies may also be found. While in such cases genotype alterations (by selection) and climatic influences may play a role, this is not the case with the phase effects mentioned above.

2.10. Uniformity and variation

2.10.1. *The one-dimensional structure of the chromosome*

The maintenance of the fundamentally one-dimensional structure of the chromosome from virus to the highest organisms may seem unexpected. It is clear, however, that for reduplication, for separation of the chromatids at anaphase and for recombination, this structure offers obvious advantages over a branched secondary and certainly over a tertiary structure. In spite of a far-reaching internal complexity and the development of a large number of specialized functions (movement; regulation of transcription, condensation and spiralization; nucleolus; pairing, exchange) the highly evolved chromosomes functionally behave like the "simple" bacterial chromosomes, even though the way this behaviour is effected is quite different.

2.10.2. *Three moments of central importance*

There are three central moments in the normal course of meiosis of all eukaryotes: (1) chromosome pairing, (2) exchange, (3) orientation (segregation). In all three, phenotypic variation can have far-reaching genetic consequences. In a normal diploid the effect of variation in pairing is observed only through its effect on exchange. In normal cases variation in orientation as long as it remains random, has no consequences (see, however, section

7.2.2). When the karyotype deviates from that of the diploid (structure and number of the chromosomes), however, variations in pairing, exchange and segregation pattern can be of considerable importance. With the treatment of chromosome variants these three central processes will therefore be brought up repeatedly. In a sense they can vary independently but eventually the pairing pattern determines the limits and sequences of the exchange system. Orientation is highly dependent on the number, location and terminalization of the chiasmata. From this hierarchy results a close connection between these three central moments of meiosis.

As will be discussed in more detail in chapter 7, meiotic processes may show considerable variation in apparently crucial elements. This demonstrates that what is usually considered to be the normal course of meiosis is merely the sequence of events that has proven to be the most universally adaptable system.

Chapter 3

SPECIALIZED CHROMOSOMES

3.1. Adaptational forms of normal chromosomes

3.1.1. *Polytene giant chromosomes*

Chromosome form and structure can adapt to a temporary or permant special function of the cell in which the chromosome occurs. The condensation of the chromosomes in the normal mitotic and meiotic cycles is an example of adaption to the requirements of nuclear division. This particular relation between form and function will not be discussed further. Several more instances are known of sometimes extreme specialization. The most extensively studied are the giant polytene chromosomes that occur in the salivary and other (often less well analysable) glands and organs of the larvae of Drosophila and other Dipterous flies. In some species of these flies such giant chromosomes have even been observed in the bristle-initials of the puppae. The function of all cells in which giant polytene chromosomes are found is the rapid production of large quantities of specific substances.

These chromosomes are in principle normal interphase chromosomes. The two homologues are paired and each has undergone eight to nine reduplications. Non-separation of the strands results in *polyteny* of the interphase chromosomes with 1024 or 2048 strands. The combination of polyteny and pairing leads to broad bands of great length (fig.3.1). Longitudinally they are not homogeneous: dense (stainable) and less dense parts alternate, leading to a specific and constant pattern of transverse bands. The interband regions are much lower in DNA and histone content than the bands. The condensed bands are called *chromomeres.*

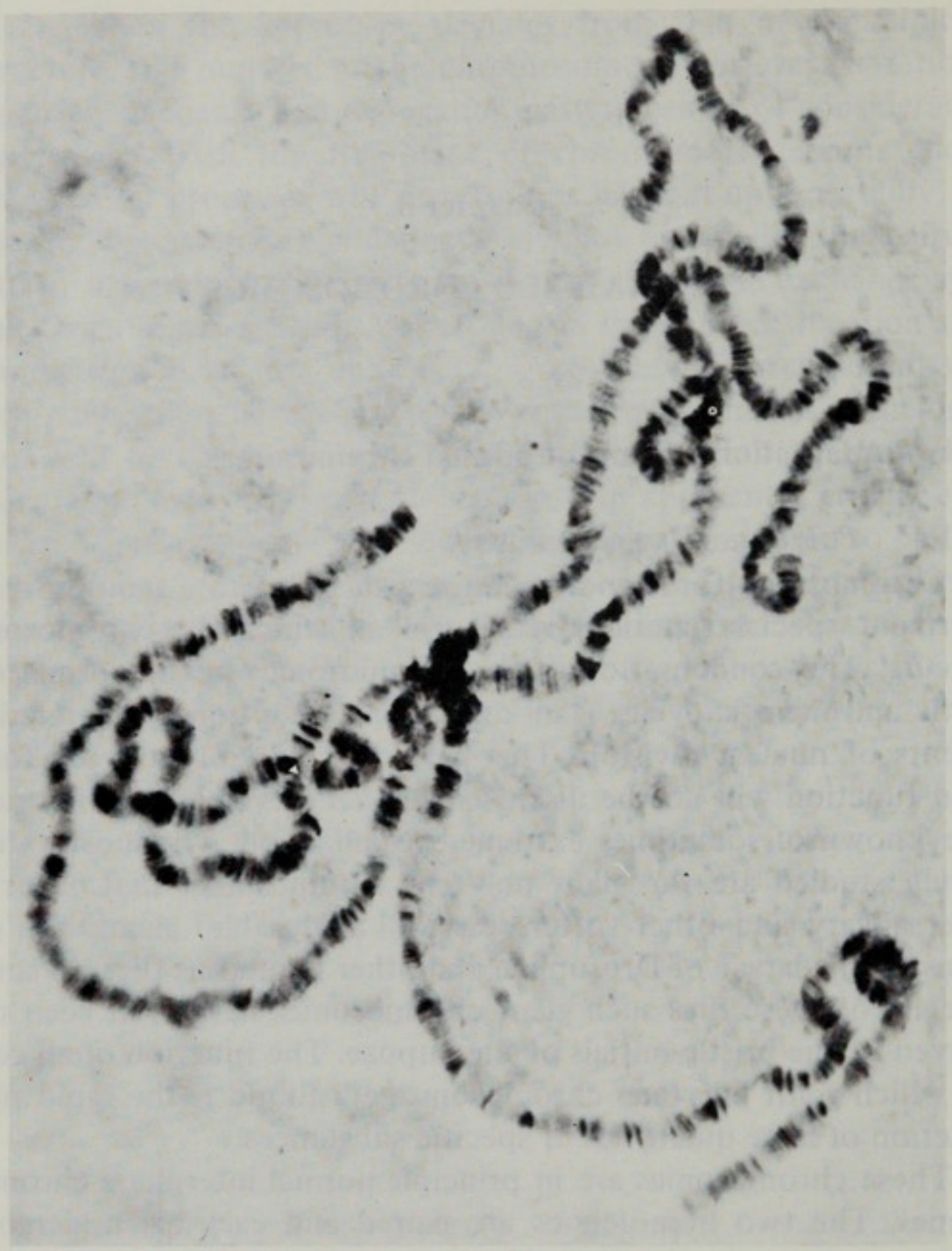


Fig.3.1. The giant polytene interphase chromosomes in the salivary glands of the larvae of *Drosophila melanogaster*. There are four: the acrocentrix X-chromosome, the two metacentric chromosomes 2 and 3, and the very short chromosome 4. The homologues have paired and only five long arms and one very short arm are present. All heterochromatic chromosome segments have fused into the *chromocentre* which contains all centromeric regions, and in male flies almost the entire Y-chromosome. Since these heterochromatic segments do not have the same high degree of polyteny of the rest of the chromosomes, the chromocentre is not especially large. From it extend the five long arms and one very small arm. (Courtesy Mrs. Dr. A. Koopmans, Institute of Genetics, Groningen.)

They appear to have a considerable individuality. In respect to DNA synthesis, for instance, they behave like a unit, and can perhaps be considered to coincide with a replicon, although several bands together usually show some coordination in replication. Many good arguments have been advanced for the view that one chromomere is one gene. The interband regions then would merely contain spacer DNA (with histones), as is found between the repeated rRNA cistrons in nucleoli (section 3.2.6). Definite proof for the one band–one gene hypothesis is not yet available.

In 1934 Painter made a complete map of the salivary gland chromosomes of *Drosophila melanogaster*. This was repeated later for several more species of Drosophila, Chironomus, Sciara and other Diptera. In some of these, like in *D. melanogaster*, all heterochromatic segments fuse into one *chromocentre*. This includes all segments immediately adjacent to the centromeres, and also the entire Y-chromosome in the males. Since these segments are not polytenic to the same degree as the euchromatic segments, the chromocentre is not especially large. From it extend (in *D. melanogaster*): the single non-heterochromatic arm of the X-chromosome, two arms of chromosome 2 and two arms of chromosome 3, and the single very short arm of chromosome 4: a total of five long arms and one very short arm.

The fact that the finest structure of these paired polytene chromosomes can be recognized makes them a very important tool in cytogenetic analysis: even minute variations in chromosome structure can be recognized and correlated with genetic phenomena. The exact pairing of the homologues is of special importance, as small structural differences that are not easily detected as variations in the banding pattern become conspicuous by irregularities in pairing. Analysis of giant salivary chromosomes will be encountered on several occasions in the following chapters.

In the specialized organs in which polytene chromosomes occur, not all genes need to function: the majority remains repressed and only a few are activated. Several external factors, for instance a brief period of high temperature, or the introduction into the larvae of substances with a hormonal function can induce transcriptional activity in specific genes. The molting hormone ecdy-

Table 3.1
Changes in the puffing pattern of *Drosophila hydei* observed at 30 min after injection of ecdysone (Berendes, 1967).

Puffs which originate			Puffs which increase in activity			Puffs which decrease in activity		
Region	Average activity value		Region	Average activity value		Region	Average activity value	
	C	E		C	E		C	E
31C	–	3.0	8BC	1.1	3.2	4CD	3.5	2.4
58B	–	3.0	19B	0.1	2.3	13D	1.3	0.6
90AB	–	1.5	26B	1.2	2.3	47B	1.9	0.6
			31B	1.8	3.4	64A	1.9	1.3
			35A	0.7	1.6	64C	2.6	1.0
			37C	1.1	1.7	85A	1.7	0.4
			47C	0.5	0.8	93A	0.2	–
			52C	0.6	2.0	98A	1.1	–
			63A	0.2	0.9	110C	0.3	–
			63C	2.8	3.8	111B	1.5	0.8
			78B	0.6	3.9	115A	2.5	0.1
			87C	1.6	3.3	122A	1.1	–
			89A	0.5	1.5			
			95D	2.5	4.0			
			97A	2.3	3.5			
			97C	2.4	3.6			
			100C	0.9	1.7			
			115C	1.1	1.9			

C = control larvae, E = ecdysone treated larvae.

sone is of special interest in this respect (table 3.1). The activation, natural or induced, of a gene is accompanied by a striking extension of the chromomere in question. The adjacent chromomeres may join in the morphological change, but it is not certain whether they are actually activated. The extended structure is called a *puff* (fig.3.2) and its size can vary from hardly distinguishable from a normal chromomere to more than double the usual diameter of the chromosome. Some puffs can become excessively large and are then often maintained during relatively long periods of larval life. Such puffs are called *Balbiani rings*. Puffing, except in a

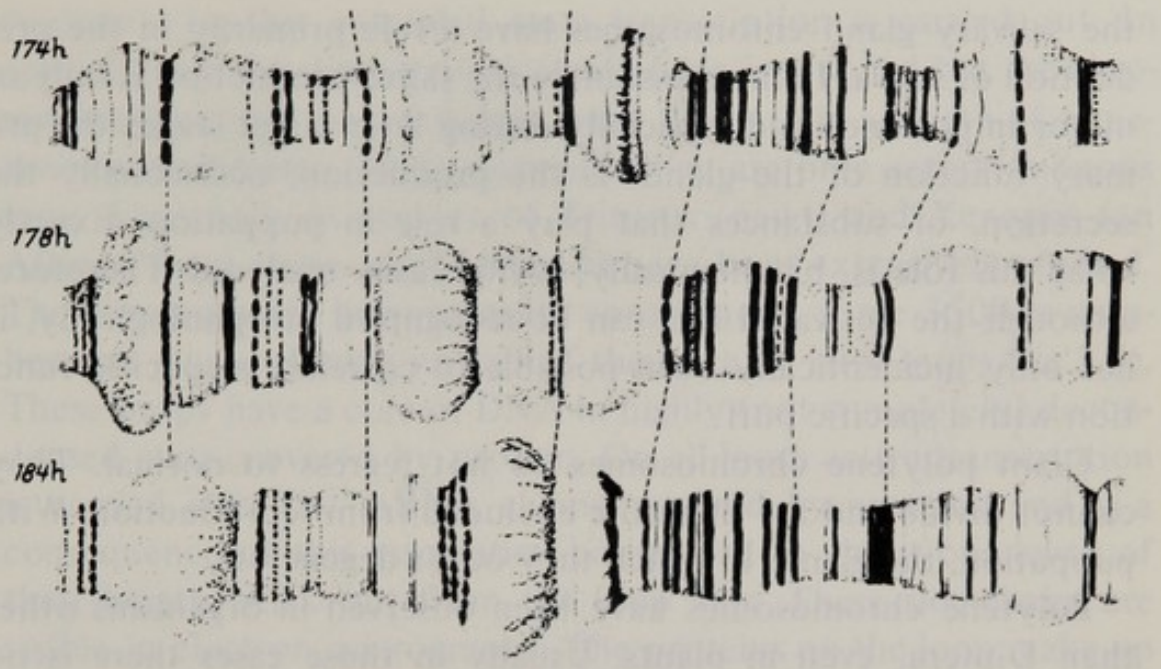


Fig.3.2. Puffs in the polytene salivary gland chromosomes of *Drosophila hydei*: centres of high transcription activity. This is a segment of chromosome 5 at different moments of development of the larvae, with corresponding different patterns of puffing. Age in hours at left (Berendes, 1965).

few DNA-puffs with special function, does not involve an increase in DNA content. Specific staining reactions have shown it to be an accumulation of acidic proteins derived from unspecified sources outside the chromosomes. The RNA content increases also, and much of this is newly synthesized mRNA. The greatly extended DNA loops in the puffs do not shed their histones, but the DNA–histone complex assumes a loose conformation that permits transcription of all available DNA strands of the puff. The messenger RNA produced in the puffs is packed into small protein particles that serve to transport the mRNA through the nuclear membrane into the cytoplasm, where translation is carried out. A considerable fraction of the mRNA, however, never leaves the nucleus. Its role is uncertain.

The study of the biochemistry of polytene chromosomes has been and still is of great importance for the understanding of gene action and regulation. For a review see Berendes and Beermann (1969).

It would not be correct to suppose that the genes activated in

the salivary gland chromosomes have a role primarily in the production of saliva. In most instances the saliva production is only of minor importance and especially during later larval stages the primary function of the glands is the production, occasionally the secretion, of substances that play a role in puppation. Exactly what this role is, biochemically, is not easily analysed. Therefore, although the activated loci can be recognized morphologically, it has only incidentically been possible to correlate a specific function with a specific puff.

Giant polytene chromosomes do not regress to normal. They cannot divide and are therefore excluded from reproduction. With puppation, the glands in which they occur degenerate.

Polytene chromosomes have been observed in organisms other than Diptera, even in plants. Usually in those cases there is no somatic pairing and except in the centromere and some heterochromatic segments the association of the strands is not as good as in the Diptera and the bands are not quite as clear. They usually occur in what are called nutritive tissues. In the suspensor of the kidney bean *Phaseolus vulgaris* polytene chromosomes are relatively well accessible to analysis (Nagl, 1969). In other instances (antipodal cells in the embryosac of several plant species; endosperm cells) the structure is less well organized and then approaches the condition of endopolyploidy (section 1.3.1) where the chromosomes also duplicate repeatedly, but do not remain connected (fig.1.8).

3.1.2. *Lampbrush chromosomes*

Meiosis in the oocytes of most vertebrates and some lower animals is blocked at a stage corresponding with diplotene. At ovulation, the process is resumed. In humans this stage is reached before birth and in most oocytes lasts till the end of the reproductive period: it may span a period of 40 years or more. In most animals it is shorter but still relatively long. During this period or at least large parts of it, an important biochemical activity is required (and observed) for the growth of the egg and for the developments immediately after fertilization. Frequently the bivalents decondense completely and an interphase appearance is acquired (*dic-*

tyotene). In this extended state transcription is carried out. In special cases the diplotene bivalent shape is retained but the chromosomes extend tremendously until they attain a length of sometimes a millimeter, while normally they are only several microns long. Especially in species of Triturus (newt) and Xenopus (an African frog) these giant bivalents have been extensively studied. The chromomeres become quite conspicuous, some 3500 in number and many, though not all of them, carry large loops (fig.3.3). These loops have a core of DNA in highly (not completely) decondensed state, covered by protein. On all loops active transcription is carried out. The mRNA chains, covered by proteins, and as a consequence of this association contracted to about one-tenth of their length, stick out from the loop axis. These side-chains are visible in electron micrographs. The proteins on the loop make up about 98% of the total mass. The microscopically visible loops give the bivalents a brushy appearance from which the name lampbrush chromosome is derived. Some loops have characteristic, sometimes peculiar shapes that make their recognition possible (fig.3.3). The loop is part of the continuous DNP thread of which the chromosome consists: the chromomere apparently consists of two parts one on each end of the loop, which parts in some way are kept together by connections other than the DNA continuity. The chromomere apparently represents a gene, part of which is in the loop. It is understood that the loop is not a specific portion of the gene: the loop segment of the gene is gradually released from one half of the chromomere and regresses into the other half until the entire gene or most of it has passed through the loop stage. Partly on the basis of the fact that lampbrush chromosomes are clearly single stranded (with respect to each chromatid) and yet contain five to six times the quantity of DNA of somatic chromosomes, Callan (for instance in Lima-de-Faria, 1969) has suggested that the DNA in the chromomeres and loops does not represent a single DNA sequence, but one main sequence ("master") followed by a number of copies ("slaves"). The "master" would remain inside the chromomere and the "slaves" would pass through the loop to be transcribed.

The chiasmata in the lampbrush chromosomes are never formed

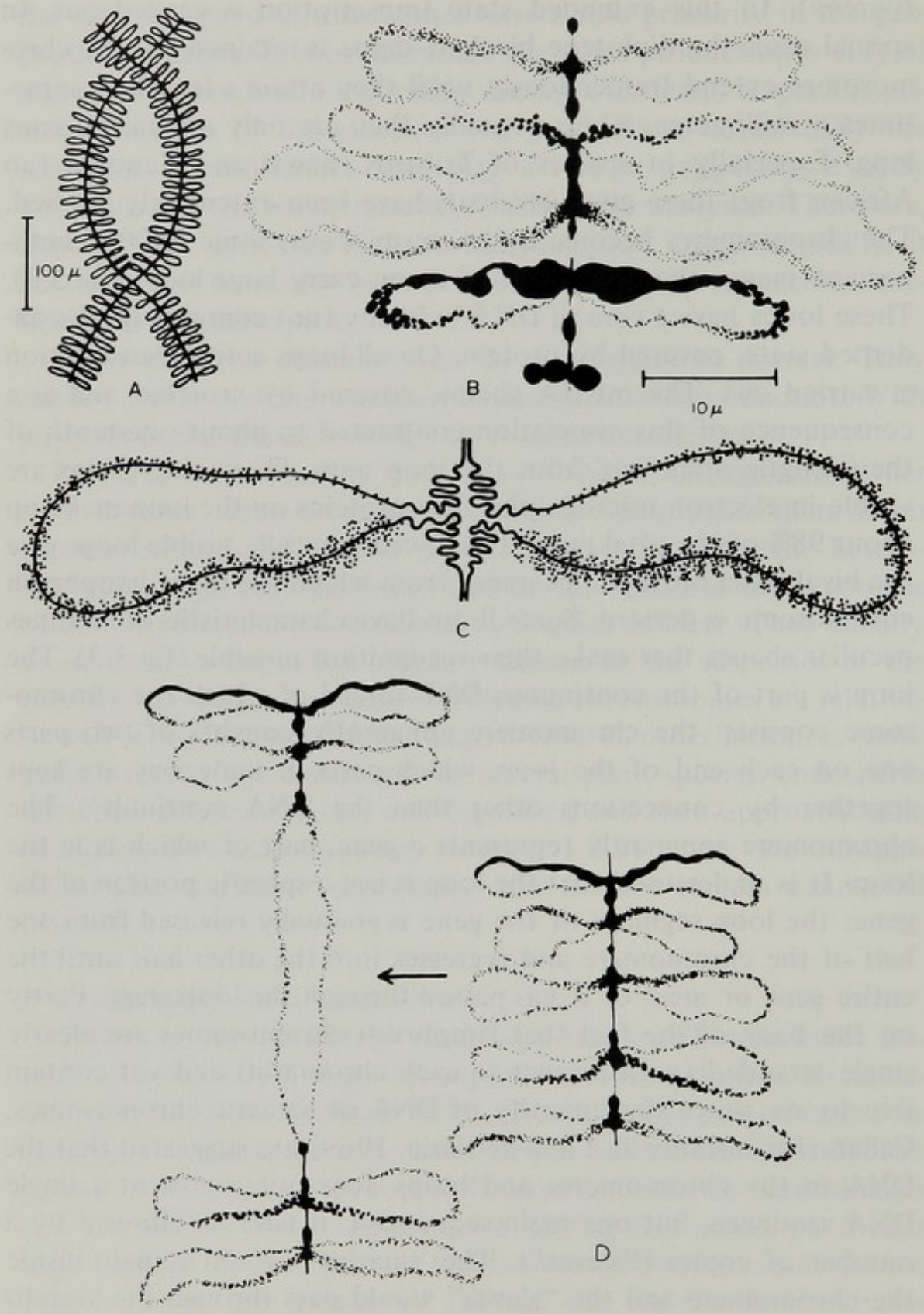


Fig.3.3. (A) Diagram of a lampbrush bivalent with two chiasmata and numerous side-loops. (B) More detailed drawing of a few loop pairs in a lampbrush chromosome, differing from each other in a characteristic way (*Triturus viridescens*). (C) Diagram of the postulated structure of a chromomere and it attached loop pairs. (D) Callan's stretching experiment. (From Gall, 1956.)

in the loops but in or between the chromomeres. It is probable that, like in the giant polytene chromosomes the unit of replication (replicon) is the chromomere, with some coordination between neighbouring chromomeres.

3.2. Permanently specialized chromosomes

3.2.1. *Sex chromosomes. Genetic sex determination*

In most species of animals, but also in several plants and lower organisms, two sexes are distinguished: male and female. The primary function of sexual differentiation is to prevent the combination of gametes derived from the same parent, and the maintenance of a high degree of heterozygosity and genetic exchange. Only few species, especially plants, thrive with complete or almost complete homozygosis as a result of regular self-fertilization. Cross-breeding populations have a high degree of genetic flexibility (with the disadvantage that excellent genotypes are not usually fixed) and can also make use of interallele interactions that are possible only in heterozygotes. At the same time, they carry a load of recessive alleles that in a homozygous condition have a decidedly unfavourable effect: combination of gametes from the same parent leads to inbreeding depression. Although this is an important reason to prevent self-fertilization in cross breeders, it is only secondary: in established predominantly self-fertilizing species such a genetic load does not build up and is not a threat to the population.

To avoid the combination of genetically closely related gametes, many plant species have more or less complex genetic systems of self- and cross-incompatibility. Such systems may even be more efficient than sexual systems in maintaining high levels of heterozygosity. Since they not only involve self-incompatibility but at the same time a high degree of cross-incompatibility, they fail in small populations: either the fertility is severely limited or the system breaks down. Since incidental severe restriction in population size is an important factor in evolution, it is possible that this limitation has been a reason for the minor role incompatibility systems have in animals.

There is considerable variation in the way sex-differentiation (*dioecy*) is realized. In a few cases, for instance the worm Bonellia and the plant Equisetum (horse-tail) the environment of the young organism determines whether it will develop into a male or a female. In the Hymenoptera (bees, wasps, etc.) males develop from unfertilized eggs (they are haploid), females from diploid fertilized eggs (*arrhenotoky*). It seems that heterozygosity for specific genes induces femaleness: the haploid can never be heterozygous. Arrhenotoky has genetic consequences: there is no recombination in the male since all his gametes are identical. For complete sexual differentiation, interaction with the environment (especially the composition of the diet) is important. With all such systems, the numerical relationship between the two sexes (the sex-ratio) depends on non-genetic factors.

Generally, however, sex determination is regulated by a genetic mechanism. In the simplest form there is a single gene or gene-complex. An example of such monofactorial sex determination is asparagus. Several fishes probably also have very simple systems. In principle, three genotypes are possible: AA, AA' and $A'A'$, but since there are only two sexes, only two of these three may be realized. The system resembles a test cross: one of the sexes is heterozygous (the *heterogametic* sex AA'). The homozygote AA forms nothing but A gametes, the heterozygote two types of gametes: A and A'. The progeny is either AA or AA', in a 1:1 ratio. The segregation from which the two types result is realized in the heterogametic sex. In principle, it is not important which of the two sexes is homogametic and which heterogametic. A prerequisite for the proper functioning of the system is that two A' gametes cannot be combined into a $A'A'$ zygote: dioecy and therefore sex-differentiation must be perfect in order to function.

In maize, a monoecious species with female and male reproductive organs in different parts of the same plant, a simple artificial sex determining mechanism has been constructed, that in principle is based on a single factor. The plants of both sexes should be made homozygous recessive for the factor *ba* (barren stalk, also referred to as *sk*, silkless): the plants do not have cobs (the female inflorescence) and exclusively the normally male flowering tassel. There is

a factor *ts* (tassel seed) that in homozygous condition results in tassels that have ovaries instead of stamens: *ts ts* plants are purely female. Combination with homozygosity for *ba* results in *baba tsts* plants that are female (homogametic) and *baba Tsts* plants that are male (heterogametic). With complete sex differentiation one parent produces only *ba ts* gametes, the other *ba ts* and *ba Ts* gametes: male and female progeny arise in a 1:1 ratio. In the twenties and thirties the system has been developed by Emerson (1924) and Jones (1939). In nature it would not be sufficiently efficient in such a primitive form (the sex differentiation is not always perfect, seed production is low, etc.), but it is probable that many natural differentiating systems have developed along comparable lines. Besides a major factor, there are a large number of modifying factors that affect sex expression and that can act in male or female direction. By selection for the proper modifying factors it is even possible to override an otherwise efficiently functioning system of sex determination. In asparagus, where a single main factor determines the system, selection can change the system from dioecy to monoecy. Something similar has appeared possible in the fish Lebistes, where after selection hermaphrodites can be obtained.

In most cases a complex of genes (probably a major factor with several secondary factors) is, as a unit, responsible for sex determination and then occurs in a single chromosome, often combined with fertility factors. For proper functioning the complex may not be broken up: recombination with the homologue (in the heterogametic sex) is highly undesirable. Some pairing and chiasma formation, however, must remain possible, in order to warrant proper metaphase I orientation and anaphase segregation. By this way two differentiated specialized *sex-chromosomes* originate that consist of at least two parts: (1) a sex-differentiating part that is free of exchange and that often is structurally different for the two chromosomes; (2) a homologous part for pairing and chiasma formation. In man, Pearson and Bobrow (1970) could show that the short arm of the Y-chromosome contains the pairing segment: Making the *long* arm fluorescent with quinacrine mustard or quinacrine dihydrochloride shows it to be away from the X-chro-

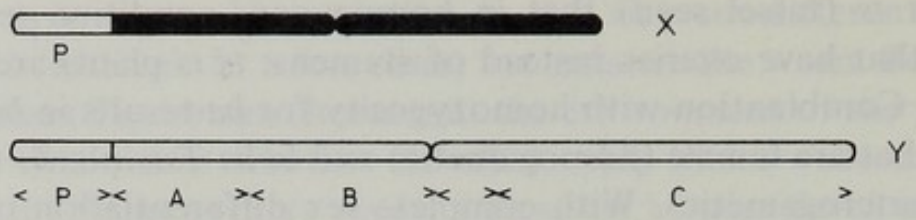


Fig.3.4. The sex-chromosomes of Melandrium: one X and one Y-chromosome. The X is probably largely heterochromatic. Both have the pairing segment P. In the Y-chromosome A and B are male-stimulating segments, C suppresses female development. (From data of Westergaard.)

mosome in X-Y bivalents. Often more segments than the pairing and differentiating segments can be distinguished (fig.3.4).

Usually the sex-chromosomes of the homogametic sex are called X-chromosomes. Then the heterogametic sex has one X-chromosome and a second one that is called the Y-chromosome. In most animals and plants the female is homogametic (XX), the male heterogametic. In birds and butterflies this tends to be reversed. Then, another terminology is often used: two W-chromosomes in the homogametic sex (WW) and WZ chromosomes in the heterogametic sex.

The X-chromosome which occurs in both sexes, usually contains numerous genes that do not play a role in sex determination. The Y, limited to one sex only, does not carry these genes, except for a few in the pairing segment. It is often more heterochromatic than the X and can be significantly smaller (compare fig.1.20), but this is not necessarily always so: In *Drosophila melanogaster* for instance the Y-chromosome is slightly larger than the X, although it is almost entirely heterochromatic. In the plant Melandrium again the Y-chromosome is larger than the X (fig.3.4) but is not entirely heterochromatic. Occasionally, for instance in Locusta and other locusts (fig.2.5) there is no Y-chromosome at all: there is an XO mechanism. Here the single X-chromosome manages to orient itself in meiosis without forming a bivalent, perhaps because of a weak neocentric activity in the end (see section 7.2.1). In anaphase the X moves to one of the two poles and unlike most univalent chromosomes in meiosis, is recovered in 50% of the gametes. There are many, often complicated variations on the sex

Table 3.2
Chromosome constitution and sex in *Drosophila melanogaster* and Melandrium (Warmke, 1946).

Drosophila Chromosomes	Sex	X/A ratio	Melandrium Chromosomes	Sex	X/Y ratio
2A 3X	super ♀	1.5	2A 1X 2Y	♂	0.5
3A 4X	super ♀	1.33	2A, 3A, 4A 1X 1Y	♂	1.0
2A 2X(Y)	♀	1.0	4A 2X 2Y	♂	1.0
3A 3X	♀	1.0	4A 3X 2Y	♂	1.5
4A 4X	♀	1.0	2A, 3A, 4A 2X 1Y	hermaphrodite (predominantly ♂)	2.0
4A 3X	intersex	0.75	4A 4X 2Y	hermaphrodite (predominantly ♂)	2.0
3A 2X(Y)	intersex	0.67	3A, 4A 3X 1Y	hermaphrodite (predominantly ♂)	3.0
2A 1X(Y) (Y)	♂	0.5	4A 4X 1Y	hermaphrodite (predominantly ♂)	4.0
4A 2X	♂	0.5			
3A 1X	super ♂	0.33	2A 2X	♀	~

A represents one complete set of autosomes. In Drosophila, sex is determined by the X/A ratio, in Melandrium by the X/Y ratio.

chromosome mechanism. In certain Orthoptera and Heteroptera, for instance, complexes of up to six sex chromosomes can be formed, apparently originating from reciprocal translocations between an originally single sex chromosome (either X or Y) with an autosome (autosomes or A-chromosomes are the normal chromosomes that have received this name to distinguish them from other types of chromosome). In such complex situations, orientation in meiosis is surprisingly good (compare section 4.5.5).

According to the balance-theory of Bridges, in *Drosophila melanogaster* sex is determined by the ratio of the X-chromosomes and the autosomes, the Y-chromosome not being of any importance in this respect. It does, however, contain male-fertility factors: XO flies are male, but sterile. Hess (1968) and others have demonstrated the presence of lampbrush-loop-like structures with specific shapes, in the Y-chromosome of spermatogonial cells of male Drosophila flies. Deficiencies or even structural abnormality of these loops resulted in sterility. In other organisms such fertility genes are not usually present in the Y-chromosomes. In Drosophila

the X-chromosomes have a feminizing effect, autosomes act in male direction (table 3.2). In man, on the other hand, the Y-chromosome has an important role in sex determination: XXY individuals are male, although not quite normal. In the plant Melandrium, the only plant of which the sex-chromosomes have been studied as extensively as in animals (especially by Westergaard) the Y-chromosome is also important: sex is determined primarily by the X-Y ratio (table 3.2). The autosomes are of more limited importance, as are environmental conditions.

In spite of this variation, chromosomal sex determining mechanisms are generally quite stable. According to Löve, the rather complicated $XX:XY_1Y_2$ mechanism of Acetosa (=Rumex) has not changed in 40 million years. The sex chromosomes can frequently, although not universally, be distinguished, not only from each other, but also from the autosomes. There may be characteristic size differences, and differences in heterochromatin content. Large heterochromatic segments are especially frequent in the Y-chromosomes. These segments carry little or no genetic information and can vary considerably in size without any consequence. A certain type of Y-chromosome, however, may be maintained almost indefinitely and is carried over from one generation to the other: a specific size of Y-chromosome is characteristic for certain families. These differences occur in man also. As far as the difference can indeed be shown to be significant, it is possible to use this characteristic to settle questions of paternity (Chapelle et al., 1967).

In interphase the heterochromatic Y-chromosomes are often visible as condensed bodies. In man, because of their small size, these are usually not clearly distinguishable. Since the long arm of the human Y-chromosome can be made visible by specific fluorescence with quinacrine hydrochloride and quinacrine mustard, it is possible with this technique to distinguish cells with a Y-chromosome from those without (Barlow and Vosa, 1970). It is thus possible to determine the sex ratio in sperm, and even sperm with more than one Y-chromosome can be recognized.

3.2.2. *X-chromosome heterochromatinization. Sex chromatin. Barr-body. Drumsticks*

In many animals the female has two X-chromosomes, the male only one: all X-chromosome genes in the female are present in a double dose, those in the male in a single dose, except for the very few that have an allele in the Y-chromosome. For a number of the X-chromosome genes this difference in dosage would have an effect on gene expression, leading to unbalance in one of the two sexes. In most mammals this is corrected by inactivation by means of heterochromatinization of the greater part of one of the X-chromosomes of the female. Transcription of the majority of genes in this particular chromosome is impossible. With heterozygosity for genes in the X-chromosomes, one of the alleles will be expressed in some cells, the other allele in other cells. For instance, women heterozygous for an abnormality of the gene coding for glucose 6-phosphate dehydrogenase, have two classes of erythrocytes, one with normal and one with low levels of the enzyme. The classes are of approximately equal frequency: the probability of being inactivated is equal for the two chromosomes.

In humans the heterochromatinized X-chromosome is visible as a condensed body of approximately 1 μ diameter in the later stages of interphase in several tissues and is usually positioned on the inside of the nuclear membrane. This body, the *sex-chromatin*, is also named *Barr body* after M.L.Barr, who, in the early fifties, recognized it as the heterochromatinized X-chromosome. In several species of rodents there is so much heterochromatin in the autosomes that the demonstration of Barr bodies is difficult. With high mitotic rates, the number of cells with a visible Barr body is low. Heterochromatinization starts rather early in the development of the individual, and once it has been determined which of the two X-chromosomes will be heterochromatinized, it remains the same chromosome in all succeeding cell generations. Consequently, the expression of the alleles occurs in patches of tissue: in respect to X-chromosome genes, the female mammal is a mosaic. This is especially striking when the X-chromosomes are heterozygous for a colour factor in the skin as in the tortoise shell cat: some sectors have one colour, other sectors have the other colour.

There are no male tortoise shell cats, with the exception of scarce XXY males that have an extra X-chromosome as a result of non-disjunction in one of the parents. Such XXY males also have one Barr body. The same is the case for XXY men. XXX and XXXX females have two and three Barr bodies respectively: there is always only one X-chromosome that remains active. In occasional tetraploid body cells, however, two Barr bodies are observed: apparently doubling of the chromosome number has occurred after X-chromosome differentiation.

In 1961, Lyon, on basis of variegation patterns of X-chromosome genes in female mice, postulated that chance decides which of the two chromosomes remains active. Abnormal chromosomes, however, are generally preferentially heterochromatinized. In women with one normal X-chromosome and one iso-X (consisting of two long arms connected at the centromere), the iso-X is always the heterochromatic one and the Barr body is much larger than normal.

Heterochromatinization is also expressed in late DNA-replication: when tritiated thymidine is applied late in the S phase, only late-replicating chromosomes will take up the label and will be recognizable as such in autoradiographs of metaphase chromosomes. They follow the same rules as Barr bodies: all X-chromosomes minus one are late-replicating. Abnormal X-chromosomes are preferentially late-replicating. These abnormal chromosomes are usually more easily recognized by their replication pattern than as Barr bodies. X-chromosome–autosome translocations, however, are not necessarily preferentially heterochromatinized. Cattanach et al. (1967) described such a translocation in mice in which the dominant allele of the albino locus on an autosome was transferred to the X-chromosome. There was also a corresponding normal autosome with the recessive allele. The female mice were spotted: the originally autosomal dominant allele was inactivated together with the X-chromosome to which it had been transferred, permitting the recessive allele to be expressed. In other patches on the same mouse, apparently the normal X-chromosome had been inactivated, the translocated one with the dominant allele remaining active. In other translocations in the mouse, however, Lyon

found that the translocated chromosome segment always remained active. It appears that the X-chromosome has one, perhaps more centres that regulate the inactivation. It spreads from these centres into translocated A-chromosome segments, but only for limited distances and dependent on temperature (see also Lyon 1972).

In hybrids the X-chromosomes do not behave identically. In the female mule, for instance, the (recognizable) X-chromosome of the donkey is more frequently late-labelling than that of the horse. Cohen and Ratazzi found that also the horse isozyme of G-6-PD was present in the blood cells more frequently than the donkey isozyme.

In certain types of blood cell, the polymorpho-nuclear leucocytes, the sex chromatin sticks out of the body of the nucleus and consists of a head of 1.5 μ in diameter, connected to the nucleus by a filament. It is called *drumstick*. The frequency of detectable drumsticks is low: approximately 1 in 38 cells, against 35%-90% for Barr bodies in other tissues.

In meiotic cells heterochromatinization of the mammalian sex-chromosomes often follows a different pattern: in males both the single X and the Y appear to be heterochromatic, the two X's of the female are both euchromatic. In somatic cells the male Y is usually also heterochromatic, but, at least in men, too small to be detected in the resting nucleus.

In birds and butterflies where the male is the homogametic sex, no sex chromatin has been observed. Late labelling of the single Z chromosome has been noticed in females. For further details see Mittwoch (1967) and Ohno (1969).

3.2.3. *Hemizygosity*

Besides for sex determination the special genetic system of the sex chromosomes has consequences for the segregation and recombination of the genes in them. Usually, one sex has two chromosomes of one type (XX), the other sex only one, but then it is combined with a chromosome that is genetically empty (XY) or it is without companion (XO). Therefore, in the heterogametic sex, recessive alleles in the X-chromosome do not have dominant alleles to suppress their expression: they are *hemizygous* (compare defi-

ciencies, section 4.2.2). One important consequence is that there is no recombination in the sex chromosomes of the heterogametic sex. In most Diptera the autosomes similarly, but for other reasons do not recombine in the heterogametic male. This, however, is a special property of this group and is observed in only few other taxa.

3.2.4. *Deviating segregations of genes in sex chromosomes*

Genetic factors in the sex chromosomes follow these chromosomes in their transfer from one generation to the other: they are *sex-linked* factors. As shown in fig.3.5, the X-chromosome of the male is always derived from the mother, the Y-chromosome from the father. The female receives one X from her mother and the

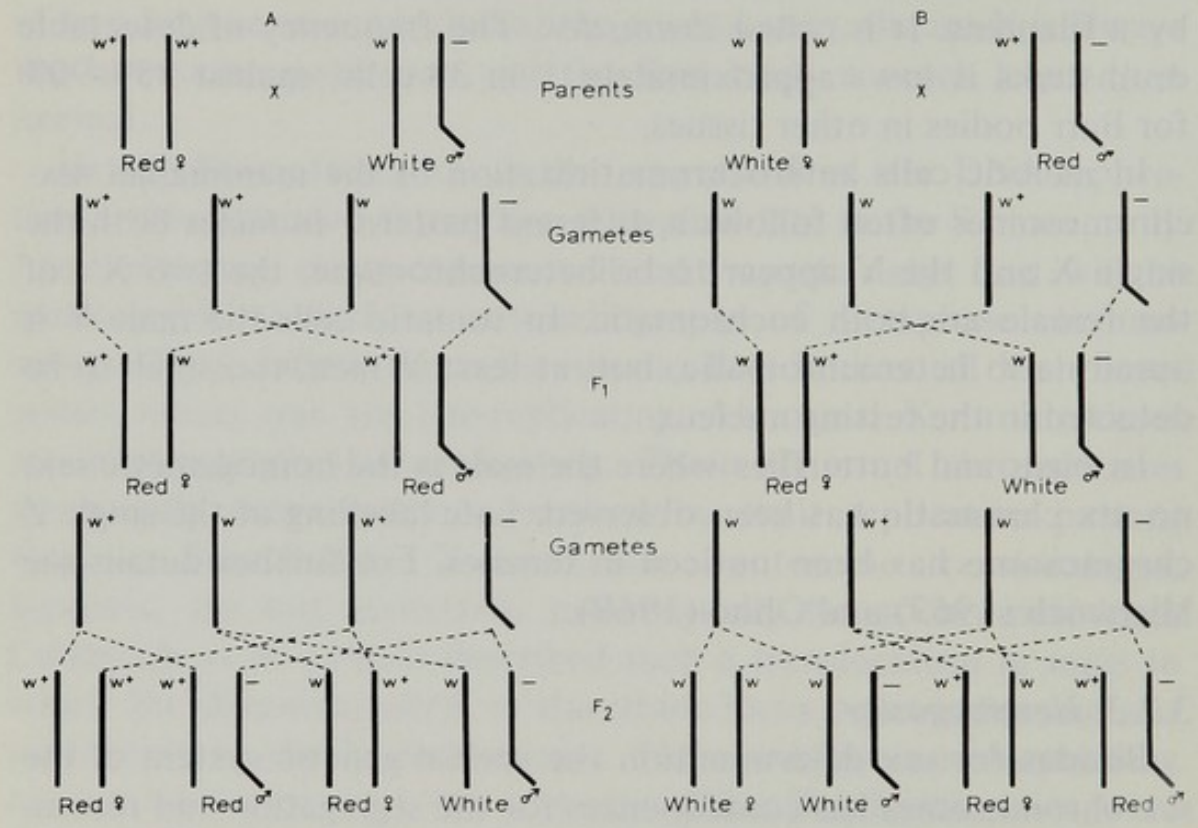


Fig.3.5. The segregation of sex-linked factors in *Drosophila melanogaster* according to T.H. Morgan. The eye colour factor *w* (white eye, recessive; wild type red eye, dominant w^+) is in the X-chromosome and follows the sex determination system. The X-chromosome is represented by a straight rod, the Y-chromosome by a broken rod, the Y does not have an allele of the *w* factor. In A there is a 3:1 segregation, but males all have the recessive allele in the F_2. In B there is criss-cross inheritance (F_1) and a 1:1 segregation in the F_2.

other from her father. One of the earliest sex-linked factors studied was the gene for white-eye-colour (w) in *Drosophila melanogaster*. The red (wild) type (w^+) is dominant. Already in 1910, T.H.Morgan reported a cross between a white-eyed male ($w-$; $-$ indicating absence of a second allele: hemizygosity) with homozygous red-eyed females (w^+w^+). The F_1 had red eyes, both for males and females (fig.3.5). The F_2 (brother–sister crosses) showed 3470 red-eyed and 782 white-eyed flies: a common deviation from the normal 3:1 segregation which is due to reduced viability of w gametes and ww or $w-$ (= hemizygous w) zygotes. The white-eyed flies were all males. A white-eyed ($w-$) male, crossed with heterozygous (w^+w) females gave: 129 red-eyed females (w^+w); 132 red-eyes males (w^+-); 88 white-eyed females (ww); 86 white-eyed males ($w-$). Again a considerable deviation from the expected ratio (here 1:1:1:1) but the rule of sex-linkage is obeyed. While in the first example linkage with sex was indeed observed, this is not so in the second example: here also white-eyed females and red-eyed males appear. The term sex-linked, therefore, is somewhat misleading. Starting from the opposite situation: w^+- (red) males × ww (white) females, the female F_1 flies have red eyes (w^+w) and the male flies white eyes ($w-$): *criss-cross inheritance* (fig.3.5). The F_2, however, again produces red- and white-eyed flies for both sexes, in the 1:1:1:1 ratio.

Not all genes in the X-chromosome follow this rule. Very few have an allele in the Y-chromosome, in Drosophila for instance bobbed, that in recessive condition makes the hairs of the fly shorter and thinner. It is a mutation of the nucleolar organizer. A male with bobbed in the X-chromosome but with the wild-type allele in the Y-chromosome, is normal. Crossed with a homozygous bobbed female, it has sons that are normal and daughters that are bobbed. Sex-linked factors have been studied in a large number of organisms, including man and occasionally indications of genetic activity in the Y-chromosome have been found.

3.2.5. *X-chromosome mutations*

Because of *hemizygosity*, recessive mutations in the X-chromosome are fully expressed in the heterogametic sex. This property

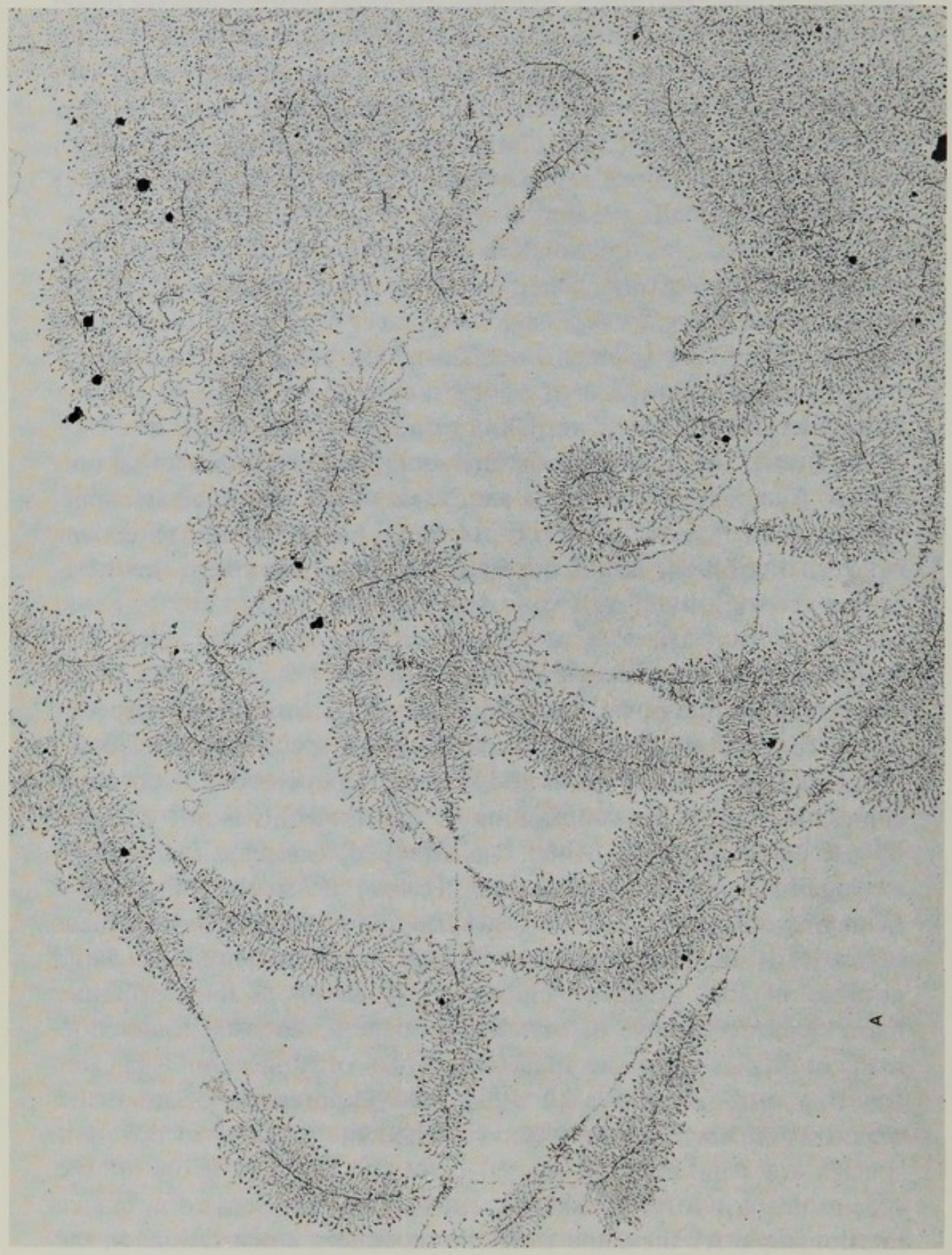
has been used extensively in experiments on artificial mutagenesis with various agents, applying different conditions and often with different pre- and post-treatments. The simplest mutations studied are the *recessive lethals*. Already in the late twenties, Muller developed the *ClB* method in which irradiated male flies were mated with females of a very special genetic constitution. They had, in addition to one normal X-chromosome, an X-chromosome with several markers: (1) an inversion (*C*) that served to suppress recombination with the normal chromosome; (2) a lethal factor (*L*) leading to the death of animals lacking the normal allele; (3) a dominant, actually intermediate, factor *B* (Bar) in the heterozygote causing a bar-shaped eye, homozygous Bar having a very narrow eye. The F_1 of this cross has the *l*-factor in the X-chromosome of half of the males: there is no compensating allele in the Y, so these males do not develop. The sex ratio is 1:2, males:females. None of the males, of course, have an X-chromosome from their irradiated father, and these, therefore are not of interest for the experiment. Of the female flies in this F_1, half has the normal chromosome of the mother in addition to one irradiated chromosome of the father and is normal in respect to recessive mutations. The other half of these females has a *ClB* chromosome of the mother and can be recognized because of their Bar eyes. They have one irradiated chromosome from their father and because of the inversion recombination with the *ClB* chromosome is restricted. These Bar-females are isolated and used in the next step: they are mated individually with normal males and the progenies are bred separately. In these progenies again, a male: female ratio of 1:2 is observed as half of the males has a *ClB* chromosome and dies at an early stage. In some cultures, there are no males at all: these represent the irradiated X-chromosomes with a recessive lethal mutation. Simply counting the cultures without males gives the frequency of this kind of mutation. To find other recessive mutations, a more close observation is required. Since all males of the population represent the same mutation, the detection is still relatively simple and quite reliable.

The *ClB* method has certain disadvantages: for instance, the possibility of some crossing-over between the *ClB* and the mutant

X-chromosome in the intervening generation is not entirely excluded. Muller, therefore, developed several more methods based on comparable principles. The most frequently used is the *Muller-5 method.* Here, the females have one X-chromosome with two inversions (to prevent crossing-over) and three marker factors: Bar (*B*) (dominant), scute (sc^8), a hair factor and white-apricot (w^a), an eye colour factor, both recessive. The method runs as follows: (1) The Muller-5 females are mated with irradiated males and from the F_1, the Bar females are isolated: they have one Muller-5 and one irradiated chromosome. They are not phenotypically scute nor apricot, since these are recessive factors; (2) the F_1 Bar females are individually mated to normal males and the progenies bred separately. In these cultures, half of the males have *B*, sc^8 and w^a, the remainder have the irradiated X-chromosome. When only *B* sc^8 w^a males occur, the irradiated chromosome had a recessive lethal mutation. Again, vital mutations can be detected. The recessive marker genes sc^8 and w^a serve to detect possible crossing-over between the Muller-5 and the irradiated chromosome in the intervening generation.

3.2.6. *Nucleolar chromosomes*

Every genome normally contains one, occasionally more nucleoli, that are attached to a fixed place in a specific chromosome. Nucleoli follow the division and segregation cycles of the chromosomes. Normally the functioning of the nucleolus is not affected by the chromosome to which it is attached, as long as the *nucleolar organizer* remains unimpaired. It may be freely translocated from one chromosome to another. Loss of the nucleolar organizer often leads to the formation of very small nucleoli on a large number of loci in all chromosomes. It seems as if in principle many loci are capable of organizing nucleoli, but that under normal conditions only the major nucleolar organizer performs this function and suppresses all other loci. Suppression of nucleolar organization may also be observed when the genomes of different species are combined: in several cases the nucleoli of one of the species are not formed. There seem to be rather strict dominance relationships. At the same time the secondary constrictions in the



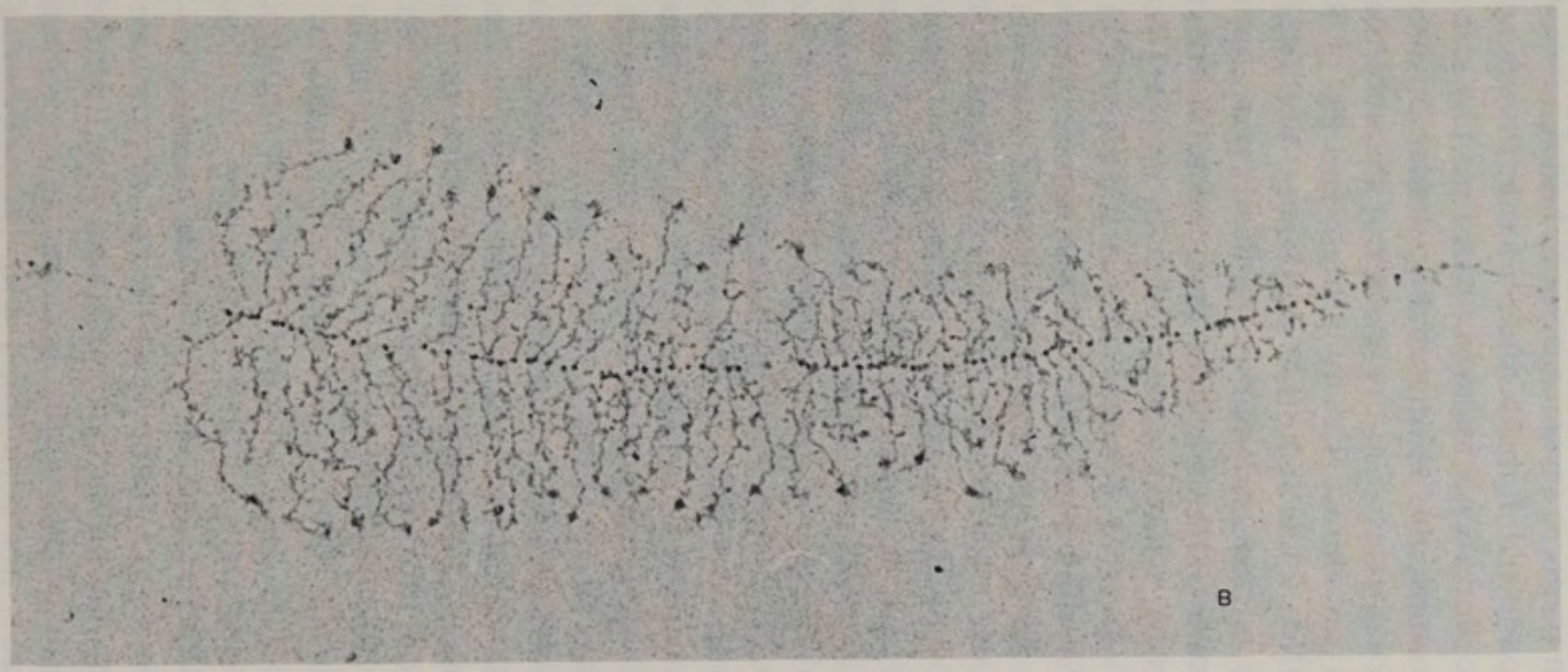


Fig.3.6. (A) Electron micrograph of a portion of the highly reiterated nucleolar (40S rRNA) gene in an oocyte of the spotted newt *Triturus viridescens*. The fibrils are 40S rRNA precursor molecules in the process of being read off the DNA axis. The length of the fibrils reflects the stage of transcription: there is a polarity that is the same for each gene. The rRNA is condensed by a factor ten as a result of its association with proteins. Granules of 125 Å diameter on the DNA may be RNA polymerase molecules. Numerous RNA molecules are synthesized simultaneously on each genes. The successive genes are separated by a segment without transcription. (B) A single nucleolar gene at higher magnification (×50000). The rRNA is equivalent to mRNA transcribed on a normal gene. (Courtesy Dr. O. Miller.)

chromosomes, usually associated with nucleoli, do not appear. It has been observed in plants in several species hybrids and allopolyploids. In man–mouse cell hybrids only mouse ribosomes are made, probably also due to nucleolar suppression. The structure and function of the nucleolus is remarkably similar in different cells, organs and even species. The dry matter of the nucleolus on the average consists for almost 92% of protein and 8% of RNA. There are traces of DNA, lipids and other substances. Yet this DNA is the essence of the nucleolus: it is the practically pure, approximately 1000-fold reiteration of a gene that codes for the ribosomal RNA. The gene is serially repeated with spacer DNA in between (fig.3.6). In the frog *Xenopus laevis* with 800 rRNA cistrons, each is transcribed by some 100 RNA polymerase molecules: there are 80.000 in total. In oocytes of the newt Triturus at the lampbrush chromosome stage, a large number of small nucleoli are successively produced by the nucleolar organizer and released into the nuclear sap. This results in a 3000-fold increase in the number of rRNA cistrons relative to the rest of genome. The RNA formed is a 45S precursor of rRNA and it is coated with protein immediately upon formation. In several steps this large molecule is split up into two smaller fractions (18S and 28S) and a residual fraction that remains in the nucleolus. The 18S and 28S fractions, associated with protein and in rather compact form, move through the nuclear membrane into the cytoplasm to form the ribosomes. The rRNA cistrons are quite stable; mutations apparently are efficiently removed. Occasional unequal crossing-over seems to lead to variations in redundancy. Sometimes it appears possible to split up the nucleolar organizer into two functional parts. A probable mutation in an rRNA cistron is the bobbed mutant in the nucleolus-carrying X- and Y-chromosomes of *Drosophila melanogaster*: hair formation, requiring very rapid protein formation is impaired by insufficient ribosome activity. For a brief review of ribosome synthesis see Perry, 1969.

Nucleoli practically always disappear at the end of prophase and reappear at telophase before the chromosomes have completely despiralized. In the interphase nucleus, nucleoli can fuse. Then the observable number does not correspond with the number of nu-

cleolar organizers. When the number per genome is known, the maximum observed number is an indication of the degree of ploidy. A second reason why the number of nucleoli may be lower than the number of genomes, in hybrids and in allopolyploids, is that the nucleolus of one species can suppress the formation of that of the other species.

3.2.7. *B-chromosomes*

A large number of plant and animal species, besides having autosomes (A-chromosomes) and perhaps sex-chromosomes, possess a special catagory of chromosomes without obvious genetic function. These occur mostly in only part of the population and between individuals can vary in number per cell. In animals they occur most frequently in insects but may even be found in some small mammals.

These B-chromosomes (supernumerary chromosomes, accessory chromosomes, accessory fragments, etc.) usually have a normal structure, are often somewhat smaller than the autosomes and can be predominantly heterochromatic (many insects; maize, see fig.3.7A) or predominantly euchromatic (rye, see fig.3.7B). In maize, their number per cell can vary from 0 to 30 (in nature infrequently over four). Their effect on the genotype is small when they occur in low numbers. Only with higher frequencies there is a clearly unfavourable effect on development and fertility.

Occasionally, especially in animals, the B-chromosomes disappear from the non-reproductive (somatic) tissues and are maintained only in the cell-lines that lead to the reproductive organs. It has been suggested that in populations in which B-chromosomes

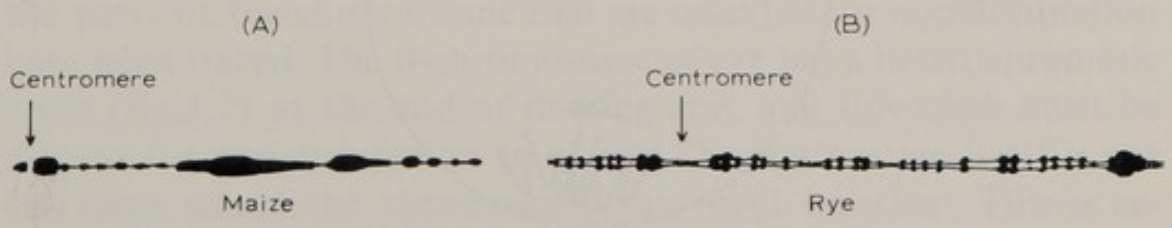


Fig.3.7. B-chromosomes of maize and rye at pachytene, semi-diagrammatically after Rhoades, 1965 (maize) and Müntzing and Lima-de-Faria, 1949 (rye). Stage and magnification slightly different.

are frequent, they have a selective advantage by way of the effect they have on the regulation of recombination (section 2.9.6). The most important factor in their survival in spite of a lack of obvious benefit to the organism or even a clearly deleterious effect, is their unusual behaviour in meiosis or, in plants, in the nuclear divisions of the gametophyte (pollen and/or embryo sac). This behaviour results in an accumulation. The fact that such systems of accumulation are necessary to maintain the B-chromosomes, without leading to excessive numbers, shows that they have negative rather than positive consequences for the organism in which they occur: in a sense they are parasitic. In highly selected populations they disappear without exception.

In animals, accumulation is usually restricted to the females: the basis is non-disjunction, either at first or at second division. The two chromosomes (in MI) or the two chromatids (in MII) remain together and are included in the single cell that develops into the egg (compare section 2.4.1). When there is only one B-chromosome, it moves to the pole where the functional nucleus is formed. Instead of one, the egg-cell receives two B-chromosomes. In the spermatocyte divisions, B-chromosome distribution is often random. Accumulation of B-chromosomes in plants has been studied most extensively in the male parent. Two examples will be given: rye and maize. In rye, PMC meiosis is normal and

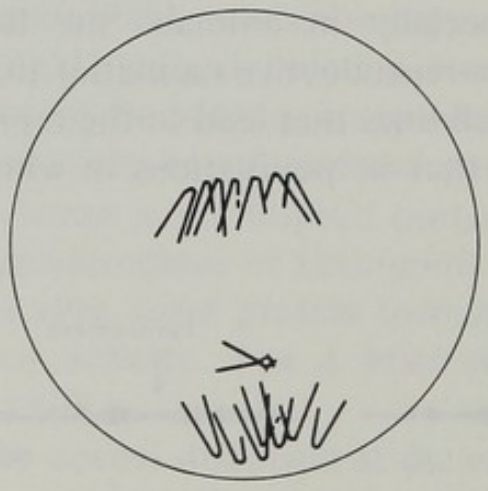


Fig.3.8. Non-disjunction of a B-chromosome at the first pollen mitosis in rye. The unsplit B stays in a peripheral position where it is included in the generative nucleus. The chromatids separate during interphase.

non-disjunction occurs at the first pollen mitosis (fig.3.8). The dividing nucleus occupies a position at the perifery of the cell. At anaphase the two chromatids of the B-chromosome do not separate but stay together and remain at the position of the original nucleus. This is also where the daughter nucleus stays that passes through a second mitosis to produce the two generative nuclei. Thus, the vegetative (pollen tube) nucleus is without B-chromosomes, the generative nuclei each have two. In the development of the embryosac, a similar phenomenon can be observed. With special breeding methods, rapid accumulation is possible. In "natural" populations, however, even the occurrence of four B-chromosomes in one plant is relatively infrequent. In maize, non-disjunction takes place at the second pollen mitosis: one of the two generative nuclei receives two, the other no B-chromosomes. Now *selective fertilization* is required for accumulation: the orientation of the mitotic spindles in respect to the porus of the pollen grain is such that the vegetative nucleus goes first into the pollen tube, followed by the nucleus with two B-chromosomes, while the one devoid of B-chromosomes is the last. The egg nucleus is situated at that micropylar side of the embryosac, where the pollen tube penetrates. The first generative nucleus (the one with the B-chromosomes) will therefore in most cases fertilize the egg nucleus.

In some species of Lillium and Trillium where usually single B-chromosomes occur, at EMC meiosis unpaired B-chromosomes move towards the pole which later organizes the functional tetrad cell. In these species nothing special occurs in pollen development: the single B's are distributed at random in meiosis, and pollen mitosis is normal.

With the use of incomplete and translocation B-chromosomes, the parts of the chromosome that are essential for non-disjunction have been traced. The B-chromosome of rye has a heterochromatic knob (fig.3.7) at the end of its long arm, and this knob must be present for non-disjunction to occur. The centromere itself is not the locus where the chromosomes are held together: This is accomplished by a point in each arm at some distance from the centromere. These two points are equivalent: an iso-B-chromosome consisting of two long arms joined by the centromere, shows

non-disjunction. A similar chromosome without the heterochromatic knob, however, does not. When the knob is present in another B-chromosome in the same cell, then the non-disjunction mechanism functions also in the deficient chromosome. In translocations between A and B chromosomes in maize, an A-chromosome occasionally shows a tendency to non-disjunction when the distal heterochromatic portion of a B-chromosome has been transferred to it. As long as this is somewhere present in the cell, the B-chromosomes too, like in rye, will perform regular non-disjunction. When it is not, non-disjunction occurs nowhere in the cell. In maize, the chromatids stick together quite close to the centromere. A-chromosomes with heterochromatic knobs tend to be eliminated at second pollen mitosis where they often fail to split properly.

The origin of the B-chromosomes is uncertain. In some animals they may be derivatives of the sex chromosomes, but this is not the rule. They generally do not show any pairing affinity with the A-chromosomes. Gibson and Hewitt (1970) found the base composition of the DNA of the B-chromosomes of the grasshopper Myrmeleotettix to be different from that of the A-chromosomes. In species hybrids tendencies to non-disjunction somewhat similar to that in Bs are regularly observed in some or all chromosomes of one of the two species, often leading to gradual elimination, not to accumulation. The cause may be delayed separation of chromatids, due to uncoordinated timing of chromosomal processes. Possibly B-chromosomes are stabilized remnants of interspecific hybridization with a non-disjunction system restricted to critical stages where accumulation instead of elimination results.

3.2.8. *Holokinetic chromosomes*

The chromosomes of most plants and animals have centromeres that are situated at one specific position in each chromosome. In a number of animals, especially insects of the order of the Hemiptera and a few, mostly monocotyledonous plants (Juncales, Cyperales), the kinetic activity is distributed over the entire chromosome: holokinetic chromosomes. The term diffuse centromere has been used as an alternative, but is not quite logical. In 1966 Flach

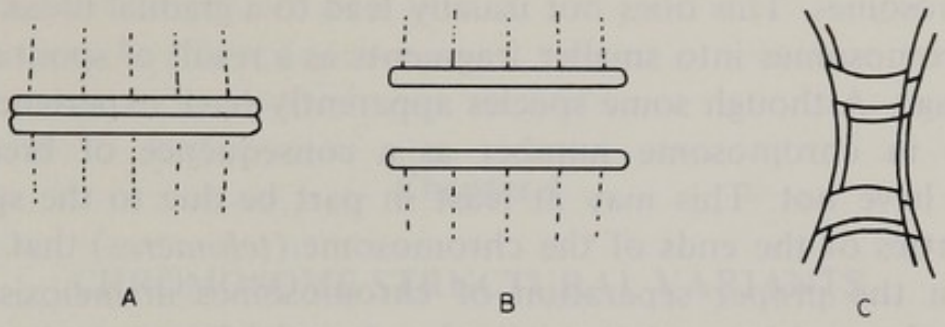


Fig.3.9. (A) Holokinetic chromosome at mitotic metaphase, (B) at anaphase (diagrammatic). (C) Meiotic metaphase I bivalent of a holokinetic chromosome pair (diagrammatic). The kinetic activity and the connection between the separating chromosomes both concentrate at the ends, giving the bivalent its peculiar shape. The two chromatids of each chromosome may be considerably separated.

found strong indications that this type of centromere occurs also in some primitive Dicotyledons (Ranales: Myristica). Comparable chromosomal types in the nematode Ascaris and in the pseudo-scorpion Tityus are possibly polycentric: in some developmental stages (Ascaris) or in related species (Tityus) the same chromosomes are found to be split up in many, perhaps each monocentric, small chromosomes. It has been suggested that in all other cases too the chromosomes are polycentric rather than holokinetic, but with the methods presently available, the distinction cannot be made.

In mitotic metaphase, the chromatids orient parallel in the equator: one chromatid towards one pole, the other towards the other pole (fig.3.9A). This is also the way they separate in anaphase (fig.3.9B), and they maintain this orientation until they arrive at the poles. Such metaphase orientation requires considerable coordination in order to prevent the formation of bridges in anaphase such as are regularly observed in dicentrics with widely separated centromeres. Probably the kinetic activity starts at one point and proceeds from thereon, orienting each unit to the preceding one. In a similar way the two centromeres in a dicentric with the centromeres close together tend to orient to the same pole. Chromosomal fragments have enough "centromere" activity to behave like complete chromosomes, and do not get lost as they would if the original chromosome had been monocentric. This is one of the methods to distinguish holokinetic from monocentric

chromosomes. This does not usually lead to a gradual breaking up of chromosomes into smaller fragments as a result of spontaneous breakage. Although some species apparently have experienced increase in chromosome number as a consequence of breakage, many have not. This may at least in part be due to the specific properties of the ends of the chromosome (*telomeres*) that play a role in the proper separation of chromosomes in meiosis: new breaks do not have such specialized ends and fail in meiosis.

Meiosis deviates somewhat from that in monocentric species. It also differs between species with holokinetic chromosomes. The Hemiptera especially show a considerable variation, and often they are difficult to analyse. Meiosis in Luzula (Juncaceae) is representative for some meiotic types and in those Luzula species that have reasonably large chromosomes it can relatively easily be studied (Nordenskiöld, 1962). All is normal up to the moment of spindle formation (prometaphase I). Then, however, the chromosomes must be sorted out in such a way that they do not interlock. How this is accomplished is not known, but the result is that at anaphase two chromatids move to one pole and two to the other pole. The chromatids remaining together are sister chromatids (reductional separation) at one side of a chiasma and non-sister chromatids (equational separation) at the other side.

In several insects, but also in some plants metaphase and anaphase I follow a different course (Hughes-Schrader, 1948). The chromosomal ends, perhaps because of terminalization of chiasmata, remain associated during MI while the central portions separate. The kinetic activity also concentrates in the terminal regions, which therefore are greatly stretched out, the central parts remaining condensed. This results in the peculiar structure that is diagrammatically shown in fig.3.9C. This structure often helps in the identification of holokinetic chromosomes. The main criterion for identification of course is metaphase–anaphase behaviour, but in species with small chromosomes this may be difficult to distinguish. A third criterion is the mitotic behaviour of fragments, as mentioned above.

Chapter 4

CHROMOSOME STRUCTURAL VARIANTS

4.1. Introduction

The somatically stable structural variants of section 1.4.11 can cause meiotic complications that may have consequences for the chromosomal and genetic composition of the gametes and consequently of the progeny. Some of these complications are merely variations of the normal behaviour, others are straightforward irregularities that sometimes may result in the (secondary) origin of one of the other types of chromosomal aberrations. In the following, the phenotypic effects and the meiotic and segregational consequences of the four main types of structural variants will be discussed: deficiencies (section 4.2), duplications (section 4.3), inversions (section 4.4) and translocations (section 4.5).

4.2. Deficiencies

4.2.1. *Types*

The terms deficiency and deletion in practice have the same meaning although some authors tend to use them in a somewhat different sense. This difference will be neglected here and the term deficiency will be used throughout. There are two principal types: *terminal* and *intercalary* deficiencies. In some organisms terminal deficiencies are more common (for instance in maize) than in others (for instance Drosophila) which may be related to differences in repair systems or stability of open breaks. Certain loci, such as the centromeres or the secondary constrictions produce

stabile ends rather easily. When a centromere breaks in such a way that it remains functional in at least one of the two arms, a *telocentric* (half) chromosome is produced. Although it has been observed that a centromere can be split into two functional halves, frequently the break is such that only one of the two parts is sufficiently large to function. Then one of the arms is lost and a large deficiency results. This may be tolerated in polyploids but even in heterozygous conditions it is too drastic for normal diploids. As extra chromosomes, however, telocentrics are tolerated readily by diploids, and therefore they are usually not treated with the structural variants but with the numerical variants: see chapter 5.

A special category of stabile terminal deficiencies arises as a consequence of reciprocal translocation between a normal A-chromosome and a B-chromosome. As long as the B-chromosome is present in the cell, there is no net deficiency. Only when the B-chromosome, that by itself is not essential, is lost, the A^B chromosome can be considered as a deficiency chromosome. Distal of the break, of course, there is a segment of B-chromosome: the deficiency is not truly terminal.

4.2.2. *Phenotypic effect*

With all heterozygous deficiencies, the chromosomal segment involved occurs in a single dose in the diploid: the genes in such segments are *hemizygous* (compare sex-chromosomes, section 3.2.3). Then recessive alleles can be expressed: they become *pseudo-dominant* as a result of the deficiency (fig.4.1). In an extensive experiment on deficiencies in maize, McClintock in 1941 used a breakage-fusion-bridge cycle (fig.1.16; section 1.4.11) to induce deficiencies. One chromosome 9 had a short, non-lethal terminal deficiency in the same arm in which closer to the centromere, the dominant allele of the factor *c* (colourless aleurone; *C*: coloured aleurone) was situated. Through the pollen it came into the endosperm, where it was combined with two normal chromosomes 9, each with the recessive allele *c*. Because of *C*, the aleurone was coloured. Although in the embryo the break appeared to be stable, it lead to a breakage-fusion-bridge cycle in the

endosperm. The new breaks could occur anywhere in the bridge. If the break occurred between the locus of *C* and one of the two centromeres, then the cell this centromere went to, did not receive *C*. As it had only the two *c* alleles of the mother, it lacked colour. The sector of the endosperm developing from this cell was also colourless: variegation of the aleurone. When the location of the deficiency can be cytologically determined, the location of the hemizygously expressed factor is known. This is an efficient method of localization of genes that also in man has led to determination of the chromosome segment in which recognizable genes occur. It will be further discussed in chapter 6.

Very small deficiencies that comprise little more than one factor locus, sometimes resemble *recessive mutations*. When a known recessive factor is nothing else than a non-functional version of a normal factor (i.e. it does not have an independent expression) the deficiency for that factor will result in the same recessive phenotype. In Drosophila, for instance, a recessive mutation, not accompanied by a detectable change in the chromosome is known, that causes the body colour to be yellow: yellow body, *y*. A very small deficiency, comprising only a few bands including the locus of this factor, caused the same phenotype. In maize too, deficiencies have been found that phenotypically completely resemble the corresponding mutants: white seedling, pale-yellow seedling, brown midrib. Most genes involved in a deficiency cannot be classified qualitatively: they lack a recognizable phenotype and are expressed as *negative changes* in quantitative characters (rate of development, size, fertility, etc.). Large deficiencies give a serious unbalance of the genetic equilibrium and are in general deleterious even in heterozygous condition. When homozygous, they behave as many small deficiencies do, like *recessive lethal factors*. Demerec and Hoover have studied a series of apparently terminal deficiencies of the X-chromosome of *Drosophila melanogaster*. Loss of 10-11 bands, including the factors *y* (yellow body), *ac* (achaete), and *sc* (scute) when homozygous was lethal for the organism and even for individual cells. Eight bands with *y* and *ac* when homozygous deficient were lethal for the fly, but not for individual cells. Loss of four bands without known genes did not

cause lethality. There also are deficiencies that behave like dominant mutations. They are not viable when homozygous: these too are in fact recessive lethals. In Drosophila several are known: Pale, Beaded, Notch. Of some of these, such as Notch (notched wings; located in the X-chromosome) dominant mutations on the same locus with the same phenotype are known, but these are not caused by a recognizable deficiency. The "cri-du-chat" syndrome in man is based on heterozygosity of a deficiency of a large segment of the short arm of chromosome 5. Besides the sound of the voice, several other characters are abnormal, which is not unexpected with a rather large deficiency. Many deficiencies in man have been described, several with specific effects and most of them probably lethal when homozygous.

In special cases deficiencies may have an adaptive advantage: in some cancer cell-lines certain deficiencies, sometimes even involving entire chromosomes, appear to contribute to the very specialized adaptation of the tumor. The surrounding normal tissue can take care of a large number of vital functions, so that the loss of several factors, accompanying the disappearance of regulating functions, does not present difficulties.

4.2.3. *Chromosome pairing. Recombination. Genetic consequences*

At the place of the deficiency, where only one of the two homologous chromosomes is complete, this forms a loop (fig.4.2) when pairing is exact. In the polytene salivary gland chromosomes of the Diptera, the pairing of homologues is quite exact and the location of the deficiency can be accurately determined, while the banding pattern makes the boundaries clearly recognizable. In pachytene bivalents, as far as they are accessible to analysis, the location is much less easily determined because fewer details of the chromomere pattern can be recognized, and also because the boundaries of the loop can move as a result of non-homologous pairing. Small deficiencies do not even appear as loops.

Within a deficiency exchange is impossible. Disturbance of pairing causes a reduction of exchange in a segment around the deficiency. Alteration of the interference pattern may increase ex-

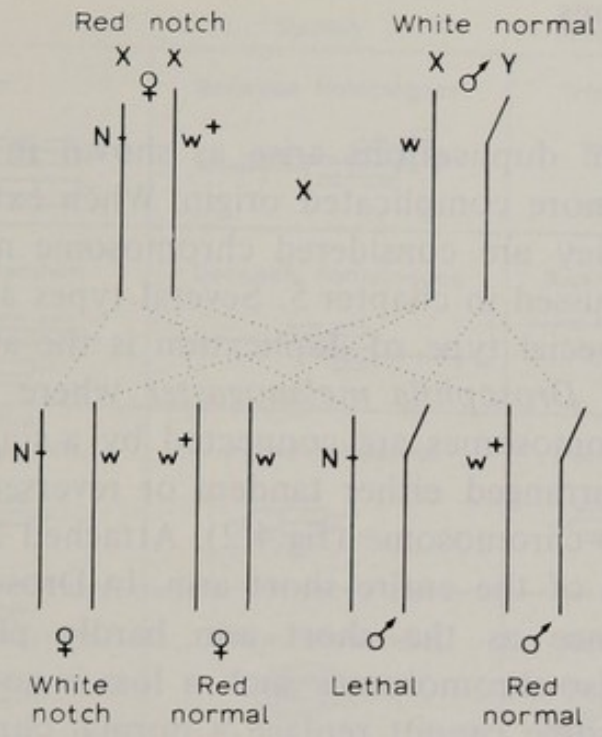


Fig.4.1. Segregation involving the deficiency Notch in the X-chromosome of *Drosophila melanogaster*. It contains the locus for white eyes *w* (red eyes *w*⁺). The Notch type is dominant, but homozygous (in the female) and hemizygous (in the male) it is lethal. The *w* gene in heterozygous Notch females is hemizygous (pseudo-dominant).

change in adjacent parts of the same chromosome. The frequency by which a deficiency is transmitted from one generation to the next depends on its length, the indispensability of the genes involved and the autonomy of the haplophase: in plants this phase is rather sensitive to unbalance as a certain development has to be completed. In addition competition in fertilization results in a strong selection for normal types of pollen grains. In animals the genetic composition of the nucleus has little effect on the haplophase. Here, however, zygote and embryo lethality are of more importance, The deficiency Notch in Drosophila (compare section 4.2.2) behaves like a sex-linked dominant factor in the female, but is lethal in the male (fig.4.1). Only through heterozygous females the deficiency can be maintained.

4.3. Duplications

4.3.1. *Types*

Simpe tandem duplications arise as shown in section 1.4.11. Others have a more complicated origin. When extra centromeres are involved, they are considered chromosome number variants and will be discussed in chapter 5. Several types are distinguished (fig.4.2A). A special type of duplication is the attached-X ($\widehat{XX}$) chromosome of *Drosophila melanogaster* where two practically complete X-chromosomes are connected by a single centromere. They may be arranged either tandem or reversed: in the latter case it is an iso-chromosome (fig.4.2). Attached X-chromosomes involve the loss of the entire short arm. In Drosophila this is of little consequence as the short arm hardly plays a role. In other cases of iso-chromosomes such a loss is not tolerated, and the iso-chromosome cannot replace a normal chromosome. It is then found only as an extra chromosome. Such cases are discussed also in chapter 5.

4.3.2. *Phenotypic effect*

The phenotypic effect of duplications is not as striking as that of deficiencies and larger duplications are tolerated than deficiencies. In plants again the haplont is more sensitive than the diplont. For genes that are sensitive to dosage, a specific phenotypic expression is expected to result from a duplication. Whenever the breakage-fusion-bridge cycle (mentioned above) causes a deficiency by which the dominant allele *C* is lost, the opposite cell receives a duplication for *C* (see section 4.2.2 and fig.1.17). The gene-dose is increased in this cell and the colour of the endosperm sector grown from it is more intense than the colour of the surrounding tissue. When both cells manage to form a sector, there are an uncoloured and a dark coloured sector side by side: *twin sector.* Repetition of the process in the dark sector can lead to spots with very different intensities.

Some duplications do not merely result in an intensification of an existing, recognizable effect, but show a new phenotype: they behave like *dominant mutations.* The best known is the Bar mu-

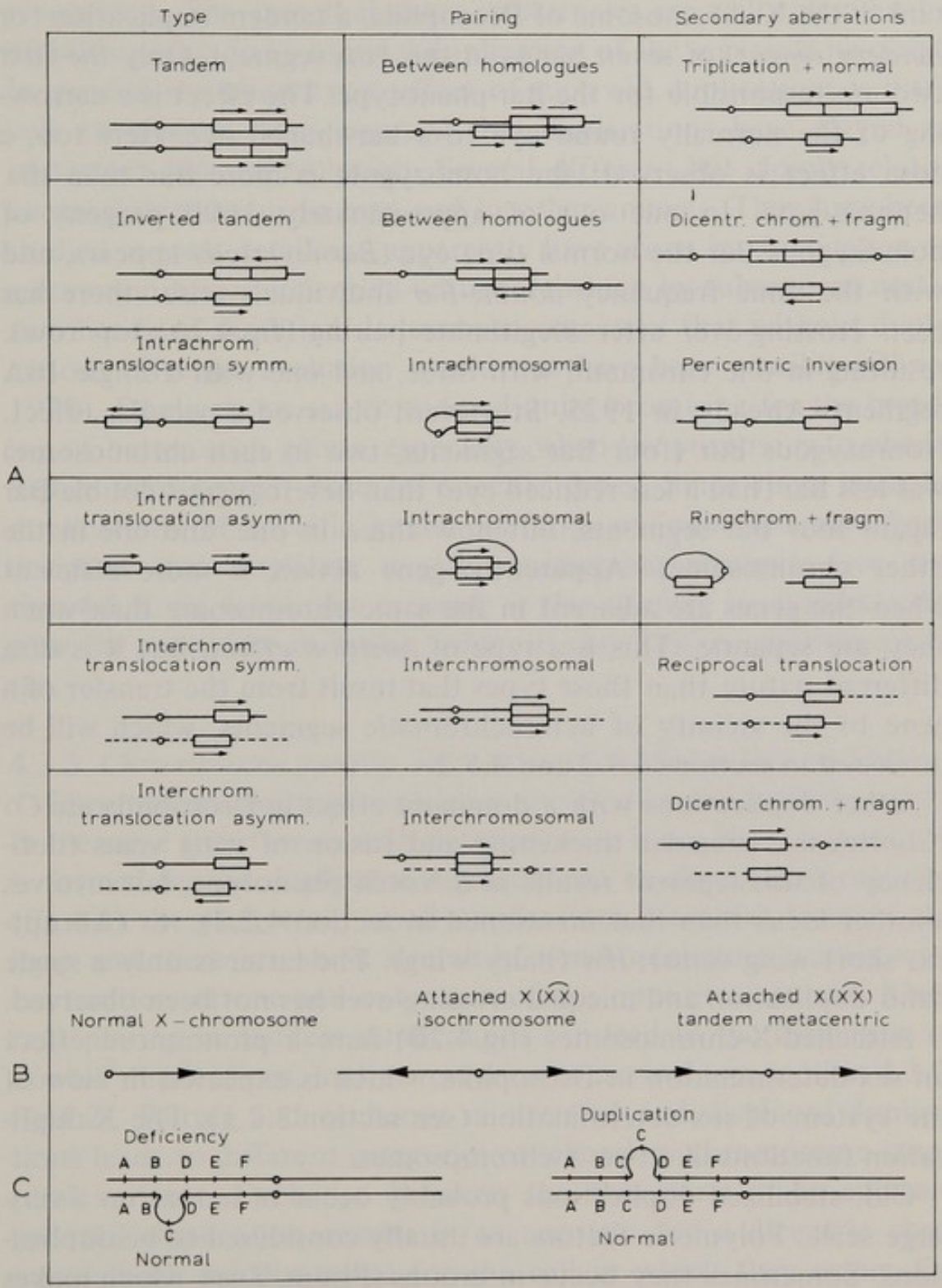


Fig.4.2. (A) Diagrams of some of the possible duplication types, with a number of possibilities of pairing, and the resulting secondary aberrations after exchange has taken place in deviating pairing situations. In the top row the origin of double-Bar and Bar-reverted in *Drosophila melanogaster*. (B) Diagrams of a normal X-chromosome of Drosophila and the two forms of attached X(XX). (C) Diagrams of pairing in a deficiency- and in a duplication heterozygote.

tant in the X-chromosome of Drosophila, a tandem duplication (or *tandem repeat*) of seven bands in the 16A segment. Only the first two are responsible for the Bar-phenotype. The effect is a narrowing of the normally round eye to a bar-shaped eye. Here too, a dose effect is observed: the homozygote is more Bar than the heterozygote. In one out of approximately 1600 progeny of homozygous Bar the normal type eye (*Bar-reverted*) appears, and with the same frequency *double-Bar* individuals arise: there has been crossing-over after illegitimate pairing (fig.4.2A, top row), resulting in one chromatid with three, and one with a single 16A segment. Already in 1925, Sturtevant observed a peculiar effect. Homozygous Bar (four Bar segments, two in each chromosome) was less Bar (had a less reduced eye) than heterozygous double-Bar (again four Bar segments, but now three in one, and one in the other chromosome). Apparently gene action is more efficient when the genes are adjacent in the same chromosome than when they are separate. This is a type of *position effect*, but it is of a different nature than those types that result from the transfer of a gene to the vicinity of heterochromatic segments, which will be discussed in sections 4.4.2 and 4.5.2.

Other duplications with a dominant effect in Drosophila are *Co* (Confluens), irregular thickening and fusion of wing veins (deficiency of this segment results in a Notch phenotype, but involves another locus than that mentioned in section 4.2.2); *Ax* (Abruptex, short wing veins); *Hw* (hairy wing). The latter is only a single band duplication and unequal crossing-over has not been observed.

Attached-X-chromosomes (fig.4.2B) have a pronounced effect on sex-determination in Drosophila, which is expected in view of the system of sex-determination (see section 3.2.1). The X-duplication functions like two X-chromosomes.

Old, stabilized duplications probably occur in nature on a very large scale. Polymeric factors are usually considered to be duplications. Sometimes they occur in groups (Pisum, Zea), which makes their duplication character even more probable. When several of such groups are encountered, an ancient allopolyploid origin is probable. In Drosophila, where accurate analysis of the polytene chromosome is possible, numerous duplicated segments have been

recognized. The fact that some deficiencies are well tolerated by diploids points to an original duplication of the segments involved. In general the phenotypic effect of such old duplications is difficult to analyse. It is assumed that duplications have played an important role in evolution. Several different but closely related haemoglobins occur in man and in other animals. They have been studied in great detail and apparently have arisen by duplication of a common basic nucleotide sequence, which by subsequent mutation has been modified. It has been suggested that without duplications effective evolution would not have been possible (Ohno, 1970). Duplications are considered quite promising for the breeding of better crop plants, especially when the purpose is to obtain higher than normal doses of specific genes. Since these must be duplications of specific genes, methods of induction have been designed that duplicate exactly predictable segments (see section 4.5.8, fig.4.16). An example is the attempt to duplicate the gene for α-amylase in barley, where it may improve malting qualities.

4.3.3. *Chromosome pairing. Recombination. Secondary variants*

Chromosome pairing involving heterozygous duplications results in the same structure as with deficiencies, a loop. Here, however, it is formed by the duplicated segment, not by the normal segment (fig.4.2). In pachytene, small duplications often fail to show the loop and there is a short stretch of non-homologous pairing with contraction of one and extension of the opposite segment. Aberrant pairing in tandem duplications can lead to the formation of double duplications, and to reversion to normal (see section 4.3.2, fig.4.2). Pairing and exchange in inverted and displaced duplications leads to different catagories of secondary chromosome structural variants. Exchange between the two parts of an adjacent inverted duplication results in an acentric and a dicentric chromatid, both usually lethal for the cell in which they occur. Exchange between a displaced duplication and the original segment results in a reciprocal translocation. Depending on the orientation of the duplicated segment, it will be either symmetric or asymmetric. In a comparable way, inversions, rings, etc. can arise. Gen-

erally, in heterozygotes recombination is reduced inside and around the duplication as a result of disturbed pairing. This may be compensated by an increase elsewhere.

Stabilized natural duplications generally have little tendency to pair with the original segment, either because they have a reduced capacity of long-distance attraction or because their specificity of attraction has been modified. Yet in haploids one frequently observes pairing and exchange apparently involving duplicated segments that have retained at least some capacity to associate.

The transmission of duplications from one generation to the other resembles that of deficiencies, but the complications are less severe. One disadvantage that deficiencies do not have is that large duplications can reduce the fertility as a result of meiotic complication, and in this way reduce their own probability of survival. Although duplications in the gametes are generally better tolerated than deficiencies, in plants competition between male gametes (certation) can give the bearer of a duplication a definite disadvantage.

4.4. Inversions

4.4.1. *Types*

Two types are distinguished: (1) *Paracentric* inversions: the inverted segment is included in a single arm of the chromosome. It is the only type possible in telocentric chromosomes. The shape of the chromosome remains unaltered. (2) *Pericentric* inversions: the inverted segment contains the centromere and when the centromere is not exactly in the middle of this segment, chromosome shape changes.

Two different inversions in the same chromosome can have the following mutual relations: (a) independent (there is no common segment in the two); (b) overlapping (they have a common segment); (c) included (one is confined in the other) (fig.4.3).

4.4.2. *Phenotypic effect. Position effect*

An uncomplicated inversion does not have primary phenotypic effects other than on chromosome shape. Frequently, however,

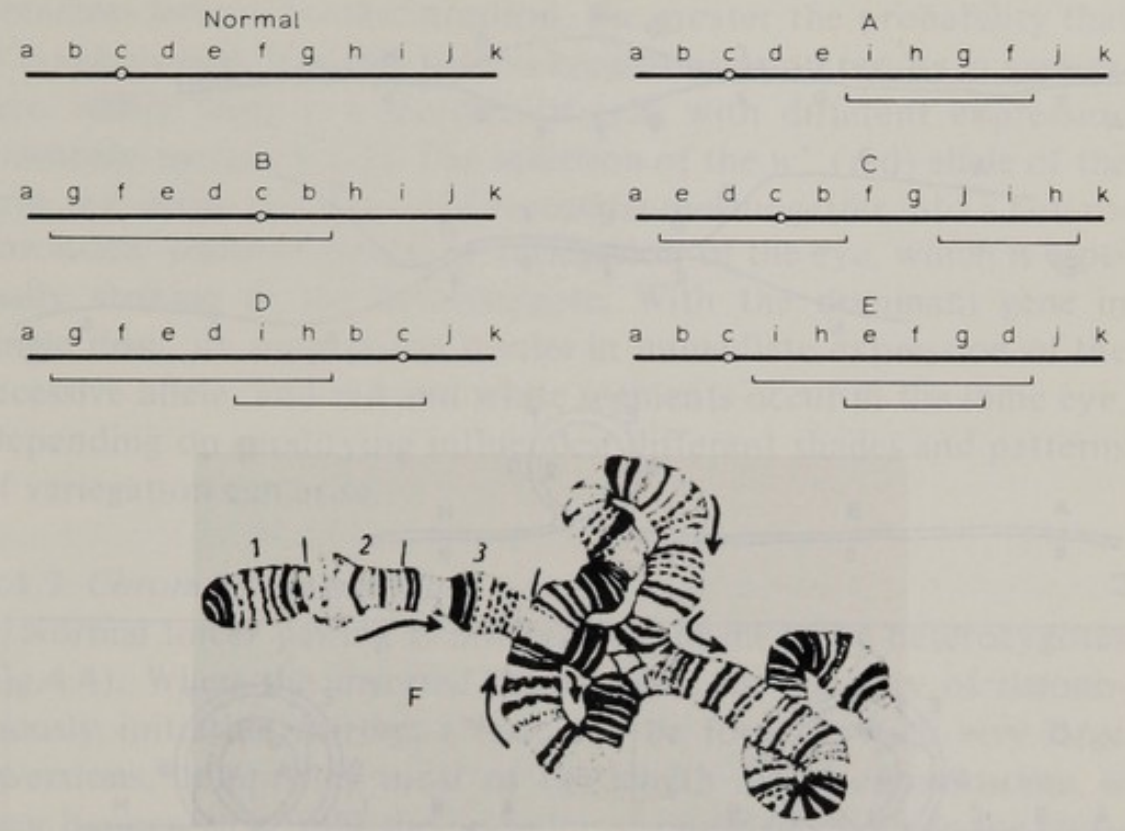


Fig.4.3. Diagrams of a paracentric inversion (A), a pericentric inversion (B), two independent inversions (C), two overlapping inversions (D), one inversion included in the other (E) and the polytene chromosome pairing in an inversion-heterozygote of *Drosophila melanogaster*, according to Painter (F).

some DNA at a break point has been damaged, and this may result in an observable mutation, often recessive. Sometimes these are quite striking mutations, among which *recessive lethals* may occur, that will then indefinitely accompany the inversion. This may be the origin of the recessive lethal in the *C1B* chromosome of Drosophila (see section 3.2.5). A secondary effect, rather often observed in Drosophila but not in most other organisms, is a *position effect*. In section 4.3.2, one type of position effect involving duplications has been mentioned. Here it is quite a different phenomenon. It is caused by the transfer of a gene from a euchromatic segment to the vicinity of a heterochromatic segment. Heterochromatinization may then extend into the displaced, originally euchromatic region and suppress the transcription of the gene in it. Heterochromatinization is variable and the nearer the gene to the centre that

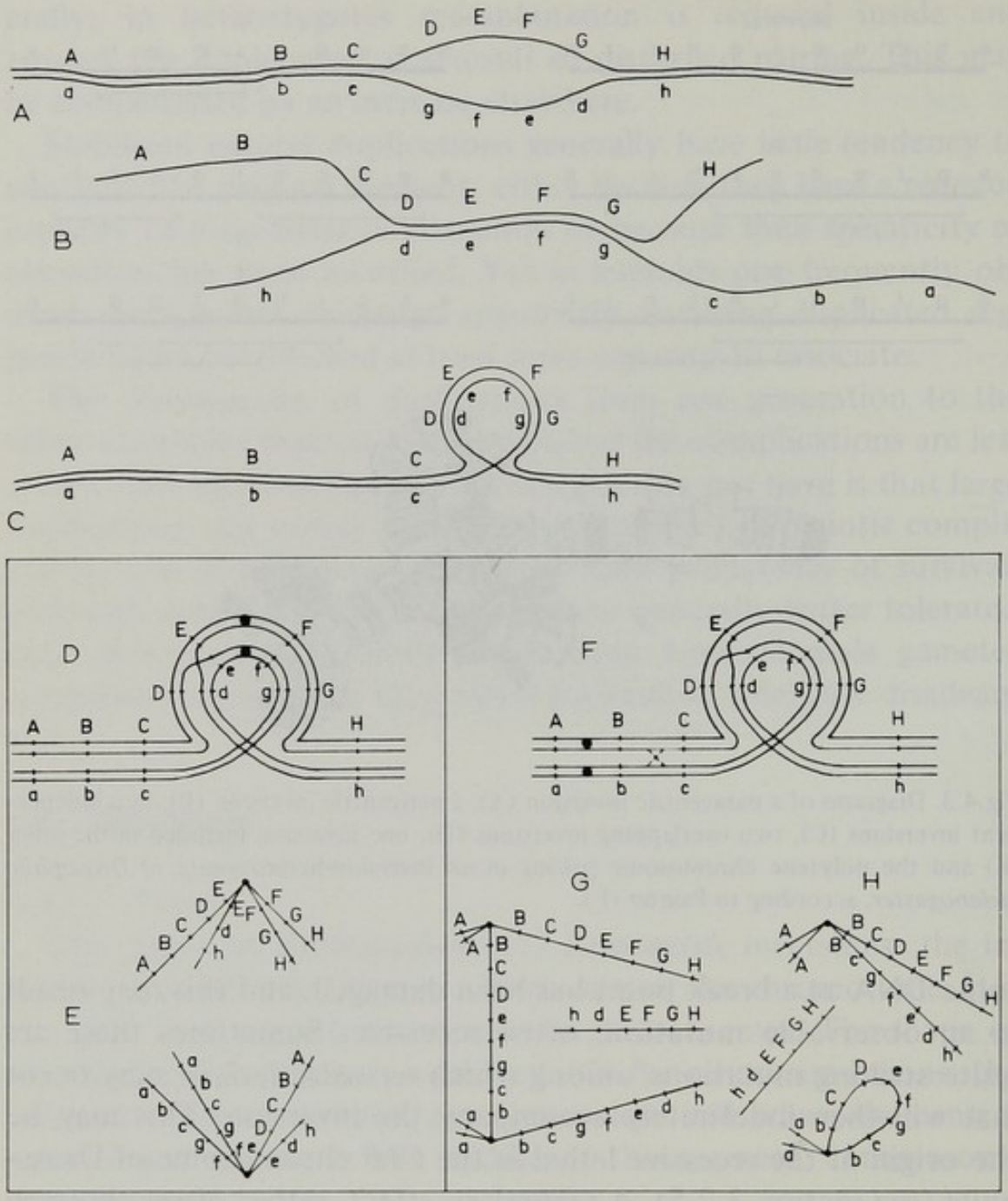


Fig.4.4. Pairing in an inversion heterozygote. (A) Only the non-inversion segments have paired. (B) Only the inversion segment has paired. (C) The entire bivalent has paired, but this requires the formation of a loop. (D) Pericentric inversion, pairing with loop, diagrammatic, with one chiasma in the loop. (E) The same inversion at anaphase I: on both sides there is a deficiency–duplication chromatid; the non-crossover chromatids are unaltered. (F) Paracentric inversion, pairing with loop, diagrammatic, with one chiasma in the loop. (G) The same inversion at anaphase I: a chromatid bridge and an acentric fragment result from crossing-over in the loop. (H) The same inversion again at anaphase I, but now after an additional chiasma had been formed between the centromere and the proximal break-point (the two chiasmata are disparate). Now there is a loop in one of the chromosomes, but no bridge. The bridge appears in anaphase II when the centromeres of the loop chromatid separate. There also is a fragment at anaphase I.

organizes heterochromatinization, the greater the probability that it is suppressed. Variable heterochromatinization results in variegation rather than in a mixture of cells with different expression (compare section 3.2.2). The insertion of the w^+ (red) allele of the gene for white (w) eyes of *Drosophila melanogaster* into a heterochromatic segment results in variegation of the eye, which is especially striking in the heterozygote. With the dominant gene in single dose, its suppression results in immediate expression of the recessive allele, and red and white segments occur in the same eye. Depending on modifying influences, different shades and patterns of variegation can arise.

4.4.3. *Chromosome pairing*

Normal linear pairing is not possible in inversion heterozygotes (fig.4.4). When the inverted segment has the capacity of autonomously initiating pairing, a loop wil be formed. With very large inversions, comprising most of the length of the chromosome, it may happen that only the inversion segment pairs, while the chromosome ends remain unpaired. With small inversions it often is the inverted segment that remains unpaired. In the very exactly pairing polytene chromosomes of the Diptera the course of pairing in the loop can be studied in detail. In the usually less exactly pairing pachytene chromosomes it frequently occurs that small inversions are not recognizable because the inversion segments pair in non-homologous association. When in larger inversions no point of pairing initiation (zygomere) occurs in the segment involved, then here too the inversion segment remains unpaired. When pairing is possible, it is often reduced around the break-points because of the change of pairing partners.

4.4.4. *Recombination. Consequences of exchange*

The difficulties encountered with pairing cause a reduction of exchange in and around the inversion. Because of *interchromosome effects*, an increase may then result in other parts of the genome. These effects have been quite carefully studied, but a satisfactory explanation has not yet been given (section 2.8.4). (For a review up to 1951, see Schultz and Redfield, 1952.)

Table 4.1
Recombination percentages in two segments of the X-chromosome of *Drosophila melanogaster*, without inversions in the autosomes, and with inversions in chromosomes 2 and 3. (According to Redfield, from Burnham, 1962.)

Segment	Recombination %	
	Without inversion	Inversions in 2 and 3
$y^2 - w^a$	0.79	4.76
w^a – spl	0.59	2.64

Usually the increase in crossing-over is confined to specific segments of chromosomes that are not involved in the inversion (table 4.1). Following Green, several investigators have used these effects to drastically increase crossing-over in chromosome segments in which recombination is normally too low for fine analysis (compare section 6.1.3). There are also within-chromosome interference effects. In some species of locusts, White and Morley could demonstrate an increase in chiasma-frequency in distal segments of bivalents where pericentric inversions had reduced chiasma frequencies in the proximal segments of the same chromosome (Lewis and John, 1965).

Exchange inside the inversion loop can have drastic consequences. Different aberrations are induced, depending on the type of inversion, the number of exchanges, the number of chromatids involved in the successive exchanges and the location of the exchanges outside the loop (the latter being important only in the case of paracentric inversions):

(1) Pericentric inversions (fig.4.4D,E). Exchange inside the loop leads to the formation of two *deficiency–duplication chromatids* (Df–Dp chromatids). A reciprocal chiasma can cancel the effect of the first. Two complementary chiasmata give four Df–Dp chromatids etc. Partial sterility therefore is always a consequence of pericentric inversions that have chiasmata in the inversion segment. In plants this is expressed as pollen and ovule abortion as a result of disturbed gametophyte development. In animals, Df–Dp gametes are often functional and lethality appears later in the

form of non-hatching of eggs of insects, of embryo lethality (small litters) with mice, of reduced fertility in humans etc.

(2) Paracentric inversions (fig.4.4F,G). A single exchange in the loop leads to a dicentric chromatid and an acentric fragment. The dicentric chromatid forms a bridge at first anaphase. The consequence again is sterility. Two reciprocal chiasmata cancel each other's effects and two complementary chiasmata give a double chromatid bridge with two fragments. When in addition to a chiasma in the loop, one is formed between the centromere and the inversion segment (interstitial chiasma, fig.4.4H), then the chromatid bridge is converted into a loop between the sister chromatids of one chromosome. In AI, there appears a fragment, but no bridge. The bridge shows up at second anaphase. This happens only in those combinations of chiasmata that have one chromatid in common: the disparate type. Therefore in only 50% of the combinations of one chiasma in the loop and one in the interstitial segment are AII bridges formed.

Chiasmata distal of the inversion (between inversion and chromosome end) have no effect on the course of meiosis but can cause the fragment to remain attached to the bivalent in metaphase and to one of the separating chromatids at anaphase. Then detection of the fragment may be difficult. The anaphase bridges usually break and this break can be the initiation point of a breakage-fusion-bridge cycle.

The occurrence of AI and AII bridges are indications of the presence of paracentric inversions, but no proof. Genetic unbalance as can occur in species hybrids or with inbreeding of cross-breeding species, often leads to chromosomal aberrations in premeiotic divisions, producing bridges at meiotic anaphase. In such a case, the accompanying fragments are *variable* in size, while with inversion bridges the fragment has a *constant* size (equal to the length of the inversion-segment plus twice the distal segment, see fig.4.4G). Similarly, the absence of anaphase bridges does not prove absence of paracentric inversions, since exchange can be greatly reduced or even absent in the inverted segment. Therefore, there is often uncertainty in respect to the presence or absence of inversions in populations. Such cryptic inversions, especially when they

are small, can readily maintain themselves and behave like single (unclassifiable) factors. The genes contained in the inversion are maintained as a block: there is no recombination between inverted segment and normal segment.

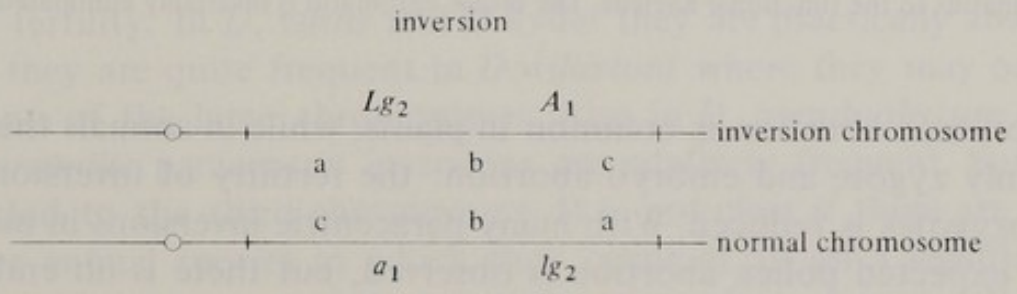
Exchange inside and around the loop is reduced as a consequence of incomplete pairing. The products of exchange are mostly inviable and are not recovered. It appears, therefore, as if the inversion segment is entirely free of crossing-over. For this reason, in the early days of genetics, inversions have been called *cross-over reducers*, when the effect on crossing-over was known, but the cytogenetic cause not. The *C* in *ClB* (section 3.2.5) stands for cross-over reducer and dates from the time that it was not known to be an inversion. Estimating true crossing-over percentages in the inversion segment is difficult, but an approximation is possible by determining the frequency of reciprocal double crossing-over between three factors in the loop: there is no bridge and the recombination products can be recognized. The same is possible with pericentric inversions that otherwise give (Df–Dp) chromatids. AI and AII bridges can be counted in paracentric inversion heterozygotes and from their number the crossing-over percentage in the loop can be derived (section 6.4.3).

There is an example by Rhoades and Dempsey (1953) of estimating crossing-over in an inversion beterozygote. It concerns a paracentric inversion in the long arm of chromosome 3 of maize. In the heterozygote, the normal chromosome had the recessive factors lg_2 (liguleless) and a_1 (colourless aleurone) in the inversion segment. The inverted segment in the other chromosome had the dominant alleles Lg_2 and A_1. The gametic ratios were determined by back-crossing the F_1 as female to the normal double recessive as father (table 4.2). Recombination between lg_2 and a_1 is normally 28%. From the 84 lg_2 A_1 recombinants in the testcross, 51 were analysed more closely, genetically as well as cytologically. Only 16 were found to have received a complete chromosome from the original heterozygote, and must have been the result of reciprocal double crossing-over. One of those two cross-overs must have occurred between the two genes (or else there would not have been recombination) and the other outside the two but inside

Table 4.2

Gametic ratios (from testcross data) of a paracentric inversion heterozygote with the two factors lg_2 and a in the inversion segment. The inversion chromosome carried the dominant alleles, the normal chromosome the recessive alleles. When the F_1 is used as the male parent, only double cross-overs are recovered (either in segments a and b, or in b and c). When the F_1 is the female parent, in addition deficiency–duplication gametes are recovered in which crossing-over only in b had occurred: the apparent crossing-over is higher in the female.

	Parental types		Recombination types		
	Lg_2A_1	lg_2a_1	Lg_2a_1	lg_2A_1	%
F_1 as male parent	1410	1215	6	8	0.5
F_1 as female parent	3015	2482	24	84	1.9



the inversion, and between the same chromatids (as otherwise there would not have been normal, non-deficient recombination types). The remaining recombinants had Df chromosomes arisen after breakage of AI or AII bridges, and loss of the fragment. There was rather a great variation between these chromosomes depending on the location of the break in the bridge. Apparently, when the distal inversion break-point is close to the chromosomal end, and the bridge breaks near the proximal break point, the deficiency can be small enough to be tolerated in the egg-cell (table 4.2, fig.4.4). The table also shows the effect of the failure of the Df chromosomes to be transmitted through the pollen: there is a great difference in recombination between the two reciprocal test-crosses.

4.4.5. *Transmission*

As expected when Df–Dp chromosomes are formed, pollen and

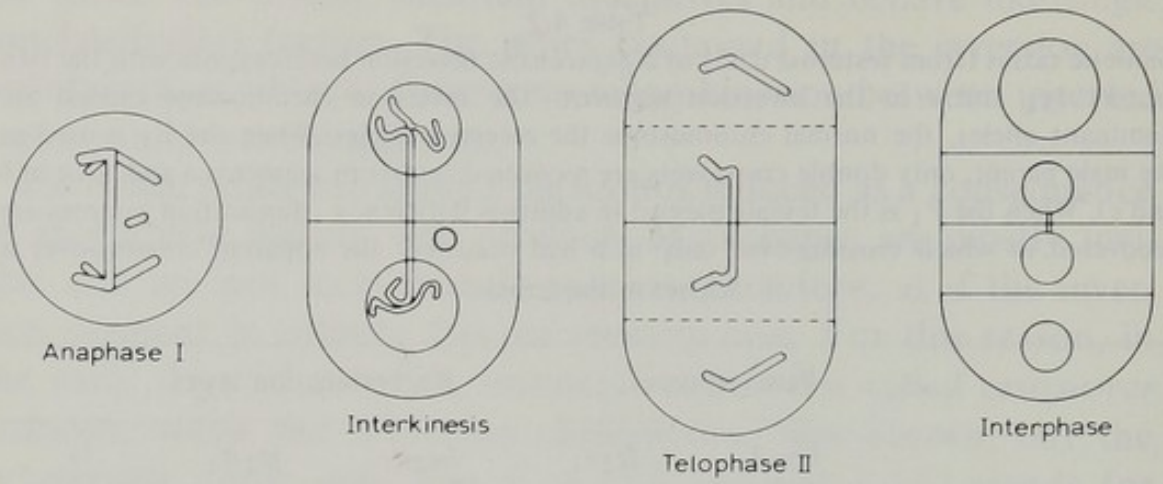


Fig.4.5. Diagram of the fate of the bridge chromatid (crossover chromatid) in the linear tetrad of maize or Drosophila where a special orientation of the anaphase II chromatids (induced by the bridge) leads to preferential segregation of one of the non-crossover chromatids to the functional nucleus. The bridge chromatid is invariably eliminated.

embryosac abortion is common in plants, while in animals there is mainly zygote and embryo abortion: the fertility of inversion heterozygotes is reduced. With many paracentric inversions in maize, the expected pollen abortion is observed, but there is no embryo abortion. In male Drosophila, there is no exchange, and consequently no sterility. In the females sterility again is not observed with paracentric inversions although crossing-over occurs. With pericentric inversions, however, the females are semi-sterile. This can be explained as follows (fig.4.5): the anaphase bridge is maintained throughout telophase and determines the orientation of the two chromatids of both chromosomes of the bivalent: the bridge chromatids, i.e. the exchange chromatids, are drawn inside, the non-exchange chromatids (one inversion and one normal chromatid) orient outwards. This orientation is maintained in the second meiotic division and determines the poles where the chromatids go. In plants, the non-exchange chromatids always end up in the two outer cells of the linear tetrad. One of these is the functional cell from which the embryosac develops: the unbalanced bridge chromatids will never be included in this cell. Similarly, in Drosophila only the non-exchange chromatids are included in the egg. In the development of pollen, there is not such a system, and partial pollen sterility is the consequence of an inversion in which

exchange can take place. However, only normal pollen functions. Therefore, although pericentric inversions are gradually eliminated in plants and animals, or in exceptional cases replace the original chromosome type, paracentric inversions usually are better tolerated in heterozygous state and in Drosophila can be readily maintained in combination with the original chromosome.

4.4.6. *Inversions in natural populations*

Paracentric inversions, mainly in heterozygous condition, are found in a high proportion of the natural populations of several species of Drosophila. These are not random inversions: in each population only a limited number of specific types occurs with appreciable frequency. As seen above (section 4.4.5) they do not affect fertility. In *D. virilis* and *D.hydei* they are practically absent, but they are quite frequent in *D.willistoni* where they may occur in any of the large chromosomes. Also in *D. pseudoobscura* and *D.persimilis* paracentric inversions are relatively frequent, but restricted to the third chromosome. It is not clear if there are any other animal species in which they occur at all on a significant scale. Of course, spontaneous random inversions occur in all animals, including man, but no other instances are known in which systematic inversion heterozygosity has been established on a scale comparable to that in some Drosphila species. At least in the thorouhgly analysed locusts and grasshoppers large inversions have never been found to occur on a significant scale. In plants, they seem to occur, but infrequently (Snow, 1969, in Paeonia).

The reason why paracentric inversions are kept floating in Drosophila populations is that they give both the carrier and the population an adaptive advantage. Absence of exchange in the male, combined with exclusive functioning of non-recombinant chromatids in the female not only prevents sterility but also leads to the maintenance of *gene blocks* free of recombination. When these contain favourable combinations of genes that exhibit heterosis in combination with a non-inverted segment, these combinations, even if they are quite complex, do not run the risk of being broken down. In the case the complex itself would be favourable, the homozygote would gradually take over the range of the spe-

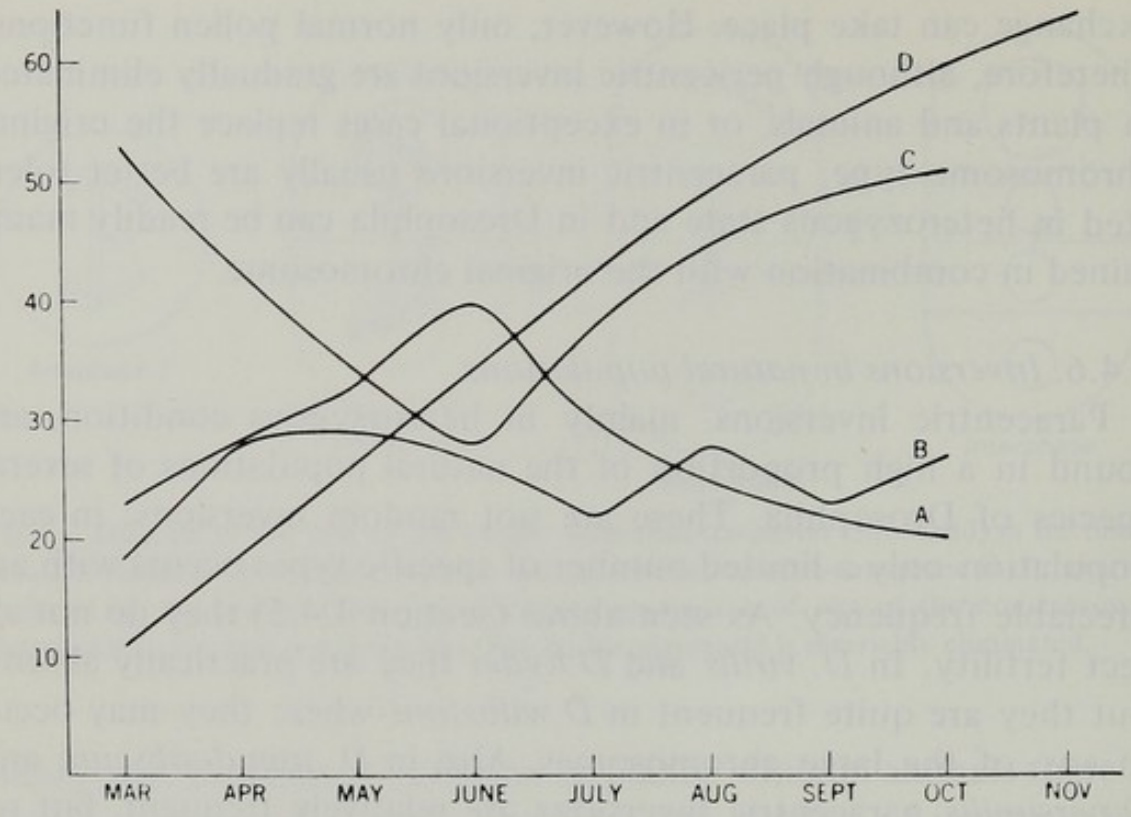


Fig.4.6. Seasonal variation in the frequencies of four inversions in natural populations of *Drosophila pseudoobscura*. (From data of Dobzhansky and others, Swanson, 1957.)

cies, which would result in a permanent karyotype alteration. With heterosis, however, the heterozygote frequency will be maximalized. There is not much chance, however, that an equilibrium will ever be attained: too many complicating factors are involved. The seasonal variation of the environment is probably the most important factor in this respect. During important periods the heterotic heterozygote will have the optimal genetic constitution, and because of genetic homoeostasis, this may rather frequently be the case. In other periods, however, either the normal or the inversion homozygote may be optimal. These are three genotypes that, because of lack exchange in the inversion segment, remain constant and are each produced continuously. For producing different genotypes with different adaptabilities, therefore, inversion polymorphism also is an advantage. The generation time of a few weeks is too long to permit adjustments to rapid changes, but seasonal variations give enough time for systematic shifts in inversion frequencies. Different inversions follow a different pattern and

will vary differently with the seasons (fig.4.6). *Drosophila pseudoobscura* and *D.persimilis* inhabit an extensive area of Western America, from Canada into Mexico and none of its inversions occurs in the entire range. There is one standard karyotype that is morphologically the same for both species and from this type a large number of karyotypes have developed by the successive formation of (often overlapping) inversions. Some thirty different inversions have been noted, all floating without being fixed in homozygous state. Because of overlap of several of them, it is possible to construct a pedigree of their origin. Much of the work on inversion polymorphism in Drosophila populations has been carried out by the school of Dobzhansky (see also Swanson, 1957; Burnham, 1962).

4.5. Translocations

4.5.1. *Types*

The two most common types (compare section 1.4.11) are: (1) *Reciprocal translocation* (*interchange*): two chromosomes have symmetrically interchanged a segment. One break in each chromosome is sufficient when the segment is terminal, which is the common situation (figs.1.13G; 1.14F; 4.7). (2) *Interstitial translocation* and *shift*, both requiring two breaks in one chromosome or arm and one in the other, followed by displacement of the segment between the two breaks in the first into the opening of the break

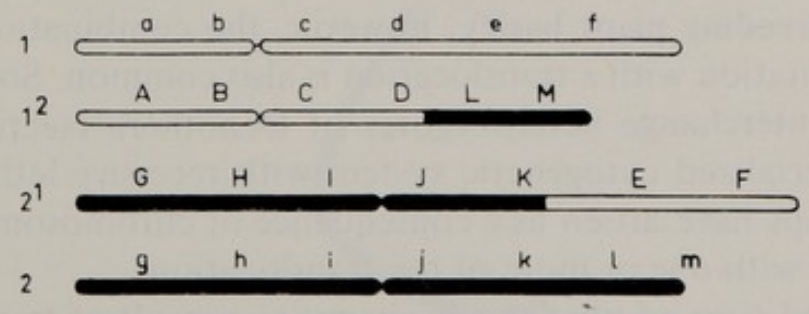


Fig.4.7. The four chromosomes of a heterozygous interchange: two normal chromosomes (1 and 2), two translocation chromosomes (1^2 and 2^1). The interchange chromosomes have the dominant alleles. The pairing situation is shown in fig.4.9; segregations in fig.4.14.

in the second (fig.1.18). The requirement that three lesions must occur within the rejoining distance, combined with a specific interaction between these lesions, is not often realized. This type of translocation, therefore, is rather scarce compared to the reciprocal type. In addition, it is somewhat less readily recognized at meiosis.

It has often been suggested that the attachment of a piece of chromosome onto an existing end is impossible, but there is insufficient reason to exclude the possibility that a lesion at a chromosome end should be capable of interacting with an interstitial lesion.

A reciprocal translocation results in an alteration of chromosome shape when the exchange segments differ in size. Interstitial translocations and shifts always result in alteration of shape, but recognition may occasionally be difficult.

4.5.2. *Phenotypic effect*

No phenotypic effects are expected, unless one of the lesions at the origin of the translocation has damaged the DNA. Translocations have indeed often been found to be accompanied by more or less clearly classifiable mutations. In Drosophila, half of the induced or spontaneous translocations are not entirely vital as a homozygote or are recessive lethals. In maize, almost all translocations are accompanied by a deficient or even recessive lethal genotype. In these two cases, both outbreeders, there is the possibility that existing recessive factors, linked but not related in cause to the translocation, produce these effects when made homozygous. In the inbreeding plant barley, however, the combination of a recessive mutation with a translocation is also common. Some of the complex interchange heterozygotes of Oenothera (section 4.5.9) have a specialized cytogenetic system with recessive lethal factors that perhaps have arisen as a consequence of chromosome damage associated with one or more of the translocations.

A second type of phenotypic change as a result of translocation is a *position effect*, observed especially in Drosophila. It is equivalent to the position effect mentioned with inversions (section 4.4.2).

4.5.3. *Chromosome pairing. Exchange. Configurations*

When all homologous segments of a heterozygous reciprocal translocation pair, a *cross-shaped* configuration of four chromosomes results (figs.4.8, 4.9). Such an association of four chromosomes is usually called a *quadrivalent*. There is a tendency to reserve the name quadrivalent for those cases where all four chromosomes are homologous. In the case of an interchange heterozygote, where the chromosomes are not equivalent, the term *quadruple* (general: *multiple*) has been introduced. This usage of the term has not yet become wide-spread.

Sometimes the paired chromosomes can be recognized (polytene chromosomes of Diptera, pachytene in maize, tomato etc., diplotene in several locusts). When with exact pairing a chromomere pattern can be analysed, the location of the interchange points can be determined: they are at the centre of the cross (figs.4.8, 4.9). At pachytene the chromomere pattern cannot be analysed as accurately as in polytene chromosomes and pairing around the interchange point is often somewhat loose. As a consequence the determination of the break points is more difficult. Partial non-homologous pairing can shift the position of the centre

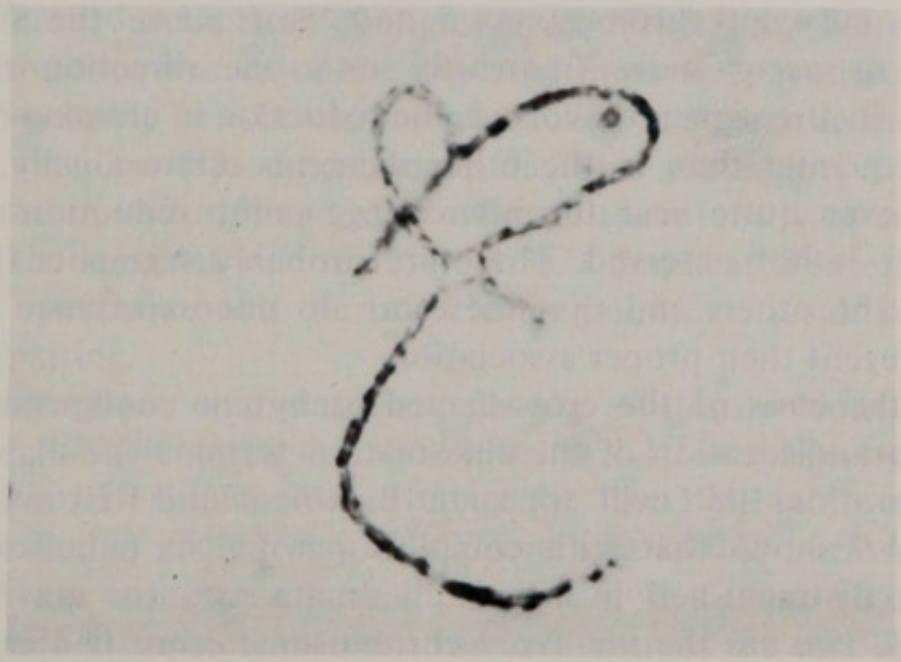


Fig.4.8. The pairing cross of a heterozygous interchange between chromosomes 8 and 10 at pachytene in maize. (Courtesy Dr. M.M. Rhoades.)

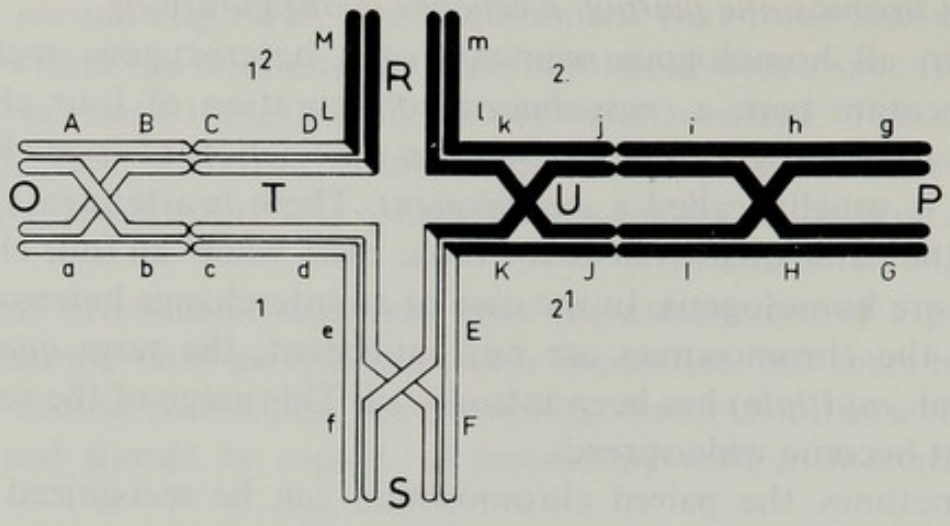


Fig.4.9. The cross-shaped pairing configuration of a heterozygous interchange. There are six segments: O, P the chromosome arms not involved in the interchange; R, S the interchanged segments; T, U the interstitial segments. Chiasmata have been drawn in an arbitrary four of the six segments; with this combination of chiasmata at metaphase I the configuration of fig.4.11B and fig.4.14D is expected (compare fig.4.7).

of the cross: then exact determination becomes quite impossible. Crossing-over around the exchange points is often greatly reduced. According to Burnham (1962) this is strongest in those cases where the pachytene cross-centre has shifted most. In an interchange between chromosomes 5 and 9 of maize the shift was found to occur more frequently into one direction than the others. In the segment involved, the reduction in crossing-over was clearly greater than in the other segments. Occasionally, in segments even quite near the interchange point, reduction in crossing-over is not observed. These are probably segments that pair before the others and that therefore do not experience torsions that prevent their proper association.

On the basis of the cross-shaped pachytene configuration, the number and location of the chiasmata determine the shape of the configurations that will appear at diakinesis and first metaphase.

Fig.4.9 shows that with complete pairing, six paired segments can be distinguished in which chiasmata may (or may not) be formed: two are the unaltered chromosome arms (O and P); two are the interchange segments (R and S): in both an interchanged segment pairs with the homologous part of a normal chromosome;

two are segments between the centromere and the interchange point (T and U). The segments O, P, R and S are called the *pairing* segments, T and U the *interstitial* segments. Without chiasmata, the homologous segments are not bound in metaphase, with chiasmata they are: these are the two possibilities for each segment. For all combinations of six segments, there are $2^6 = 64$ different possibilities, each corresponding with a particular configuration at diakinesis and metaphase I. Only 16 morphologically different types can be distinguished, since many of the 64 are homomorphic. Six of these 16 are bivalents or univalents or combinations thereof. The remaining ten are associations of more than two chromosomes and can as such be distinguished from the bivalents or univalents that are formed by the rest of the genome.

The probability that one or more chiasmata are formed in a certain segment depends on three factors: the length of the segment, the exchange level of the particular organism and characteristic properties of the segment that are relevant to chiasma formation. The latter factors form a complex that is not easily analysed: there are factors that regulate pairing, others that regulate exchange in the paired segments, etc. The probability of the separate segments to be bound at metaphase determines the frequencies of the different metaphase configurations. Therefore, determining the relative frequencies of the different metaphase configurations makes it possible to estimate the probability that each segment has at least one, perhaps more chiasmata. This probability of being bound can in its turn be used to estimate the intensity of crossing-over in the segment (see section 6.4.3). Complicated interference systems operate around the interchange point as a result of variations in pairing.

Six metaphase configurations, chosen arbitrarily from the 16 different possible ones, are shown in fig.4.10 (see also fig.4.11). Their origin is shown also. It may be seen that with at least one chiasma in each terminal segment (fig.4.10A) a closed ring arises. When only three have a chiasma, it is a chain (B). When two segments opposite in the cross are bound, the rest not (D), there are two rod bivalents. With chiasmata exclusively in two adjacent terminal segments (C) a trivalent with univalent is formed.

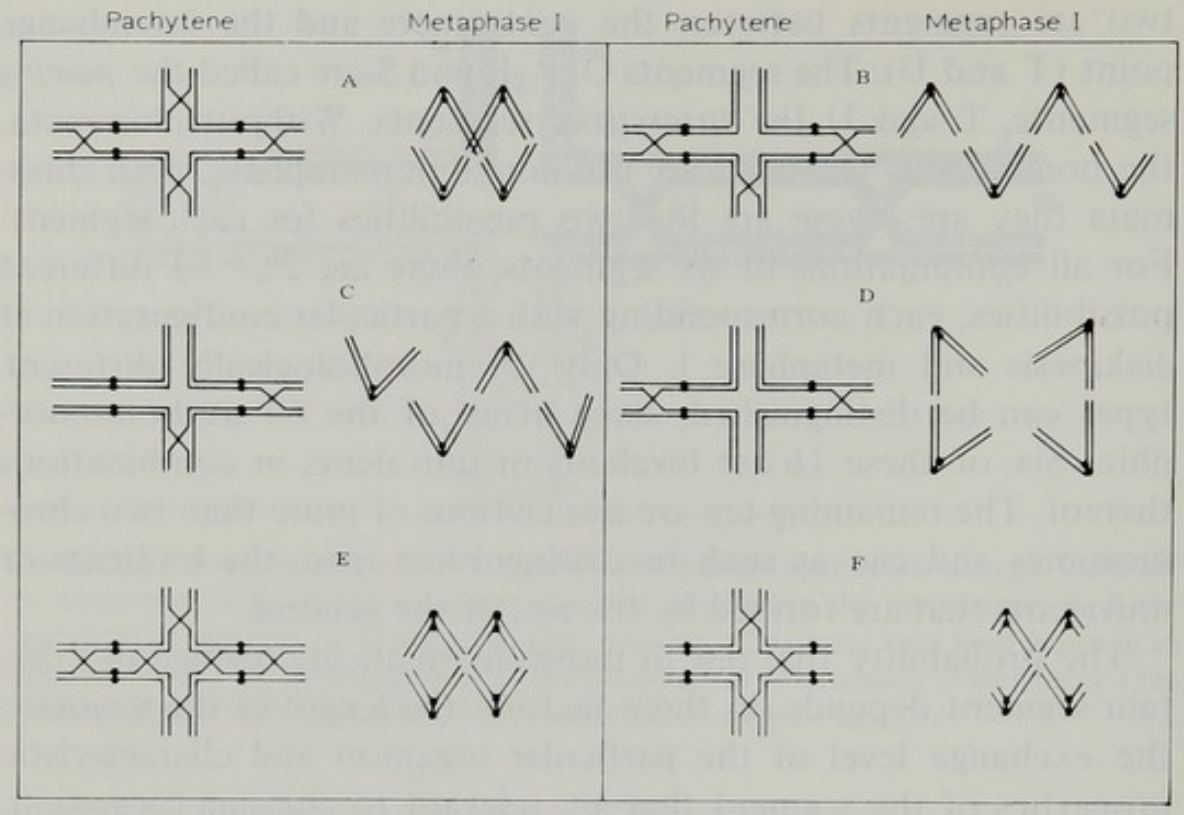


Fig.4.10. Pairing and metaphase I configurations with six different combinations of chiasmata. Usually A and B are the most common type. C is frequent in the case of fig.4.12. D is expected when both interchange segments are small, and E when the interstitial segments are large. In the case of D, the interchange may be difficult to detect. In F the non-interchange arms are small, and both interstitial and interchange segments large enough to have a chiasma. The combination of three chiasmata shown here leads to a chain quadrivalent as shown in fig.4.11C (diakinesis).

Chiasmata in all segments result in a figure-of-eight (D). This requires chiasmata in the interstitial segments, which for a number of reasons tend to be scarce: active pairing initiation is required for these segments and this is not common in proximal (=near the centromere) regions. In addition, chiasmata often are localized near the chromosome ends rather than proximally. Therefore, such figures-of-eight and other configurations with interstitial chiasmata (figs.4.9, 4.11) are relatively infrequent with most translocations. Some have quite short interchange segments: bivalents predominate, and the translocation is hard to detect. With many translocations ring quadrivalents are the most common type of configuration, followed in frequency by chain quadrivalents.

Translocations between the long arms of acrocentric chromo-

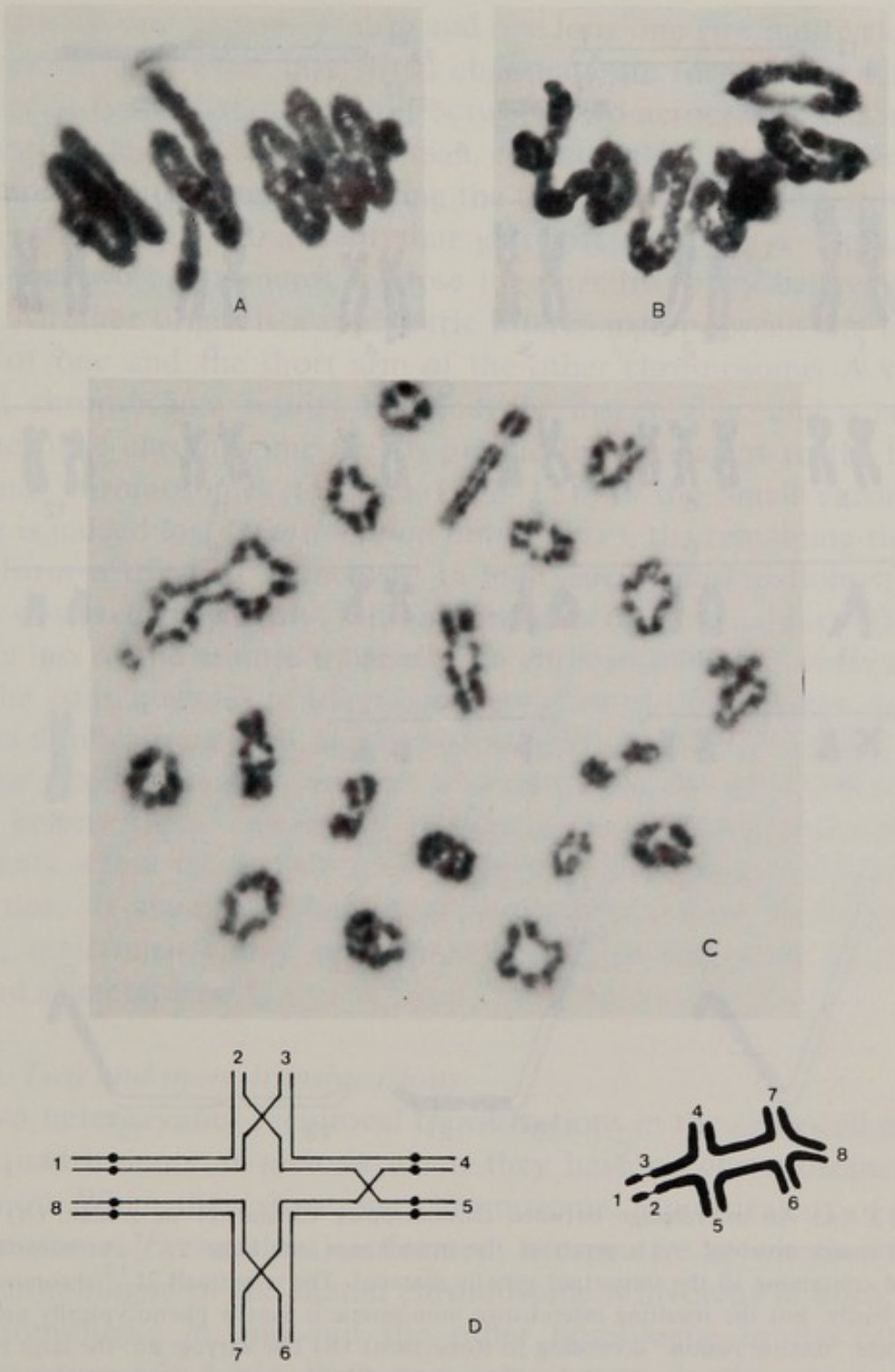


Fig.4.11. (A, B) Metaphase I in an interchange heterozygote of rye. In A there are five ring bivalents and a chain of four, compare fig.4.10B. In B there are also five ring bivalents, now with a "frying-pan" type quadrivalent, compare figs.4.9 and 4.14D. (C) Interchange heterozygote at diakinesis in the house mouse. Since the mouse has acrocentric chromosomes the interchange ring can be formed only when chiasmata occur both in the interchanged and interstitial segments, compare fig.4.10F. The origin of this chain from the pairing situation is shown in D, compare by fig.4.10F. (C: Courtesy Dr. C.E. Ford.)

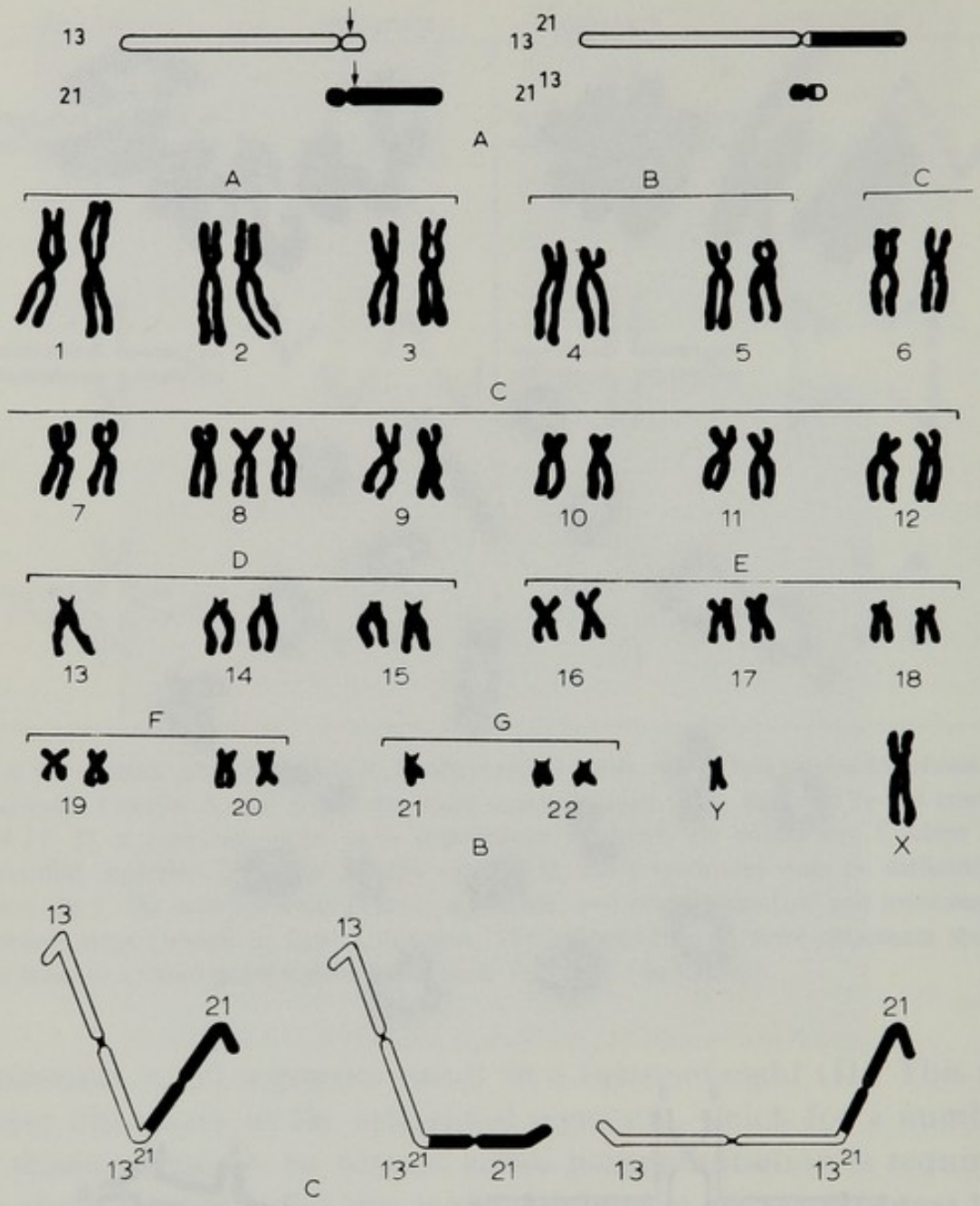


Fig.4.12. (A) An interchange between chromosomes 13 and 21 in a man. (A) The chromosomes involved. As a result of the interchange one large 13^{21} chromosome is formed containing all the important genetic material. The very small 21^{13} chromosome is lost easily, but the resulting interchange monosomic is usually phenotypically normal (Compare "centric fusion" according to Robertson). (B) The karyogram: the large interchange chromosome is classified as a chromosome 8, the small chromosome is lost and there are only one chromosome 13 and one chromosome 21. (C) The orientation of the interchange trivalent at meiosis of B. The first type is the most common and leads to two balanced anaphase groups. The second type leads to a duplication (trisomy 21) group and a deficiency (lethal) group. The third also leads to a duplication (trisomy 13) which is not viable, and to a lethal deficiency. Of the resulting six different gametic types two are balanced, one is the viable duplication type and three are non-viable. The relative frequencies, however, depend on the orientations, and therefore the balanced types are favoured (B drawn after a karyogram by Dr. T. Hustinx.)

somes with one very short arm and one long one give multivalents in meiosis only when interstitial chiasmata are formed (fig.4.11). A special type of translocation between two acrocentric chromosomes can lead to a Robertsonian centric fusion. It could be an asymmetric interchange involving the two short arms and resulting in a small acentric fragment that gets lost and a large dicentric with the two centromeres so close together that they behave like one. Another origin is a symmetric interchange between the long arm of one and the short arm of the other chromosome. A very small chromosome results that may be dispensable, and a large metacentric chromosome that is practically equivalent to the two original chromosomes together (fig.4.12). If the small chromosome is indeed lost (*translocation monosomy*), the remaining three will form a trivalent in meiosis. In man, such translocations have been observed repeatedly, with a hardly recognizable direct effect of the loss of the minute translocation chromosome, but notorious for the consequences of irregular orientation of the trivalent, leading to duplications with highly undesirable effects (section 4.5.7). Similar translocations have been observed in cattle (section 4.5.9).

A heterozygous *interstitial* translocation gives heteromorphic bivalents when the translocated segment has no points of pairing initiation. If there is pairing and chiasma formation in this segment, multivalents may be formed, but these will never be ring-shaped at metaphase I.

4.5.4. *Two and more translocations*

Two heterozygous reciprocal translocations in the same cell give two quadrivalents at meiosis when they have no chromosome in common. When they share one chromosome, a multivalent of six chromosomes (hexavalent) is formed, irrespective of whether the same homologue of the shared chromosome is involved in both, or one homologue in one and the other homologue in the other translocation (fig.4.13). With two interchanges in the same two chromosomes, a quadrivalent can result, but the shape may be somewhat complicated. When the same arms have exchanged in both translocations often just two bivalents are formed. With two translocations in three chromosomes ten different segments can be

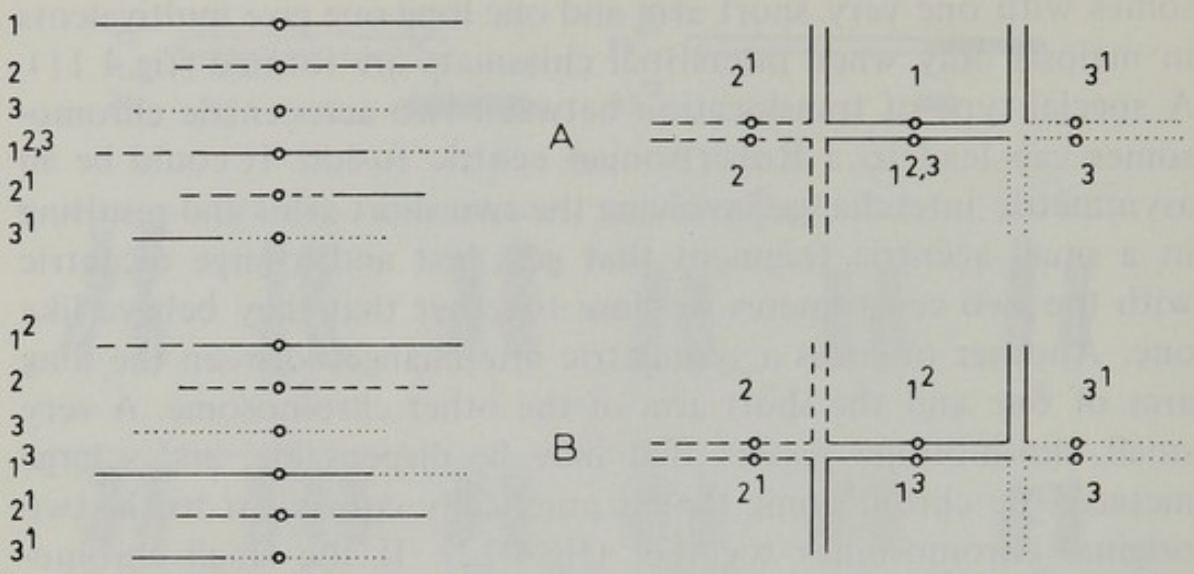


Fig.4.13. Two interchanges involving three chromosomes. (A) Of the chromosome involved in both interchanges one homologue is unaltered, the other carries both interchanges. At right the pairing configuration. After alternate orientation and anaphase separation, one pole receives both interchanges (chromosomes 2^1, $1^{2.3}$, 3^1) and the other pole only normal chromosomes (1, 2, 3). (B) Of the chromosome involved in both interchanges one homologue has one, and the other the second interchange. This chromosome has no normal homologue. At right the pairing configuration. After alternate orientation and anaphase separation, one pole receives one interchange and the normal homologue of the chromosome not involved in the interchange (2^1, 1^2, 3). The other pole receives the other interchange with the normal homologue of the chromosome not involved in this interchange (3^1, 1^3, 2).

distinguished: six pairing segments, two interstitial segments and two *differential* segments. The latter are those between the central centromere and the two interchange points. The number of possible metaphase I configurations is 1024, many of which are isomorphic (they have the same shape). The most frequent are usually chains. Chiasmata in the differential segments usually lead to unbalanced gametes, but are often relatively rare.

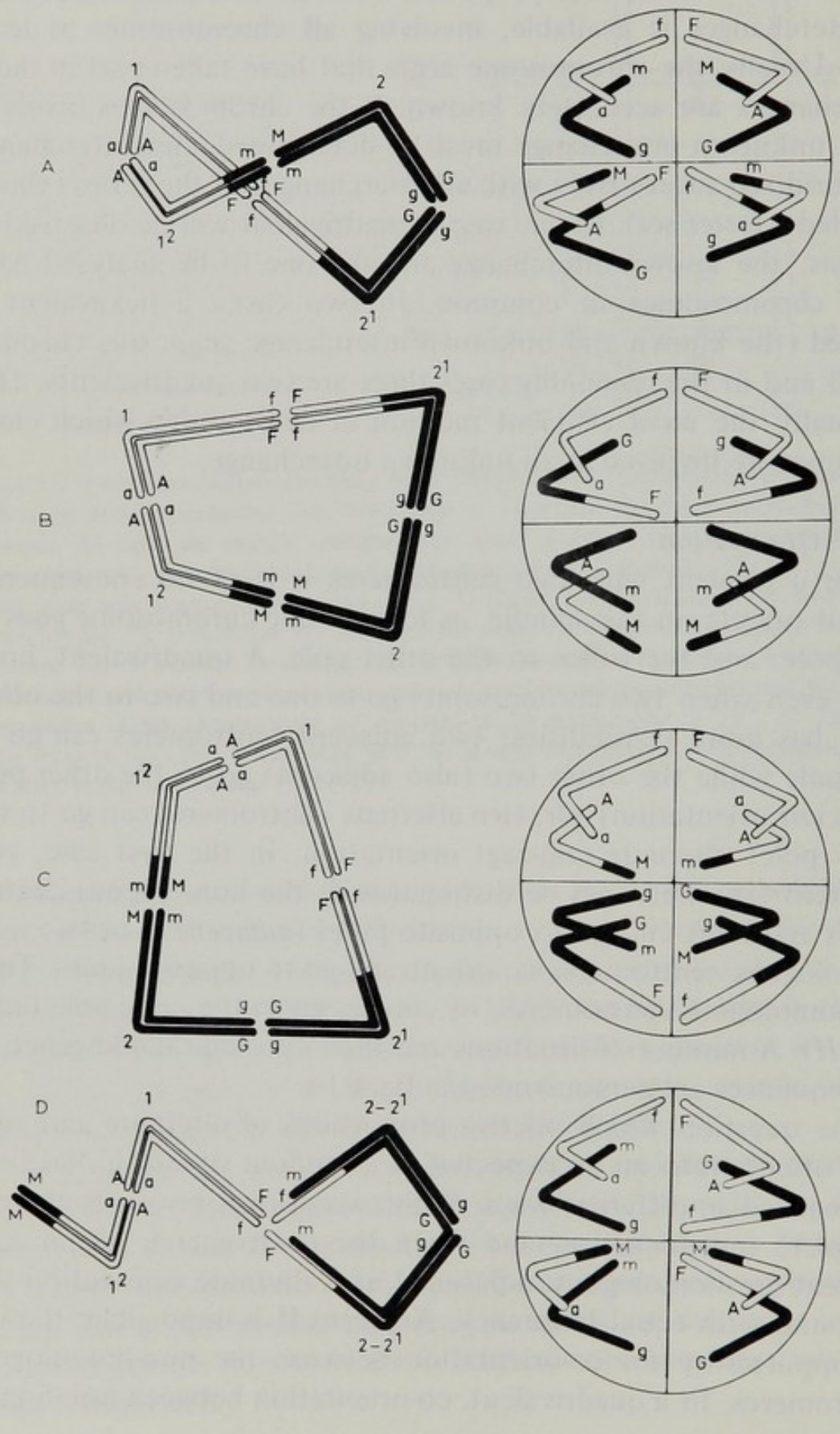
Sometimes one wishes to know whether or not two translocations share a chromosome: then it is sufficient to make the double heterozygote and to see whether two quadrivalents, a hexavalent or a single quadrivalent are formed. Since large configurations may break down into smaller ones due to failure of chiasmata in some of the segments in some cells, it is always necessary to study more then a single cell.

In maize, Datura, barley, rye and a few more organisms, a series of interchanges is available, involving all chromosomes at least once. Usually the chromosome arms that have taken part in these interchanges are accurately known. If the chromosomes involved in an unknown interchange must be determined, this interchange is hybridized successively with all interchanges of the series (which is called a *tester set*). In one case a quadrivalent will be observed at meiosis: the known interchange and the one to be analysed have both chromosomes in common. In two cases, a hexavalent is formed (the known and unknown interchange share one chromosome) and in the remaining cases there are two quadrivalents. This is usually the most efficient method of determining which chromosomes are involved in an unknown interchange.

4.5.5. *Orientation*

For a bivalent with two centromeres it is of no consequence how it orients on the spindle, as long as one chromosome goes to one pole, and the other to the other pole. A quadrivalent, however, even when two chromosomes go to one and two to the other pole, has more possibilities: two adjacent centromeres can go to one pole while the other two (also adjacent) go to the other pole (*adjacent* orientation). Or, two alternate centromeres can go to the same pole: *alternate* (zig-zag) orientation. In the first case, two different situations can be distinguished: the homologous centromeres co-orient and go to opposite poles (*adjacent I*) or two non-homologous centromeres co-orient and go to opposite poles. Then the homologous centromeres, of course, go to the same pole (*adjacent II*). A number of situations and their cytological and genetical consequences are demonstrated in fig.4.14.

The question which relative proportions of alternate and adjacent orientation can be expected in a random situation, has been approached in different ways. With two normal bivalents there is complete independence, and when the centromeres within each bivalent are homologous, adjacent I and alternate orientation will be found with equal frequency. Adjacent II is impossible: there is no opportunity for co-orientation between the non-homologous centromeres. In a quadrivalent, co-orientation between non-homo-


A
B
C
D

logous centromeres is, in principle, possible: these centromeres are connected. Adjacent II is a real possibility and there are indications that there is no preference for co-orientation between homologous centromeres, so that adjacent I and II occur with equal frequency. It has been argued (assuming a final two-by-two segregation of the quadrivalent) that the combination of the four chromosomes into two sets of two should be random. I.e. (compare figs.4.14, 4.15) chromosome 1 will go to one pole with chromosome 2 with the same frequency as it will with chromosome 1^2 or with 2^1. This supposes equal frequencies of alternate, adjacent I and adjacent II, and only 1/3 is alternate. The starting-point, however, is not valid: it neglects that chance co-orientation does not necessarily result in chance distribution. Chance co-orientation should be considered first and the expected distribution should be derived from it (fig.4.15). Excluding configurations of deviating types that would lead to other than two-by-two segregations, one can start with considering the possibilities of co-orientation of centromere 1. If it co-orients with 1^2, the centromeres 2 and 2^1 have no choice and co-orient with each other. They have two ways to do so (A and B), assumed to have an equal probability. If, however, the other possibility for co-orientation of 1 is realized, i.e. with 2, then 1^2 co-orients with 2^1, and again there are two possibilities, C and D. Of the total of four possibilities, two are adjacent, and two are alternate. Of the adjacent types, one is adjacent I, the other adjacent II. Indeed, in a number of cases, a 1:1 ratio between alternate and adjacent has been observed, but even within species there is considerable (genetic) variation. In most cases, either alternate or adjacent predominates: apparently

Fig.4.14. Different orientations of interchange quadrivalents and their consequences. (A) With alternate orientation of a ring quadrivalent four balanced tetrad cells are formed. (B) With adjacent I orientation, all tetrad cells are unbalanced (have a duplication and a deficiency). The same is true for adjacent II (C), although the chromosomal combinations are different. (D) A quadrivalent with an interstitial chiasma (compare figs.4.9 and 4.11B) results in two balanced tetrad cells (one with the normal and one with the interchange complement) and two unbalanced (duplication–deficiency) cells. Here no distinction between alternate and adjacent orientation can be made. Note the effects on the genetic segregations (gene-symbols of fig.4.9).

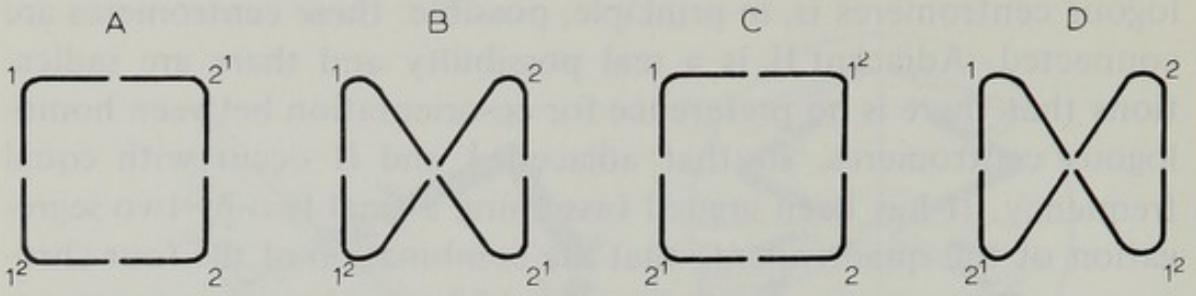


Fig.4.15. A reasonably rational way of deriving the relative frequences of different orientation types with "random" orientation of an interchange quadrivalent. There are four centromeres: 1, 1^2, 2, 2^1. 1 and 1^2 are homologous, as are 2 and 2^1. Coorientation involves two adjacent centromeres: considering 1, it may orient with 1^2 (A, B). Then all 2 and 2^1 can do is to co-orient with each other. They may do so in two ways (A and B). In B an alternate configuration results, in A an adjacent configuration (adjacent I). However, 1 with the same probability may co-orient with 2^1, and then 1^2 co-orients with 2. Again there are two possibilities, one alternate, one adjacent (adjacent II). The same reasoning with the same results may be set up for the other centromeres. This leads to the conclusion that when the two pairs of co-orienting centromeres are independent, as many alternate as adjacent orientations will result. The two adjacent types are equally frequent.

true chance orientation is not a reality. The flaccid early prophase configuration perhaps starts with randomly oriented centromeres but as soon as forces start acting on the quadrivalent, this orientation is disturbed. In principle there are two types of force acting on the chromosomes: contraction resulting in stiffness and torsion, and centromere activity. Short, stiff chromosomes, and those with little tendency for terminal chiasma localization do not have sufficient flexibility for alternate orientation: adjacent predominates and adjacent I and II occur with equal frequency. This was observed for instance by Khoshoo and co-workers in Canna and Bougainvillea interchanges. The short chromosomes of Nicotania, however, appear to have enough flexibility, especially in the centromere, for relatively frequent alternate orientation. Long chromosomes that are flexible at the centromere, can easily orient alternately.

The role of the centromere in the orientation of multivalents is not without complications. It appears that the orientation of a centromere on a pole is not necessarily stable: if there is enough time and the chromosomes are sufficiently flexible, a centromere can re-orient from one pole to the other, and it can do so several

times even during metaphase, before being fixed at the end of metaphase. Since centromere orientation is relatively well maintained as long as a counter-force is exerted on the centromere, the alternate orientation is more stable than the adjacent orientation: there are two centromeres opposite each centromere in alternate orientation. When the pull from one lapses for whatever reason, there still is the other to keep the balance. Therefore, alternate orientation will not revert readily to adjacent orientation. With adjacent orientation, however, when for whatever reason the pull from the single opposite centromere lapses, both co-orienting centromeres become unstable and resume equal probabilities to orient to either pole: the probability of re-orientation is great. When there is enough time available, there will be an accumulation of alternate orientation. It appears that chain quadrivalents are slower in this respect than rings: re-orientation of alternate chains is less improbable than of alternate rings (Sybenga, 1968). In the flexible quadrivalents of rye interchange heterozygotes, alternate rings may occur in up to 95% of the pollen mother cells in late metaphse. Re-orientation has been observed and described for living material by Nicklas and associates, and Dietz and associates. For a review see Rohloff (1970).

There are several additional factors that affect the final result: length of interchanged and interstitial segments; localization and terminalization of chiasmata (Burnham, 1956). It appears possible to select for a certain type of orientation, probably by altering the flexibility of the chromosomes, the time available for re-orientation, and the behaviour of the chiasmata.

4.5.6. *Semisterility*

The orientation of the interchange multivalent has important consequences for the way in which the chromosomes involved are distributed, and consequently on fertility. As is shown in fig.4.14A, with alternate orientation two normal chromosomes (1 and 2) go to one pole, two interchange chromosomes (1^2 and 2^1) to the other: both form a genetically complete combination. With adjacent orientation, however, one normal chromosome goes to one pole together with an interchange chromosome, and this is not

a complete set: there is a duplication and a deficiency. The same is true for the other pole (fig.4.14B). The consequence is sterility. With alternate and adjacent orientation in a 1:1 ratio, there will be 50% sterility (*semisterility*). Preponderant alternate orientation can lead to almost complete fertility. Selection for fertility of interchange heterozygotes usually is successful, although strong effects are not realized. Maize, barley and Drosophila usually show semisterility, although a few interchanges are relatively fertile. Rye heterozygotes are often quite fertile. When bivalents predominate, semisterility is always expected: there is no possibility for more than 50% alternate orientation and selection must fail. Only when the resulting deficiencies are quite small, the fertility can be high, but there may be genetic irregularities (section 4.5.8).

Chiasmata in the interstitial segment affect the orientation: reorientation becomes improbable while adjacent II occurs only very infrequently; according to McClintock, in maize never. With an interstitial chiasma between centromere and interchange point, both chromosomes involved in this chiasma will have one translocation chromatid and one normal chromatid (fig.4.14D). Alternate and adjacent I orientation now cannot be distinguished. Only in the second anaphase the chromatids separate, and not before then the combination is determined: a translocation chromatid of one chromosome and a translocation chromatid of the other gives a viable combination, but a translocation chromatid and a normal chromatid do not. There is no co-orientation but chance distribution: half of the cases give a balanced set, half do not: there is always semisterility. Chiasmata in differential segments in combinations of two or more interchanges give complications that will not be discussed.

Orientations leading to a 3:1 segregation of the quadrivalent are not at all excluded. Then one spore lacks a chromosome, the other has one in excess. Fertilization leads to trisomy in the latter case, which is regularly observed: translocations are a good source of trisomics. The frequency depends on several factors; most of which are unknown. It is usually several percent in plants when the female is the heterozygote. It is negligible when the father is the heterozygote: in diploids, pollen competition tends to prevent

spores with extra chromosomes to fertilize. Since any of the four chromosomes can be the extra chromosome, four types of trisomy are possible. These will be further discussed in section 5.3.

Simultaneous with a gamete having an extra chromosome, one is formed with a chromosome less. This gamete or the resulting zygote is usually not viable, except in polyploids or when the chromosome involved is quite small. The major cause of the elimination of such very small translocation chromosomes, is univalence (they are too small to have chiasmata regularly) rather than a consequence of irregular orientation. When such small chromosomes are lost, and the monosomic progeny is viable, a situation comparable to that of a Robertsonian centric fusion arises (fig.4.12; sections 1.5.3, 4.5.3).

4.5.7. *Translocations in man*

It has been estimated that over 0.5% of the human population is heterozygous for a detectable interchange. Since these are mostly different translocations, the probability of a homozygote being produced is small, especially as marriages between close relatives possibly carrying the same interchange, are infrequent.

Translocations have often been detected by chance in population surveys, provided they have resulted in a recognizable alteration of the karyotype. A few that were not recognizable in mitotic metaphase have been detected during studies of meiosis where they led to the formation of a quadrivalent. Since meiosis is not readily accessible in man, this is not a generally applicable method of scoring translocations. Most translocations in man have been detected in people karyotyped, because of complaints about reduced fertility or because of trisomy in the progeny. Although the correlation between fertility and translocation heterozygosity has not been studied as thoroughly in humans as it has been in plants, a clear relation has been established. Like in plants, some translocations cause a higher degree of sterility than others. In man, because unbalanced karyotypes are transferred with the sperm without much restriction, a translocation in the male has almost as great an effect on fertility as one in the female: fertilization takes place, but an unbalanced gamete usually leads to early spontaneous

abortion of the embryo. Trisomy in the offspring is not common for all translocations. Besides depending on the formation of a gamete with an extra chromosome, the incidence of trisomy also depends on the viability of the trisomic embryo, and this varies greatly with the chromosome involved. A special category of translocation is the Robertsonian centric fusion type (sections 1.5.3, 4.5.3; fig.4.12). It has been observed relatively frequently between a chromosome of the group 13-15 and the chromosomes 21 or 22. The main reason why these translocations are frequent in these chromosomes probably is the quite common association and fusion of the nucleoli that are formed on the short arms of these chromosomes: these short arms apparently are regularly so near each other that they have a great probability to interact when spontaneous lesions occur. The two short arms together form such a small chromosome that it may be lost without consequences. The total number of chromosomes is then reduced by one to 45. The loss of two nucleoli does not appear to matter. At meiosis in the heterozygote the trivalent has the large translocation chromosome (13^{21}) in the centre and the two normal chromosomes (13 and 21) at the ends. The orientation tends to be such that the central chromosome goes to one pole, the two other chromosomes to the other pole (fig.4.12). This results in balanced gametes. Occasionally, however, two adjacent chromosomes go to the same pole, i.e. the large translocation chromosome with either 13 or 21. The pole where the remaining single chromosome goes is nullisomic for the other of the two chromosomes and is not viable. A gamete with the two adjacent chromosomes in fact carries a duplication–deficiency combination as normally results from adjacent segregations. A zygote resulting from fertilization of such a gamete in practice has the equivalent of an entire chromosome in excess and carries a deficiency without genetic consequences. Compared to the parent, the normal chromosome number of 46 is restored, but because the duplication is the equivalent of an entire chromosome, these aberrant types are often considered to be trisomic. When chromosome 13 is the extra chromosome, the embryo is inviable: it is not recovered. If chromosome 21 is the extra chromosome, Down's syndrome is observed (mongolism, compare

section 5.1.1). This small chromosome is relatively well tolerated as extra chromosome, but it is probable that nevertheless a proportion of trisomic 21 embryos abort. In theory it should be possible to estimate the frequency of such abortions by subtracting the frequency of born duplication types from the frequency of duplication gametes, and this frequency depends on adjacent orientation. It has often been assumed that a random orientation of the trivalent in the heterozygote should be: 1/3 balanced, 1/3 adjacent 13^{21}-13 and 1/3 adjacent 13^{21}-21 and that consequently one out of six gametes should have a long arm of 21 in excess. Assuming lethality of the two deficient embryos and of the trisomic -13 embryos, of each three viable embryos two should be balanced and one trisomic 21, a very high frequency of trisomy. The reduction to the much lower number observed would be due to embryo lethality (abortion). These considerations, however, are based on the fallacious assumption of total absence of trivalent co-orientation. In reality the balanced type must be expected to predominate and a prediction of the frequency of adjacent orientation cannot be made. Consequently, the viability of trisomic 21 embryos cannot be estimated on this basis. It is certain, however, that trisomy 21 results from translocation heterozygosity much more frequently than true trisomy 21 from spontaneous non-disjunction. See further Turpin and Lejeune, 1965, and Court-Brown, 1967.

4.5.8. *Genetic consequences of translocations*

The genetic consequences of translocations refer to the transmission of the translocation and to the effect of translocations on gene segregation.

When only balanced segregations are considered, the normal and the translocation type arise in equal frequency: a testcross gives normal and heterozygous types in a 1:1 ratio. Selfing, or crossing of two heterozygotes results in a 1:2:1 segregation of normal:heterozygous:homozygous. The translocation behaves like any single genetic factor. Deviations may be a consequence of reduced viability of the homozygote. Two reciprocal translocations that do not have a chromosome in common, segregate independently.

When they share a chromosome, two situations may be distinguished (compare section 4.5.4 and fig.4.13). (1) The same homologue of the common chromosome is involved in both translocations (fig.4.13A). Only two types of balanced gametes can be produced: one with both translocations and one with neither. Alternate orientation is required to produce them. Selfing, or crossing of two heterozygotes then yields: 1 homozygous for both : 2 heterozygous for both : 1 homozygous normal. (2) Of the common chromosome one homologue is involved in one, the other homologue in the other translocation (fig.4.13B). This is the normal case when two independently formed translocations are combined in one individual, for instance by hybridization. Again, only two types of balanced gametes are formed: one with one translocation and the third chromosome normal, the other with the other translocation and also one chromosome of the three normal. Selfing (or crossing two heterozygotes) now yields 1 homozygote for one translocation and for one normal chromosome : 2 double heterozygotes : 1 homozygote for the second translocation and for the other normal chromosome. Single heterozygotes and normal types cannot be formed without crossing-over in the differential segment. Recombination converting one type into the other does occur, but is generally infrequent, not only because the required exchange in the differential segment is scarce, but also because the critical orientation is not frequently realized.

There are two ways by which translocations can affect linkage between genes: (1) In the homozygote, the linkage relationships have changed: genes in the translocated segment fail to show linkage with genes in the chromosome where they originally belonged. Instead, they are linked to other genes. In itself, this change may be sufficient to detect a translocation and to identify the chromosomes involved, but it requires an extensive knowledge of the material. An interesting example of transferring a gene from one to another chromosome in the silk-worm is a translocation between one of the sex chromosomes and a chromosome with a gene for egg-colour. Tazima succeeded in linking egg-colour to sex, which enabled him to separate the sexes mechanically, using photoelectric detection (Tazima, 1964). The transfer of genes from

one species to another with the use of translocations will be discussed in section 5.7.12. (2) In the translocation heterozygote, all genes on all chromosomes involved are linked. This is caused by the circumstance that usually only balanced gametes take part in fertilization or only balanced zygotes survive. These arise exclusively when either all interchange chromosomes or all normal chromosomes are combined in one spore (compare fig.4.14A). All alleles of one normal chromosome end up in the same gamete as those of the other normal chromosomes and all the alleles of the translocation chromosomes remain together.

Recombination between gene and translocation and thus between the genes of the different chromosomes occurs when exchange takes place between the interchange break-point and the locus. Crossing-over percentages between a locus and the break-point can be estimated when gene and translocation can both be classified. The translocation can often be classified by studying the mitotic chromosomes. When this is not possible, one may attempt to classify the heterozygotes by looking for multivalents at meiosis, but this is usually laborious. The two homozygous types (normal and interchange) cannot be distinguished from each other: both give merely bivalents. Heterozygotes can generally more easily be recognized because of their semisterility. The analysis of linkage between gene and translocation using heterozygotes is the least complicated in a test-cross: there is a simple 1:1 segregation for the translocation and also for the gene. Selfing, or crossing of two heterozygotes gives a more complicated situation that differs from a normal bifactorial segregation, in so far as non-corresponding types are classified: dominant and recessive for the gene, heterozygous and homozygous for the translocation. Examples where the product-ratio method was used are given by Joachim (1947). Examples for the use of the maximum-likelihood method can be found in Kramer (1954). They will not be worked out here. Genes in the interstitial segment are closely linked to the translocation: there is little exchange in this segment and at most 50% of the recombinants are balanced.

Interference across the interchange break-point between segments in the unaltered arms (O and P in fig.4.9) could not be

detected by Rhoades in 1931 in maize. More recently, however, Sybenga observed strong positive interference between the interchanged and the interstitial segments in rye, accompanied by negative interference between the two interchanged segments and between the two interstitial segments. Such interference systems probably result from torsions during pairing.

With reciprocal translocations that have only small interchanged segments, adjacent I segregation can lead to such small deficiencies that spore abortion does not follow. When a gamete from such a deficient spore is combined with one that has the recessive allele of a gene in the deficiency, the recessive phenotype is observed: *hemizygosity* (4.2.2). Examples have been found in maize where only the egg tolerated the deficiency and the recessive allele was contributed by the pollen. In animals, where there is no development of a gametophyte that by selection would remove any deficient gametes, such mutants-by-deficiency not only can be observed in a higher frequency but there also is the possibility that both parents contribute a deficient gamete and that a homozygous mutant type is produced without the introduction of a recessive allele. The impression is given that recessive mutations are produced in specific chromosome segments. Since the tolerance for homozygous deficiencies is limited, examples are scarce, but some are known for Drosophila.

The combination of two reciprocal translocations involving the same arms of two identical chromosomes can result in gametes on the F_1, that carry duplications without at the same time carrying deficiencies. Simultaneously, other gametes are formed (and are expected to abort) that do carry deficiencies, but without the duplication. Since this method had been developed by Gopinath and Burnham (1956) in maize it has repeatedly been attempted to duplicate specific chromosome segments that contain genes of economic value. The method is explained in fig.4.16. Hagberg has succeeded in duplicating the α-amylase gene in barley, important for the brewing quality. The technique is quite laborious when the proper interchanges are not already available. The duplicated segments need not necessarily be genetically identical: it is possible to select heterotic combinations that remain fixed once the duplica-

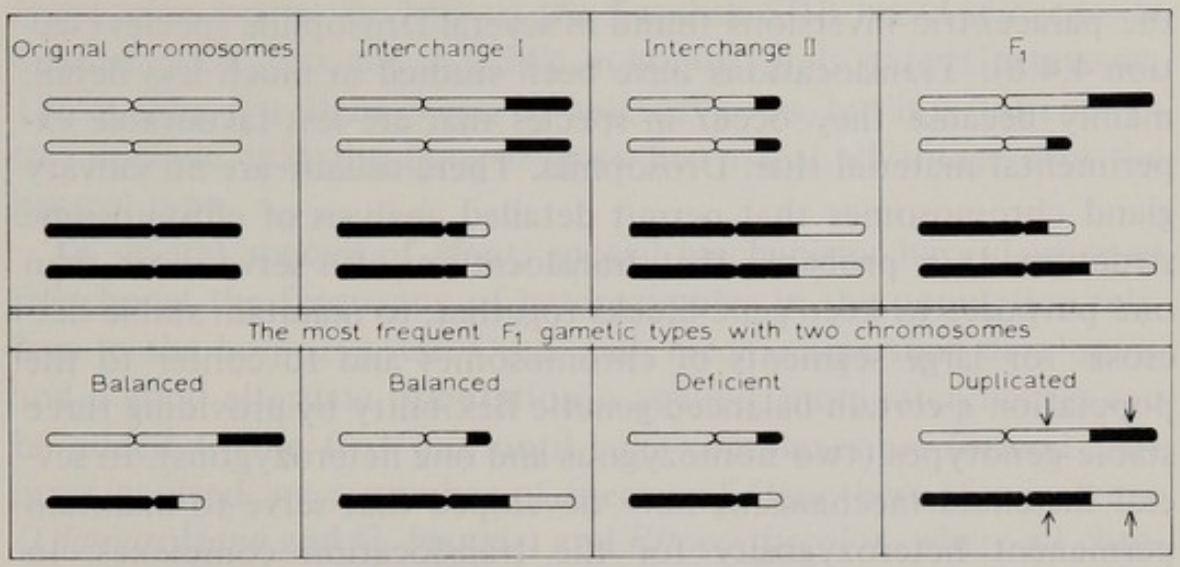


Fig.4.16. The origin of a duplication from the combination of two interchanges. The two interchanges, arisen in different individuals, involve corresponding arms of homologous chromosomes. The arrangement of the break points must be such that the interchange that has its break point closer to the centromere in one chromosome, must have it further away in the second chromosome. In the F_1 the two interchanges are combined. On the F_1 different types of gamete can be formed from these four chromosomes: some are balanced, some have deficiencies and some have duplications for specific segments.

tion has been realized. Large duplications are to be avoided as they tend to reduce vigour and fertility.

Interstitial (*intercalary*) translocations in many ways resemble reciprocal translocations in genetic behaviour but have some essential differences. They have been studied far less extensively. A rather recent example is that by Snoad (1966) in pea. Because of their relatively low frequency and often somewhat less drastic consequences as heterozygotes, they are less important than reciprocal translocations.

4.5.9. *Translocations in natural populations*

Besides translocations that occur incidentally in natural populations and that are maintained only for a limited number of generations because of a usually reduced fertility and reduced fitness of the homozygote, there are translocations that are maintained floating in populations. They have been detected in several animal and plant species and are in more than one sense comparable to

the paracentric inversions found in several Drosophila species (section 4.4.6). Translocations have been studied in much less detail, mainly because they occur in species that are less favourable experimental material than Drosophila. There usually are no salivary gland chromosomes that permit detailed analysis of chromosome structure. It is probable that translocations also serve more than one purpose: to keep gene-blocks together, to maintain stable heterosis for large segments of chromosomes and to confer to the population a certain balanced genetic flexibility by providing three stable genotypes (two homozygous and one heterozygous). In several instances mechanisms have developed that serve to maintain permanent heterozygosity for the translocation complexes. In such cases only the maintenance of gene blocks and heterosis are significant. A prerequisite is predominant alternate MI orientation and absence of interstitial crossing-over.

Quite simple cases of perpetuated translocation heterozygosity have been described by John and Lewis in 1959 for the cockroaches Periplaneta and Blaberus in small, slightly inbred populations in abandoned mines in Wales. In several more insects but also in several species of plants, for instance *Clarkia* spp., *Paeonia* spp., *Gaura biennis*, *Hypericum perforatum*, *Chelidonium majus*, *Campanula persicifolia* etc., translocation heterozygosity has been found to occur in such high (although often variable) frequency that it cannot be considered to be incidental. They always occur in populations that periodically are subject to drastic reduction in number, which implies a continuous threat of inbreeding. Clearly in all these cases the interchange has a function in maintaining heterozygosity.

When the frequency of the translocation in the population is relatively low, it may be doubted if there is any real net advantage. It is possible that the spread in the population is merely a matter of drift and then the translocation is actually part of the "genetic load". This may be the case with the numerous translocations detected in human populations (section 4.5.7). An interesting case is that of a Robertsonian translocation (centric fusion type with loss of a centromere with only very small chromosome segments) between the acrocentric chromosomes 1 and 29 in Swedish and

Norwegian cattle. In 14% of the Swedish SRB breed it occurs in heterozygous state and in 0.34% in homozygous state (Gustavsson, 1969). This is a slight excess of heterozygotes, but it is not enough to conclude that the heterozygote has a real advantage over the normal type.

In several species of plants special mechanisms have developed that boost the frequency of heterozygotes in the population relative to the homozygotes. Once such mechanisms start developing and a good alternate orientation is assured, more interchanges can be added to the first one until large complexes are formed. The most evolved are a number of species of Oenothera (for instance *O. lamarckiana* and *O. biennis*) and *Rhoeo discolor*, where all chromosomes are involved in the interchange complex. The alternate orientation starts in one part of the large prometaphase multivalent and proceeds along the ring or chain until all chromosomes have joined. Like in simple interchange heterozygotes, with alternate orientation one complete set moves to one anaphase I pole, while another, also complete but structurally different set moves to the other pole. The two sets (genomes) are not only structurally different, but also genetically. They can exchange at the chromosome ends, the interchange segments (also called pairing segments), but the interstitial (differential) segments remain unaltered: if crossing-over took place here, the entire system would break up. The differential segments contain the gene blocks that are maintained unimpaired throughout the generations. Only mutation will alter them. Occasional adjacent orientation leads to unbalanced gametes that usually are non-functional (fig.4.17).

The peculiarity of these stabile complex translocation heterozygotes and the reason that they have attained such perfection, is that under natural conditions the two homozygous types do not occur. In Oenothera the mechanisms that prevent the realization of homozygotes have been studied in detail. It has appeared that the different species with complex translocation heterozygosity use different systems to prevent homozygosity. Three principal mechanisms can be distinguished:

(1) In *Oenothera lamarckiana*, Renner in 1921 found that of the two genomes only one was able to form an embryosac: the

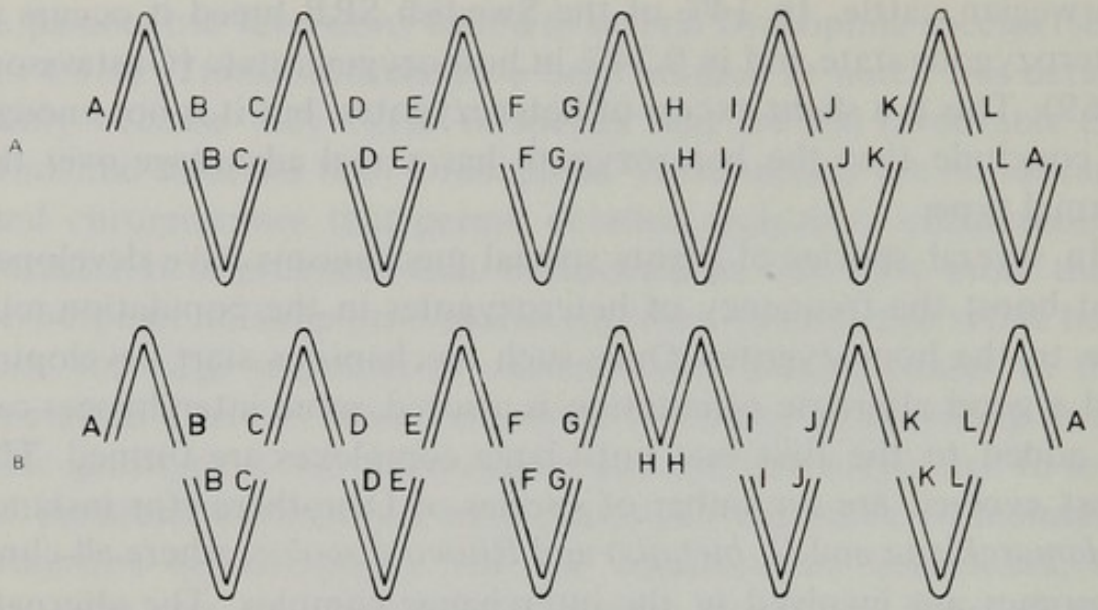


Fig.4.17. The twelve chromosomes of diploid *Rhoeo discolor* arranged in one chain at first meiotic metaphase. (A) Alternate orientation leads to the formation of two balanced groups, each with its own particular karyotype. (B) Adjacent orientation at one location in the chain not only leads to a disomic and a nullisomic gamete, but also to the combination into one gamete of chromosomes actually belonging to different genomes.

"gaudens" genome. When by chance the position where normally the developing embryosac is formed is occupied by a cell with the other (the "velans") genome, the function will be taken over by a cell that does have the right ("gaudens") genome: *Renner effect*. In the anthers, the situation is reversed: of the four tetrad cells only those with a "velans" genome develop into a pollen grain, the other two degenerate. Apparently, the "velans" genome carries a mutation that makes it unable to develop an embryo sac, and the "gaudens" genome carries a mutation that prevents it to develop a pollen grain.

(2) In other species of Oenothera, both genomes carry a (different) recessive lethal factor that, when homozygous, causes *zygote lethality*. The combination of two identical genomes is not prevented, but it does not lead to viable plants. Such recessive lethals may have originated from damage to the DNA at the breakpoints of one of the first translocations of the complex. Since such a system is accompanied by a 50% reduction in seed set, it has a definite disadvantage. A great excess of ovules, however, compensates for reduced ovule fertility.

These two systems permit continued self-fertilization without loss of heterozygosity of the differential segments. Gradually, of course, the pairing segments would become homozygous. With the third system, heterozygosity is also maintained in the pairing segment:

(3) Genetic systems of *incompatibility*. Pollen of a certain genotype does not germinate on the stigma (or does not grow into the style) in which the same allele of the incompatibility factor occurs.

With special techniques it has been possible to circumvent the systems that prevent homozygosis or to combine both genomes with an identical third genome, derived from another species. This permits the genotypes of the two genomes to be compared. The genomes indeed appeared to be genetically quite different. On the basis of such differences the names "gaudens" and "velans" were assigned to the individual genomes of *Oenothera lamarckiana*.

Darlington and La Cour in 1950 succeeded in extending an existing initial system of partial translocation heterozygosity in *Campanula persicifolia* to a reasonably efficient system of permanent translocation heterozygosity in which all chromosomes were involved. Attempts to induce such systems in Tradescantia, maize and barley have been successful in so far as complexes involving all chromosomes could be induced. Total sterility, however, resulted as multivalent orientation was random rather than alternate. This is not astonishing in the case of barley and maize, where the detection of most of the translocations had been on basis of semi-sterility resulting from a low frequency of alternate orientation.

The instability of interchange heterozygotes finds a practical application in the production of sterile hybrids of for instance the water melon, where the presence of large numbers of seeds in the fruits is undesired. A cytologically normal type is crossed with a complex translocation homozygote, that in itself is fertile. The heterozygote is heterotic and practically sterile, bearing seedless fruits. Of course, it is necessary that the fruit is formed parthenocarpically, either genetically determined or induced by growth substances.

Another promising application of translocation sterility is in

insect control: translocation individuals are introduced into the wild population in such a frequency that the general level of fertility of the total population is reduced until it is acceptable from a human point of view. There are many problems that must be solved before large scale application is to be expected.

4.5.10. *Interchromosome effects. Position effect*

Like inversions, translocations, through reduced crossing-over in the chromosomes involved, can cause changes in crossing-over percentages in other chromosomes. In this respect translocations have been studied much less extensively than inversions, and the effect apparently, but for unknown reasons, is much less general. According to Hinton (1965), in Drosophila the effect depends on the length of the interstitial segment. Reciprocal translocations between autosomes reduced crossing-over in the X-chromosome when the interstitial segment was short, but increased it when the interstitial segment was long. In a number of organisms, in the same cell low chiasma frequencies in the interchange complex are accompanied by high frequencies in the remaining chromosomes, and vice versa: there is a negative correlation between the chiasma frequencies of the different configurations within a cell. A considerable part of these correlations is merely a result of heterogenety within the cell that may have a number of causes, some even artificial, and it is clear that in such cases no true interchromosomal effects are involved. In other cases these effects may be real.

Position effects can be observed to result from translocations in Drosophila. Catcheside found a variegation of the flowers of an Oenothera translocation heterozygote, that he ascribed to a position effect.

Translocations between A and B chromosomes that can be used to induce deficiencies, have been mentioned in section 4.2.1. They are further of importance for studies of the behaviour of B-chromosomes (section 3.2.7), and for a number of other pruposes of chromosome manipulation that will not be considered here.

Chapter 5

NUMERICAL VARIANTS

Aneuploidy

Under normal conditions, aneuploidy leads to genetic unbalance that is unfavourable for the organism. When frequent, it indicates the presence of meiotic or mitotic irregularities in preceding generations. It can be quite useful for the cytogeneticist as it enables him to analyse meiotic phenomena, and to make special genetic constructions that otherwise could not easily be realized. The terminology is incomplete and often not used consistently. The most current terms will be used.

5.1. Primary trisomics

5.1.1. *Origin and phenotypic effects*

In primary trisomics, in addition to the normal chromosome complement one normal extra chromosome is present (section 1.4.4). At the diploid level they usually originate from *triploids* (section 5.5.3), the most common source in many plants: Datura, rye, barley. At all levels of ploidy aneuploids result from *asynapsis* and *desynapsis* (section 2.9.10), the most important origin in wheat, cotton (both polyploids) and maize (diploid). Spontaneous incidental *non-disjunction* for a single chromosome is the most common source of primary trisomy in man and many other animals. Further, along with tertiary and translocation trisomics (section 5.3.1), primary trisomics originate from *translocation heterozygotes* (section 4.5.6) in all organisms including man.

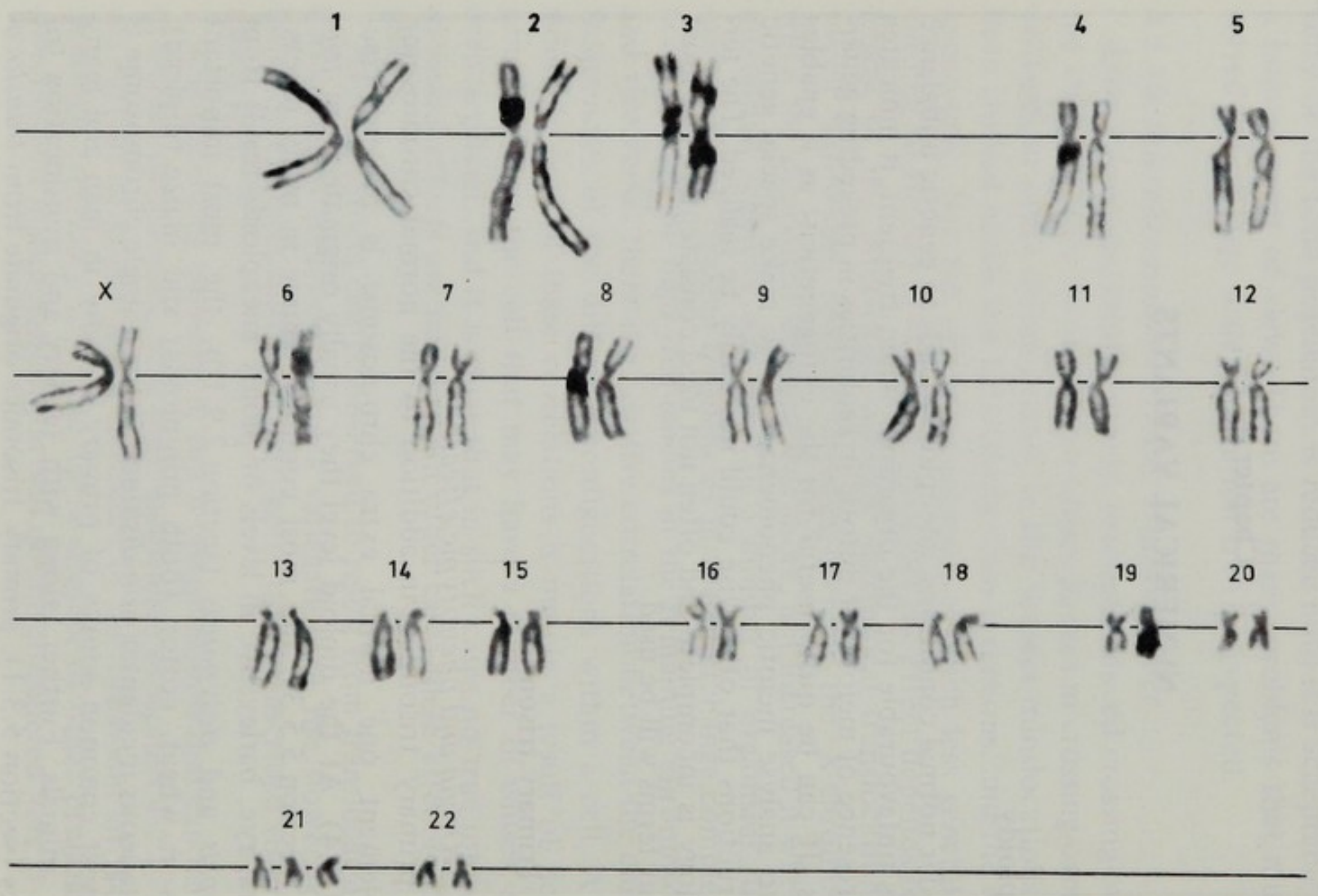


Fig.5.1. The karyogram of a female (XX) patient with Down syndrome: she is trisomic for chromosome 21. (Anthropogenetic Institute, Groningen.)

One way to obtain trisomics is by using *ionizing radiation*, which may occasionally cause spindle disturbances. Trisomics after colchicine and other *chemical treatments* have been reported.

Non-disjunction can occur during either of the two meiotic divisions in the father or in the mother. If it occurs during embryonic development in a mitotic division, mosaicism is the consequence. Because of the limited influence of the own genotype on the vitality of the male gamete in animals (including man) non-disjunction in the father (compare fig.5.2) will lead to aneuploidy almost as often as does non-disjunction in the mother. In plants the mother usually is the source.

A well-known example in man is trisomy for chromosome 21 (fig.5.1) that leads to the Down syndrome ("mongolism"): mental retardation, slanting eyes, saddle nose, etc., almost every tissue of the body being affected. Although the syndrome had been described by Down in 1867, only in 1959 Lejeune et al. recognized it as a case of trisomy: the first recorded example of a chromosome abnormality as the cause of a disease in man. Table 5.1 (Heller, 1969) shows that it is the most common case of autosomic trisomy (1 in 700). At conception the frequency is estimated to be even 1 in 140. Spontaneous abortion is the cause of the reduction in incidence at birth. A similar or even stronger reduction occurs for all types of trisomy. According to Penrose (and others), the probability of non-disjunction increases with age of the mother. Under 30 years the incidence is one in 2000, over

Table 5.1
The estimated incidence of various types of chromosomal abnormalities. (From Heller, 1969)

Syndrome	Chromosome number	Estimated incidence
Down's trisomy 21	47	1 in 700
Trisomy D	47	1 in 10.000
Trisomy E	47	1 in 4000
Trisomy X	47	1 in 10.000 females
Turner's XO	45	1 in 5000 females
Klinefelter's XXY	47	1 in 400 males
Double Y, XYY	47	1 in 250 males

45 years it rises to 1 in 40. The duplication type resulting from translocation heterozygosity is not dependent on age. It constitutes about 3.6% of all cases of Down's syndrome. The other trisomics of table 5.1 all have their own typical syndrome.

A special class of primary trisomics are the sex-chromosome trisomics, which occur with relatively high frequency. While non-disjunction of autosomes leads to trisomy in one cell and lethal nullisomy in the other, nullisomy resulting from non-disjunction of the sex-chromosomes may be viable. In man, XO females may result that have infantile characteristics, and are infertile (Turner's sundrome). YO types are not viable. When the cell with two X's develops into an egg that subsequently is fertilized, either XXY males develop or XXX females. The XXY males show incomplete sexual expression and sometimes mental retardation (Klinefelter syndrome). The XXX females do not appear to have sexual abnormalities, are often normal, but may occasionally be mentally retarded and perhaps somewhat less fertile. Non-disjunction of the Y-chromosome leads to XYY males that are slightly longer than normal and generally more impulsive, the "double Y syndrome". The impulsiveness may remain within normal limits but may also lead to agressiviness: the incidence of XYY males is especially high in penitential institutions. One of the reasons why the effect of excess of sex chromosomes is generally less drastic than excess of autosomes is their heterochromatinization: all except one X chromosomes are heterochromatinized (section 3.2.2) and recognizable as Barr bodies in resting cells. The Y's are always predominantly heterochromatic. For further details see Lilienfeld (1969) and Heller (1969).

Apart from the age of the mother, non-disjunction of normal chromosomes does not seem to be more frequent in any special genetic or social group of the human population, it can happen to anybody. In contrast, however, carriers of a translocation, especially of the Robertsonian (13-15)/21 translocations, have a greatly increased risk of non-disjunction and consequently of having trisomic progeny. The initial event of the translocation, however, is scarce.

When the parents have different alleles of a classifiable factor on

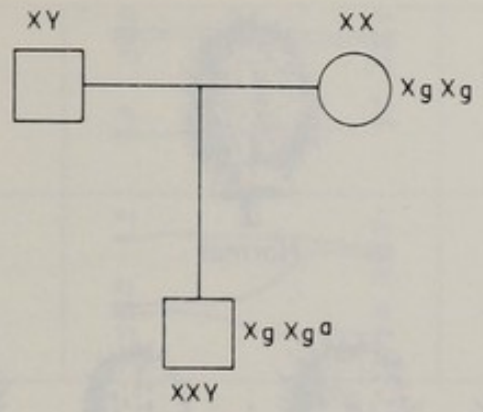


Fig.5.2. A Klinefelter son (XXY) of normal parents was heterozygous for the *Xg* blood group factor in the X-chromosome (*Xg Xg*^{a}). The mother was homozygous *Xg Xg*, and the X-chromosome with *Xg* must have been contributed by her. Therefore the other X-chromosome with *Xg*^{a} has been derived from the father, together with the Y: in the father X-Y non-disjunction must have taken place.

the chromosome involved, it is possible to determine in which parent non-disjunction has occurred. Especially the blood-group gene *Xg* on the X-chromosome is suitable for this purpose: the different alleles are present in the population with comparable frequency, which makes the probability great that the parents have a different genotype. An example is given in fig.5.2. The Klinefelter son is *XgXg*^{a}, the father being *Xg*^{a} and the mother *XgXg*. One X-chromosome must necessarily have come from the mother and this has the *Xg* allele. Therefore, the extra chromosome with *Xg*^{a} must have been contributed by the father. If the son had been *XgXg*, non-disjunction would have taken place in the mother.

McClure et al. made the interesting observation that the phenotype of a case of autosomal trisomy in the chimpanzee in all aspects resembled Down's syndrome. Chromatin-positive (Barr-body) Klinefelter male XXY sheep have been reported by Kilgour et al. to show testicular hypoplasia, but intelligence tests did not demonstrate any mental retardation.

In plants trisomics have been reported on numerous occasions. The first complete series of all possible primary trisomics with detailed descriptions of all the affected characters was constructed and analysed by Blakeslee and coworkers in the twenties and thir-

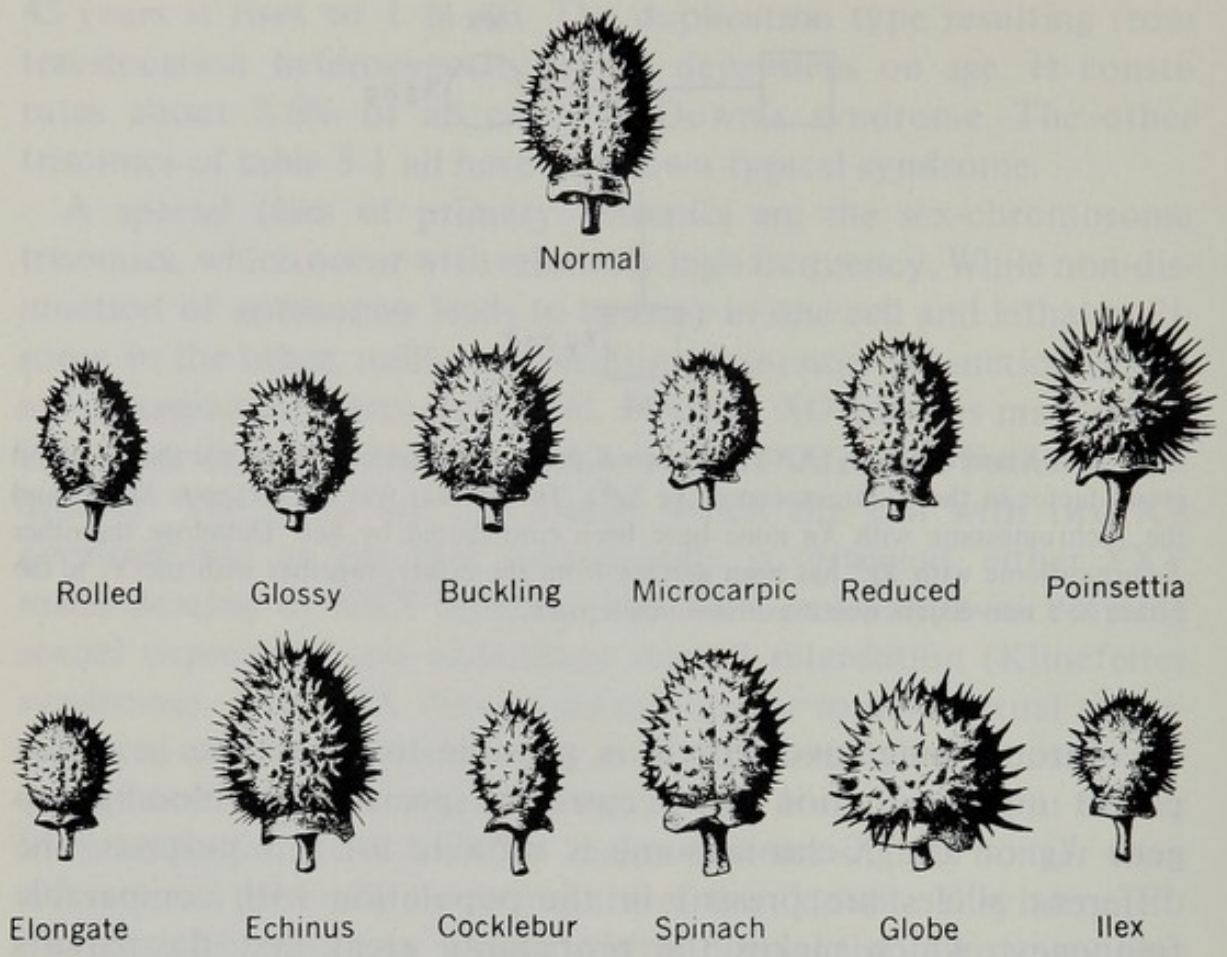


Fig.5.3. Fruit capsules of the 12 primary trisomics of *Datura stramonium*, each with its particular phenotype. (From: E.W. Sinnott, L.C. Dunn and T. Dobzhansky, Principles of genetics. After Blakeslee. Used with permission of McGraw-Hill Book Company.)

ties (fig.5.3). Complete series are now available in several crop plants such as maize, barley, tomato, rye.

5.1.2. *Chromosome pairing. Crossing-over*

In trisomics, three homologues of one chromosome are available for pairing. Pairing, however, usually involves only two at any point, but partner exchange may occur. In electron micrographs of points of partner exchange, Moens could occasionally see that over short stretches the three homologues were connected by a double synaptonemal complex. Depending on the number of points where pairing can initiate, different pairing configurations will result (fig.5.4).

(a) Pairing initiates at a single point only: partner exchange is

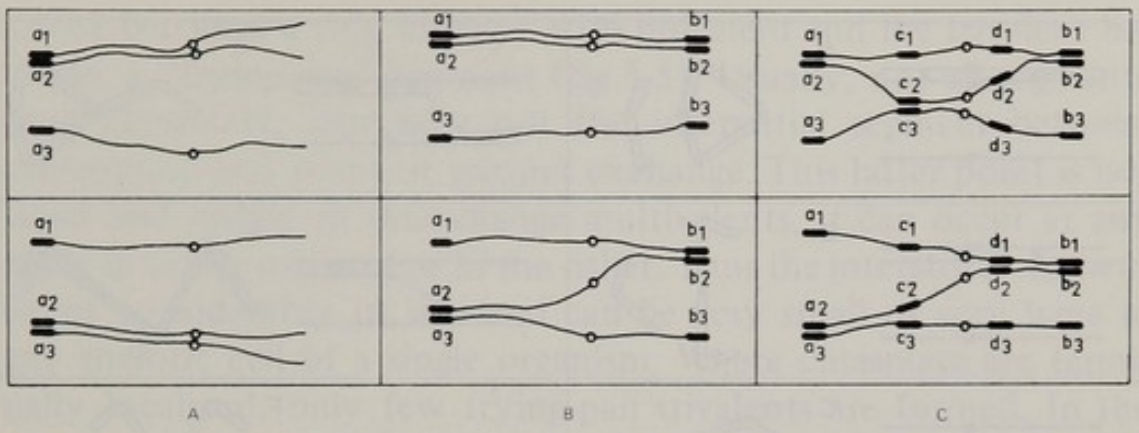


Fig.5.4. Three different systems of pairing initiation leading to different multivalent frequencies in a trisomic. (A) With only one point of pairing initiation (represented by a heavy line segment) in each chromosome, only a bivalent and a univalent are possible. (B) With two points of pairing initiation, far enough apart to permit free partner exchange between them, trivalents are possible and are expected with a frequency of 2/3. (C) With more points of pairing initiation the opportunities for partner exchange and multivalent formation increase.

excluded and a bivalent and univalent are formed. Which two of the three homologues pair, is a matter of chance. When there is a special affinity between two specific chromosomes, these two will pair more frequently than either of them with the third.

(b) More often, there are two points where pairing can start, for instance one on each end of the chromosome. Then there is an opportunity for partner exchange between these two points. In fig.5.4 ends a and b occur three times each (a_1, a_2, a_3 and b_1, b_2, b_3). When chance determines which two of the three ends pair, there are three possible combinations: a_1 - a_2 (a_3 unpaired); a_1 - a_3 (a_2 unpaired); a_2 - a_3 (a_1 unpaired). When the same is true for b, there are nine combinations, each with the same probability if there is no preferential pairing. Six result in a trivalent and three in a bivalent with univalent. This situation is reasonably well realized in rye trisomics and for trisomic 10 of maize, where McClintock and Hill in 1931 found a trivalent in 2/3 of the sporocytes and in 1/3 a bivalent with univalent.

(c) With more points of pairing initiation, scattered all over the chromosomes, the number of points of partner exchange is theoretically unlimited with the consequence of a large number of trivalents and less bivalents (with univalents). In most trisomics of

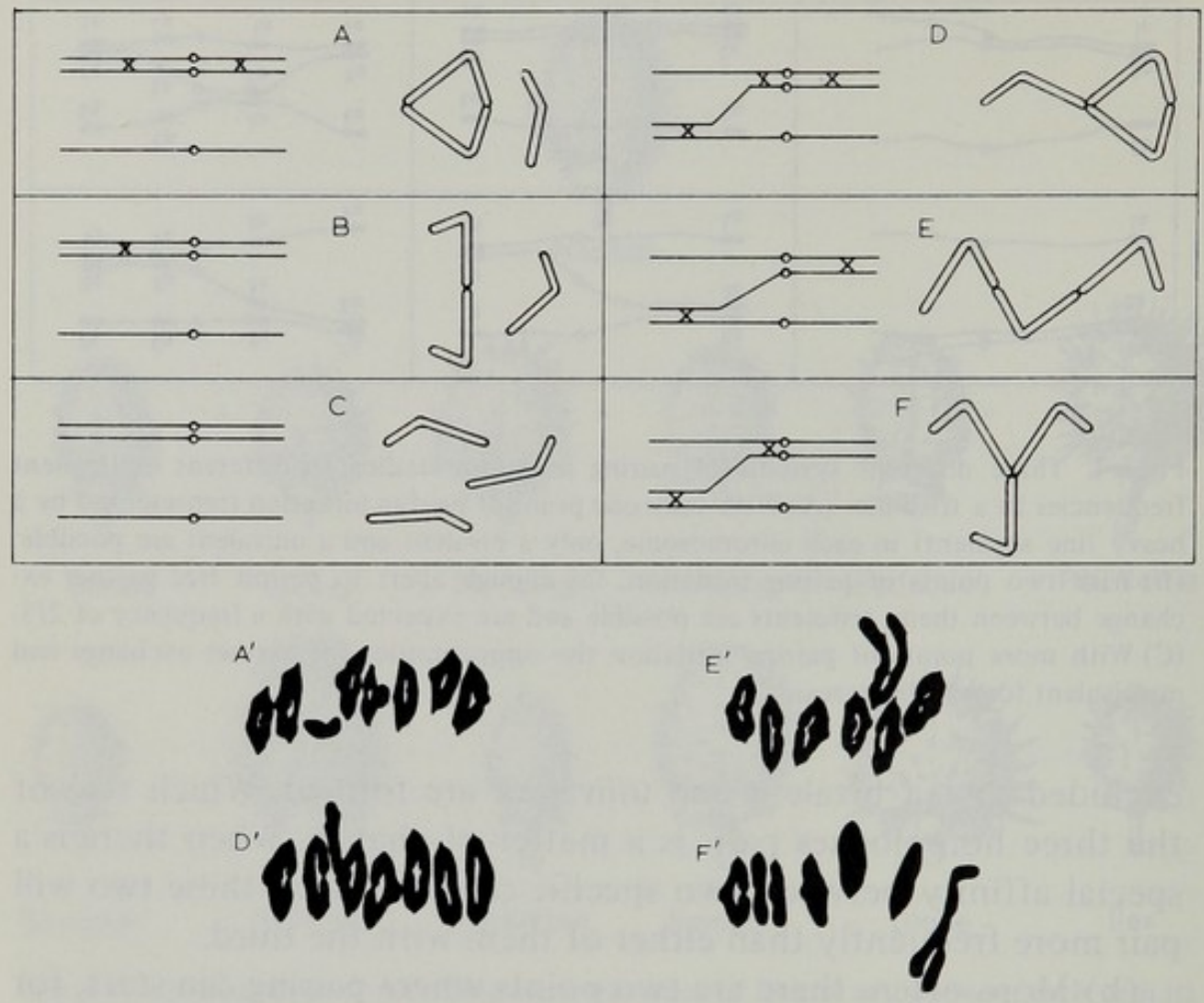


Fig.5.5. Pairing and metaphase I configurations (complete terminalization) in a primary trisomic. A, B and C: without partner-exchange, these three are the only configurations possible. D, E and F with partner exchange; there is an interstitial segment that can have a chiasma that affects the type of MI configuration. With only one chiasma the MI configuration of B is formed, without chiasmata that of C. Camera lucida drawings of these configurations in a rye trisomic in A', D', E' and F'. In addition to the trisomic configuration there are six ring bivalents in each cell.

maize this situation (with certain limitations, see below) is found.

Although on basis of these differences a rough impression can be obtained of the number of points of pairing initiation, an exact estimate is not to be expected: partner exchange always remains limited by (1) rigidity of the chromosomes; (2) pairing proceeding rapidly from any point of pairing initiation; (3) several points of pairing initiation close together attracting the same partner.

When chiasmata occur in all available segments, the metaphase configurations reflect the pairing relations: the bivalent with uni-

valent becomes a ring bivalent with univalent and the trivalent becomes a "frying-pan" trivalent (fig.5.5). Usually, not all segments have chiasmata, especially not the interstitial segment between centromere and point of partner exchange. This latter point is not fixed and unlike in interchange multivalents, it can occur at any place in either one arm or in the other. Thus the interstitial segment varies considerably in size and can be very small or very large in any meiotic cell of a single organism. Where chiasmata are terminally localized, only few frying-pan trivalents are formed. In the terminal segments too, chiasmata do not always occur, and when they do not, rod bivalents and univalents are formed at metaphase, or Y-shaped configurations (fig.5.5). A rye trisomic for the satellite chromosome had chiasmata in at least 96.0% of all its chromosome arms. In 300 cells there were 145 trivalents, among which 122 chains, 13 frying pans and 10 Y-shaped configurations. Another plant with chiasmata in only 89.6% of the chromosome arms, in 300 cells had only 106 trivalents, 91 of which were chains, 5 frying pans and 10 Y-shaped trivalents. The low frequency of trivalents ($<2/3$) is explained by low chiasma-frequencies in the shorter arm, reinforced by reduction of effective pairing around the points of partner exchange. Einset found a much lower trivalent frequency at MI of maize than at pachytene, in a trisomic for chromosome 3. At pachytene there were 2-4% bivalent + univalent; at diplotene 7-11%; at diakinesis 30% and at MI 32%. The difference between diplotene and diakinesis is partly due to loss of chiasmata by terminalization over the ends of the chromosome, and for another part by simple separation of homologues without chiasmata. Reduction of crossing-over around the point of partner exchange is often observed but not always. If it occurs, it can be compensated by an increase elsewhere in the chromosome. This may lead to changes in cross-over frequencies as studied in detail in Drosophila triploids (fig.5.12).

5.1.3. *Orientation. Centromere mis-division*

The orientation of a chain trivalent is often alternate: two chromosomes go to one pole, one to the other. The same can be said about Y-trivalents, but with frying pans sometimes the chromo-

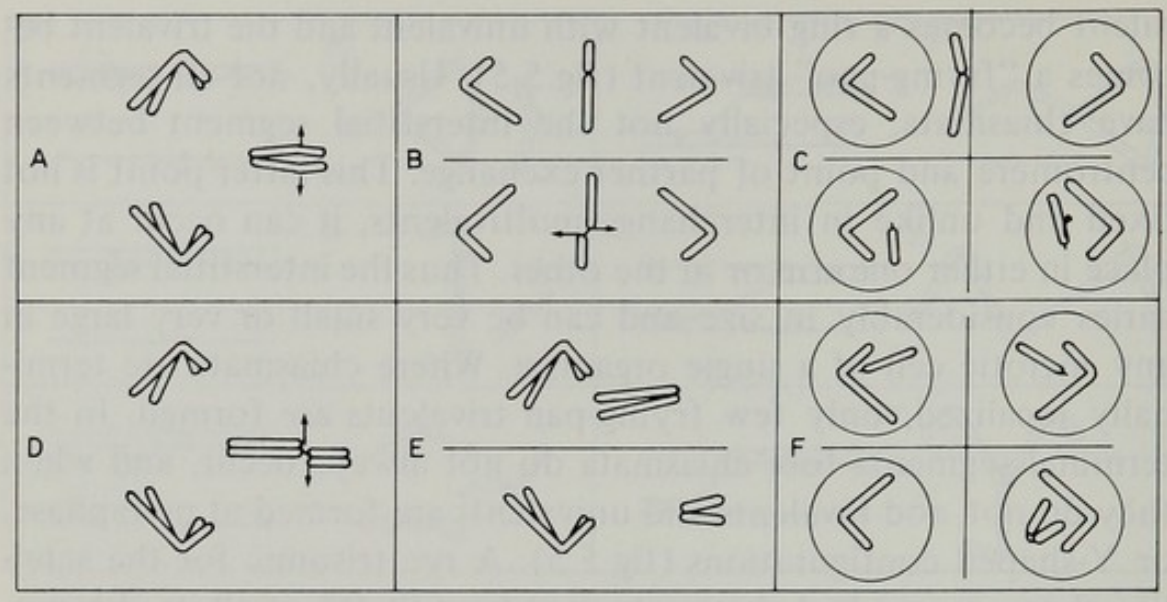


Fig.5.6. Centromere mis-division of a univalent. A normal pair of chromosomes is shown also. (A) At the end of anaphase I the univalent may divide, sending one chromatid to each pole. (B) At the second division this univalent cannot split again and remains in the equator. Quite infrequently the centromere may become active with two longitudinal parts coorienting. The centromere may break which results in two telocentric chromosomes that may be included in the daughter nuclei (C). More commonly such a centromere split occurs already at metaphase I when the chromosome is not ready to divide (D). This leads to two telocentrics each with two chromatids (E). The chromatids may separate at second anaphase and yield two telocentrics, one in each daughter nucleus (F). Or a double telocentric may undergo sister-reunion and thus is transformed into an isochromosome (F).

some projecting from the side of the ring does not reach a pole and remains on the equator. Occasionally chains can orient in a linear fashion with one chromosome in the middle that is not able to choose a pole. Univalents also tend to stay behind. Such lagging chromosomes, whatever their origin, may later in anaphase orient as if they were mitotic chromosomes, with one chromatid to one pole and the second to the other pole and separation may follow. With enough time to complete the process each chromatid may be included in an anaphase group. At anaphase II, however, there now is only a single chromatid and orientation and separation are impossible with the consequence that the chromatid lags behind and is lost (fig.5.6). In rye, this is quite common. In Datura and many other organisms the centromere of the univalent at late anaphase I is ready for orientation, but not for splitting. It then

happens occasionally that the centromeric region breaks up (mis-division), which results in two half chromosomes (the arms) each with a part of the centromere. Since the attachment of the two chromatids is not at the centromere itself but in two regions each at a side of the centromere, the break can occur even slightly outside the centromere. Then one arm receives a complete centromere, the other none. Less than a complete centromere, even half a centromere, may be quite functional, however. After centromere mis-division, functional *telocentric* chromosomes may arise, that can participate in the second division and be transmitted to the progeny. This is the way telocentric trisomics arise in the progeny of primary trisomics.

It is also possible that after breakage in the centromeric regions the two chromatids unite at the break by sister-reunion (compare section 1.4.8): a symmetric chromosome is formed with two identical arms, an *iso-chromosome* (compare attached X, section 4.3.1). This new chromosome contains only one chromatid and will meet with difficulties at second anaphase (fig.5.6). By chance it may be included in a nucleus and transmitted to the progeny. When the extra chromosome is an iso-chromosome the organism is a *secondary trisomic* (section 5.2.1).

There is a considerable difference between organisms in respect to sister reunion and consequently the relative frequency of telocentric and iso-chromosomes.

5.1.4. *Transmission*

In diploid plants, pollen competition usually prevents transmission of extra chromosomes from the father. In polyploids the chances are better. In animals there is hardly any elimination by competition. Because of loss of univalents and mal-oriented chromosomes, the frequency of trisomics in the progeny usually is much below the theoretically expected 50% and often does not exceed 20%. It can even be much lower (table 5.2). In animals, where the male transmission is good, tetrasomics can arise from crosses between two trisomics. In diploid plants, these are scarce, but in some species like maize and *Datura stramonium*, extra chromosomes are occasionally transmitted through the pollen. Accord-

Table 5.2
Progeny of three primary trisomics of Datura. (According to Blakeslee and Avery, 1938.)

Chromosome	Total number	$2n$	$2n+1$	% $2n+1$	Iso same chromos.	Iso different chromos.	$2n+1$ different chromos.	$4n$	n
1.2	2049	1780	213	10.40	6	0	27	23	0
3.4	2089	1634	452	21.64	0	0	1	1	1
19.20	4058	4498	141	2.96	7	4	100	3	1
Average all 12 trisomics				22.08					

ing to Lesley, some chromosomes are and others are not in the tomato.

5.1.5. *Genetic segregations*

When one locus with two alleles A and a is considered there are four possible combinations in the three chromosomes: AAA (triplex); AAa (duplex); Aaa (simplex) and aaa (nulliplex). The terminology follows the dominant allele. Two are heterozygotes, in contrast to the diploid where only one heterozygous type is possible. Gametes are formed with or without an extra chromosome. The simplex has six types of gamete, each expected with the same frequency when all three chromosomes are distributed to the gametes (table 5.3). Designating the two a alleles of the simplex by a_1 and a_2 the six gametic types are: Aa_1, Aa_2, a_1a_2, A, a_1, a_2. In a test-cross with aa (gametes a), like in the diploid, a 1:1 phenotypic ratio for dominant and recessive is observed as three contain a dominant allele, while three do not. When in plants, where the extra chromosome is usually not transmitted by the pollen, the trisomic parent is the father in the testcross, a 1:2 ratio may result, i.e. the ratio of the gametes without extra chromosome. A further shift in the ratio is expected because not all available chromosomes end up in the pollen. Instead of Aa gametes, a and A gametes are formed, both when the trisomic is the father and when

Table 5.3

Trisomic segregations without double reduction. (A) Simplex Aa_1a_2, gametic ratio 1:1. When in plants, disomic gametes do not function in the father, the male gametic ratio is 1:2. The F_1 segregates 3:1 when all paternal gametes function, 2:1 when disomics do not function. (B) Duplex A_1A_2a, gametic ratio 5:1; when disomic paternal gametes do not function, the male gametic ratio is 2:1. In the F_1 the segregation is 35:1 and 18:1 respectively. In practice the reduction can be stronger as a result of several factors.

A

	Aa_1	Aa_2	a_1a_2	A	a_1	a_2
Aa_1	+	+	+	+	+	+
Aa_2	+	+	+	+	+	+
a_1a_2	+	+	–	+	–	–
A	+	+	+	+	+	+
a_1	+	+	–	+	–	–
a_2	+	+	–	+	–	–

B

	A_1a	A_2a	A_1A_2	A_1	A_2	a
A_1a	+	+	+	+	+	+
A_2a	+	+	+	+	+	+
A_1A_2	+	+	+	+	+	+
A_1	+	+	+	+	+	+
A_2	+	+	+	+	+	+
a	+	+	+	+	+	–

(+: at least one dominant allele.)

it is the mother. In an F_2, like in the disomic, a 3:1 segregation is theoretically expected in the simplex, but for the same reason this is seldom realized as there is an excess of recessives.

For technical reasons segregation in trisomics is usually studied in a duplex that originated from a cross between a triplex trisomic (AAA) as the mother and a homozygous recessive disomic (aa) as the father. The trisomic F_1's are AAa. To distinguish between the two chromosomes with A the two dominant alleles are here designated A_1 and A_2. Then, in principle, the gametes A_1A_2; A_1a; A_2a; A_1; A_2; a are formed with equal frequency and a testcross gives a phenotypic segregation of $5A$: $1a$ (table 5.3). In plants, when the father is the trisomic F_1, because of general non-functioning of pollen with extra chromosomes, this is usually reduced to a 2:1 segregation. Loss of extra chromosomes during meiosis does not lead to lower ratios here: even when no extra chromosomes at all would be included in the gametes, still the gene frequency would be found in the gametes, which here also is 2:1. When the mother is the duplex trisomic F_1, loss of extra chromosomes in meiosis also leads to a drastic reduction of the 5:1 segregation.

The F_2 of a duplex F_1 theoretically gives a 35:1 segregation. Not functioning of disomic gametes of the father reduces this to 17:1 (table 5.3) and this can be further reduced to 8:1 when no trisomics at all are formed, but loss of univalents in meiosis does not again reduce the ratio.

5.1.6. *Double reduction*

There is one additional factor that causes an increase of recessive gametes: double reduction. Although quantitatively of little importance in trisomics, the phenomenon is of considerable qualitative and theoretical importance in polysomics and polyploids. It is illustrated in fig.5.7. With certain orientations of the multivalent, it is possible that two chromosomes between which a chiasma has been formed, pass to the same pole. This pole receives the extra chromosome. When there is an allelic difference between these chromosomes (*A* vs. *a*) and the exchange was between cen-

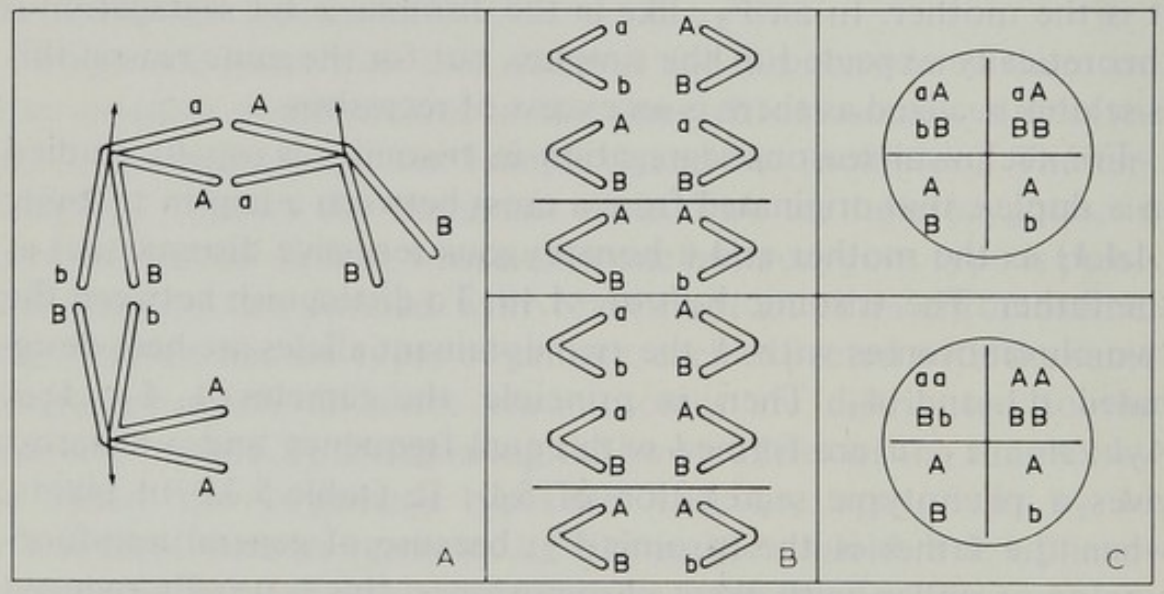


Fig.5.7. Double reduction in a trisomic. Two exchange chromosomes go to the same pole (A) both having one chromatid with the recessive allele and one with the dominant allele. At anaphase II (B) there are two possibilities for the two chromosomes that are together in the same cell: the two recessive alleles can go to different poles (top) or they can go to the same pole (bottom); the dominant alleles do the same. In the latter case, there is double reduction for both alleles, and the recessive alleles can come to expression (C, bottom). Crossing-over occurred also between the gene *b* and the centromere, but the two chromosomes were separated in AI. Now there is no possibility of combining two recessive alleles in one tetrad cell.

tromere and locus, both chromosomes have one chromatid with the *A* allele and one with the *a* allele. At second anaphase, there are different ways for the chromatids of these two chromosomes to segregate. In half of the cases one *A* and one *a* allele will go to each pole, both getting *Aa*. In the other half one pole receives *AA*, the other *aa*. Thus 1/4 of the spores has the *aa* genotype. Without this AII segregation and exchange between the centromere and the locus of *A*, *aa* spores would not be formed, which can be seen for the segregation of *B* and *b* in fig.5.7. The combination of sister alleles in gametes is called *double reduction*. It refers to the origin of *AA* gametes as well as *aa* gametes. It requires a special MI orientation that is possible only with non-alternate orientation of chains and with frying-pan and Y-shaped trivalents in trisomics. There is only one AI pole that receives the critical chromosomes. Further, the required AII segregation occurs in only 50% of the cases and then only half gets the recessive genotype that permits detection. Thus the total effect of double reduction on segregation in trisomic duplex heterozygotes is small.

The frequency of gametes with sister-alleles (*AA* and *aa*) is generally represented by α. It is an indirect measure of the recombination frequency (=genetic distance, see chapter 6) between centromere and locus. It is not to be equalized with genetic map length (section 6.1.1) as α is dependent on the frequencies of the different types of orientation.

Table 5.4
Trisomic segregations in maize (McClintock and Hill, 1931) and barley (Tsuchiya, 1959, 1960).

Plant	Trisomic	Factor	Type	Dominant	Recessive	% recessive
Maize	10	*r*	*RRr* self	396	41	9.4
Maize	10	*r*	*RRr* × *rr*	819	213	20.6
Maize	10	*r*	*rr* × *RRr*	941	486	34.1
Maize	10	*r*	*Rrr* × *rr*	679	836	55.2
Maize	–	*r*	*Rr* × *rr*	1161	1196	50.7
Barley	1	*br*	*Br Br br* self	370	25	6.3
Barley	3	*uz*	*Uz Uz uz* self	493	17	3.3
Barley	7	*ss*	*Sr Ss ss* self	145	11	7.1

As a consequence of all these factors that affect the segregation of a duplex trisomic, the F_2 ratio often drops to about 10:1. Although far below the theoretical value of 35:1, the deviation from disomic monofactorial segregation (3:1) is large enough to make it easy to decide whether or not a segregating factor is in the chromosome involved. It is one of the most frequently applied methods of determining in which chromosome a gene is located. Certainty about the duplex condition, however, is required. Examples of trisomic segregations are given in table 5.4.

5.2. Derived types

5.2.1. *Secondary trisomics*

In secondary trisomics the extra chromosome is an *iso-chromosome,* consisting of two identical arms connected by the centromere. The name simply reflects that it was the second type of trisomic discovered by Blakeslee in Datura, the first type being the primary trisomic. The origin often is centromere mis-division (section 5.1.3) in meiosis of primary trisomics, but they may also derive from occasional univalents in meiosis of entirely normal parents. This is the usual origin of the isochrome of X-iso-X women. These women are not true secondary trisomics since their iso-chromosome replaces one of the normal X-chromosomes and is not an additional chromosome. This is possible in humans since deficiency of the short arm of the X is not deleterious even though it contains segments that are not heterochromatinized with the rest of the chromosome. Only slight abnormalities result since the iso-X-chromosome is preferentially heterochromatinized. In organisms where the gene activity of the iso-chromosome is not suppressed, a fourfold dose of the genes instead of a double dose is present in the secondary trisomic, and this can have a considerable effect on the phenotype.

There are two possibilities for pairing: the two arms of the iso-chromosome pair, which result in a *ring-shaped univalent* (after chiasma formation), or the arms of the iso-chromosome pair with an arm of each of the two normal homologues. When the other

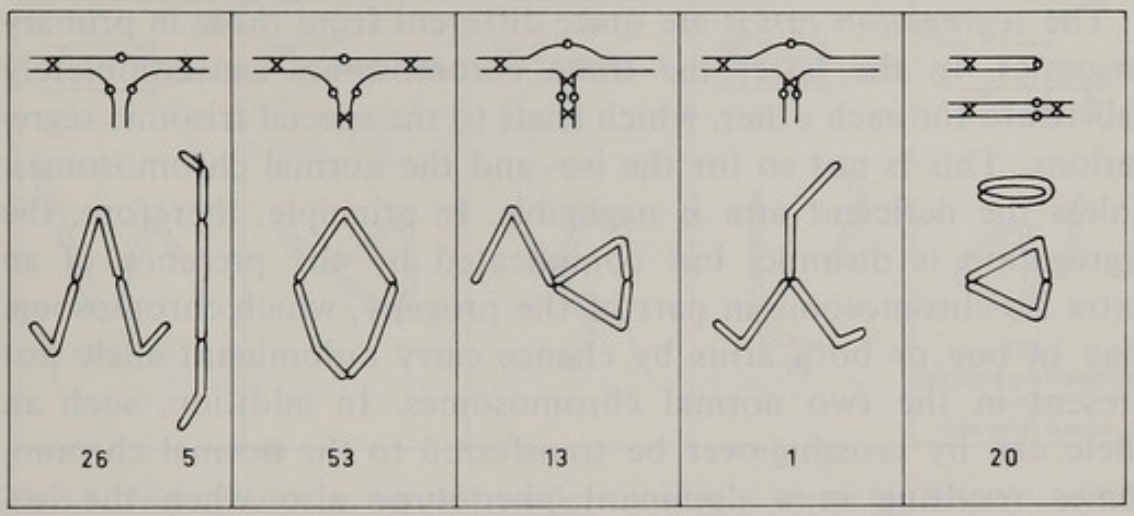


Fig.5.8. Five different metaphase I configurations in a secondary trisomic, and the explanation of their origin. The numbers refer to the frequencies of the different configurations found by Belling and Blakeslee in 1924 in a secondary trisomic of Datura (118 cells).

arms of these two chromosomes pair also, a *ring-trivalent* will be formed (fig.5.8). The relative frequencies of these two pairing types is rather variable. The two homologous arms connected by the same centromere do not usually pair preferentially, and especially when pairing initiation is concentrated at the ends of the chromosomes there is ample opportunity for pairing with the other homologous arms. With random pairing, twice as many trivalents as ring-univalents with bivalents are expected.

Exchange between iso-chromosomes and normal chromosomes does not lead to chromosomal abnormalities. When the two arms of the iso-chromosome originally were idential, they may now become allelically different. Number and location of chiasmata determine the configuration frequencies observed at first metaphase. In fig.5.8 the configuration types and frequencies as found by Belling and Blakeslee in Datura are shown.

Transmission varies with the type of iso-chromosome. In Datura, in the secondary trisomic 1.1, the iso-chromosome was transmitted to only 2.5% of the progeny; in 19.19 to 30%. The frequency of trivalents that permit an orientation leading to recovery (especially the frying pan) plays a role here, but also the effect of the fourfold dose of the arm involved. In this respect, naturally, the arms may be quite different.

The *segregation ratios* are quite different from those in primary trisomics. In the latter the three chromosomes can completely substitute for each other, which leads to the special trisomic segregations. This is not so for the iso- and the normal chromosomes, unless the deficient arm is negligible. In principle, therefore, the segregation is disomic, but complicated by the presence of an extra iso-chromosome in part of the progeny, which chromosome may in one or both arms by chance carry a dominant allele not present in the two normal chromosomes. In addition, such an allele can by crossing-over be transferred to the normal chromosomes resulting in a dominant phenotype also when the iso-chromosome is not present. Conversely, a dominant allele may be transferred to the iso-chromosome from a normal chromosome, with comparable consequence.

While the human iso-X-chromosome can only replace one of the normal X's, the attached-X of Drosophila, that may either occur as an iso-chromosome (reversed metacentric) or as tandem metacentric, can completely replace the normal X. This leads to complications in sex determination with this chromosome; there always is a double dose of the X, making the individual that carries it invariably a female.

5.2.2. *Telocentric trisomics*

The origin of telocentric extra chromosomes more often than not is by centromere mis-division (section 5.1.3 and fig.5.6). The phenotypic effect is generally less striking than that of primary and secondary trisomics. In a few cases the most typical effect of trisomy is merely due to the duplication of a few loci. The telocentric trisomic carrying these loci will then show approximately the same phenotypic deviation as the primary trisomic.

Chromosome-pairing can result in a bivalent with univalent or in a trivalent. The latter requires partner exchange, which does not have a fixed point. There is a greater probability that it occurs in one of the long normal chromosomes, somewhere around the middle, than in the single trisomic arm (fig.5.9). Ring-trivalents do not occur, but branched trivalents (frying pans and Ys) are possible. Dependent on frequency and location of the chiasmata, dif-

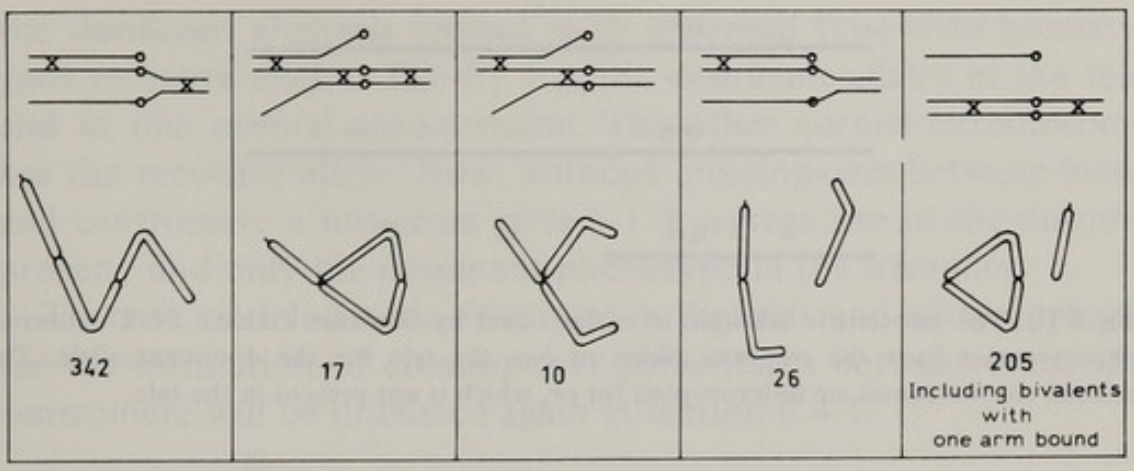


Fig.5.9. Five different metaphase I configurations in a telocentric trisomic, and the explanation of their origin. The numbers refer to the frequencies observed in a telocentric trisomic of rye (long arm of the satellite chromosome) in a total of 600 cells. The last category includes the cases in which the bivalent had chiasmata in only one arm.

ferent MI configurations arise. Chain trivalents are the most frequent, and always have the telo at one of the ends. Since several different configuration types are available and usually the telo can easily be distinguished from the metacentric pairing partners, analysis of metaphase configuration frequencies can give rather exact information on the frequency of chiasmata in the different chromosome arms.

Transmission: small extra arms have less pronounced deleterious effects than extra entire chromosomes or extra iso-chromosomes. This increases their chance of being transmitted, but their smaller size also reduces their chance of having a chiasma. This enhances univalence and subsequent loss, and therefore reduces recovery in the progeny. Small extra arms even in plants are transmitted through the pollen of diploids with observable frequency.

Segregation ratios resemble those of secondary trisomics: there are no trisomic segregations as the single arm cannot replace an entire chromosome, but in large F_2 or testcross populations there is sufficient deviation from disomic segregation ratios to detect if a segregating gene is located in the arm involved or not. This permits a more exact gene localization than with primary trisomics. It is used more often than the secondary trisomics as telocentrics are usually more common, more easily maintained and have a simpler

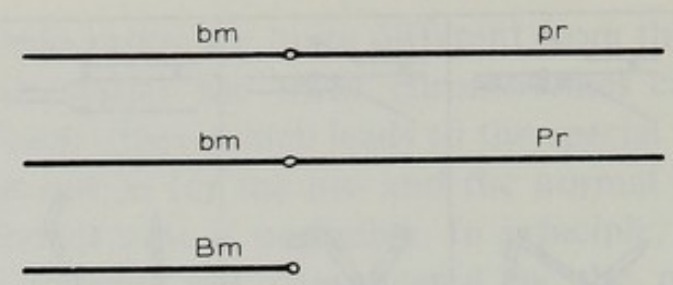


Fig.5.10. The telocentric trisomic in maize, used by Rhoades (table 5.5). The normal chromosomes have the recessive alleles of *bm*, the telo has the dominant allele. The normal chromosomes are heterozygous for *pr*, which is not present in the telo.

genetic system, as they consist of only one arm. Since the deviation from disomic segregation is not very great, the testcross is preferred. The following example from 1936 is by Rhoades (table 5.5): the telocentric trisomic of the short arm of chromosome 5 of maize has short, broad leaves and can therefore be recognized without cytological examination. A plant was obtained that had the recessive alleles (*bm*) of the factor brown midrib in the normal chromosomes, and the dominant allele (*Bm*) in the telo. The factor purple aleurone (*pr*) was heterozygous in the disomic arm (fig.5.10). Test-crossing with *bm bm pr pr* gave the segregation of table 5.5. The segregation in the disomic arm was 1:1, that in the trisomic arm 2:1. The transmission of the telo is about 30%, while with complete recovery 50% is expected. Except one recombination plant, all normal plants had the recessive allele, the trisomics the dominant allele. Apparently crossing-over is quite infrequent: the locus is close to the centromere. More common than this example is the situation that a telocentric carrying

Table 5.5

Segregation in a telocentric trisomic for the short arm of chromosome 5 in maize, according to Rhoades (1936). Compare fig. 5.10. The trisomic plants could be morphologically recognized because of their short, broad leaves.

Segregation	2 *n* (normal)	2*n* + telo short, broad leaf	Total	Ratio
Pr : *pr*	63 : 64	31 : 35	94 : 99	1 : 1
Bm : *bm*	1 : 171	85 : 0	86 : 171	1 : 2

the dominant alleles is crossed with a normal type with homozygous recessive alleles. The F_1 has the dominant alleles in the telo and in one normal chromosome. The other normal chromosome has the recessive allele. Now, without crossing-over between locus and centromere a test-cross gives a 1:1 segregation in the disomic progeny and only the dominant phenotype in the trisomics.

The use of telocentric trisomics in diploids and allopolyploids for the estimation of crossing-over percentages between locus and centromere will be discussed again in section 6.4.1.

5.3. Tertiary and interchange trisomics

5.3.1. *Types and origin*

In a *tertiary trisomic* there is a translocation chromosome in addition to a normal karyotype. Since in each translocation at least two chromosomes are involved, there are at least two possible types of tertiary trisomic for each translocation. In principle, the situation is the same when a normal chromosome is extra in a translocation homozygote. The name was given by Blakeslee: it was the third type of trisomic observed in Datura. Although such trisomics may occur both for interchanges and for interstitial translocations, the first are by far the most common, and the only type considered here.

In an *interchange-trisomic*, an extra chromosome occurs in an interchange heterozygote. Now there are four possibilities: the extra chromosome may be normal type 1, normal type 2, interchange type 1^2 and interchange type 2^1 (compare fig.4.9). There is no difference of principle between these four types.

The most important source of these trisomics is the translocation heterozygote. The two types of interchange trisomic having a normal chromosome as the extra chromosome can be experimentally produced by combining a primary trisomic with a translocation.

The phenotypic effect of these trisomics is comparable to that of primary trisomics and, of course, depends on the genetic make-up of the extra chromosome.

5.3.2. *Chromosome pairing*

As there is an excess of homology, the chromosomes must make a choice. When there is no preference for pairing between two specific chromosomes or parts thereof, and when pairing starts at the ends, there are nine (3^2) different possibilities of association: three for each of the two extra arms. It may be deduced that in a tertiary trisomic four association types are a multivalent of five, four are a quadrivalent with univalent, and one is a trivalent combined with a homomorphic bivalent. In an interchange trisomic the situation is only slightly different. The chromosomes and some of the nine possible configurations are shown in fig.5.11. Of the

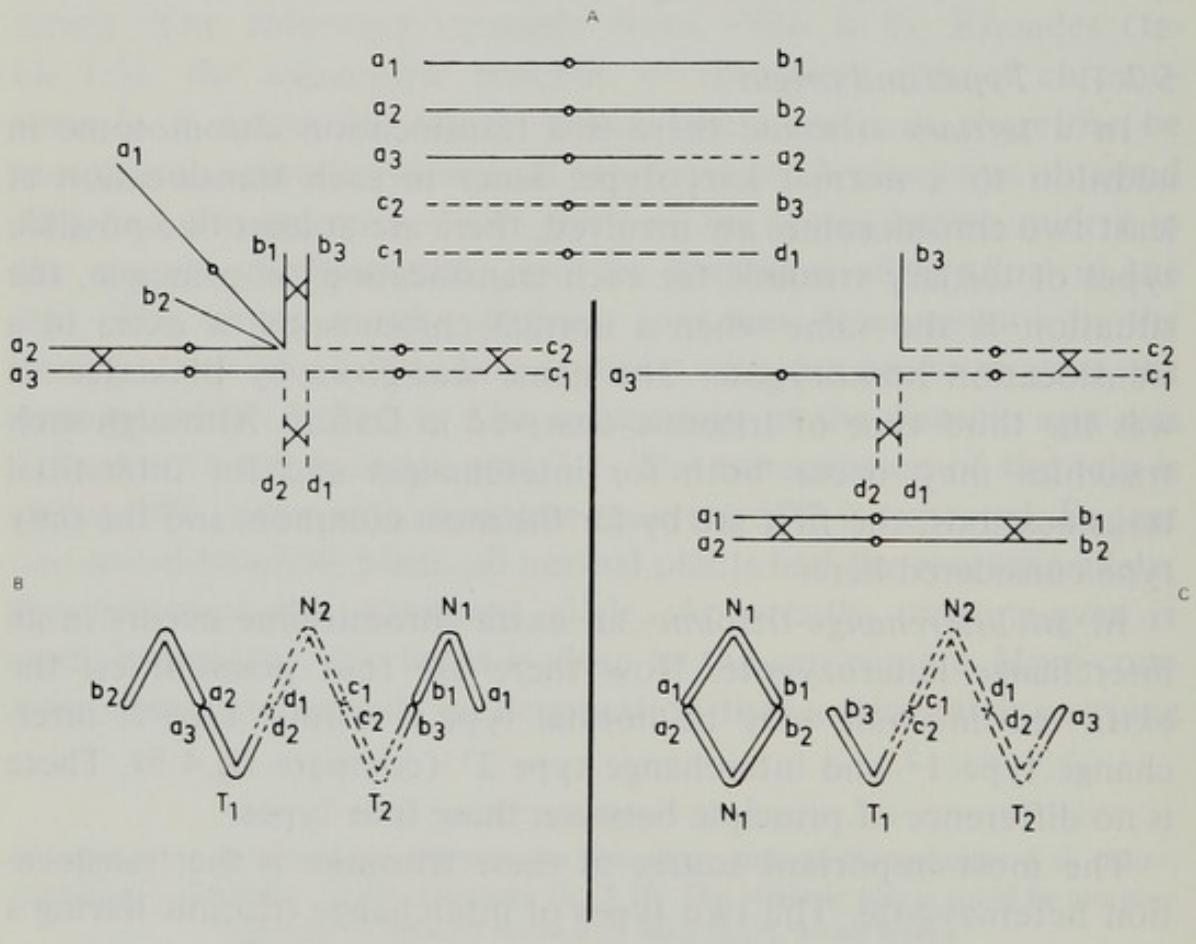


Fig.5.11. Interchange trisomic. (A) The five chromosomes of the complex. (B, C) Two of the nine pairing configurations and the corresponding metaphase I configurations that result when chiasmata have formed as indicated in the pairing configurations. In the bivalent in C two completely homologous chromosomes have paired. Merely by chance this happens once in nine times. When it is observed more frequently, there has been preferential pairing between completely homologous chromosomes. T: interchange (translocation) chromosome, N: normal chromosome.

nine possible associations six are quinquivalents (multivalents of five chromosomes), two are quadrivalents with a univalent, and one is a trivalent with a homomorphic bivalent. Number and location of chiasmata determine the type of configuration formed at diakinesis and MI. Partner exchange with chiasma formation in the interstitial segments will lead to branched multivalents. In the absence of chiasmata in some chromosome segments, large configurations fall apart into smaller ones.

5.3.3. *Transmission. Genetic consequences*

In organisms with predominant alternate orientation of multivalents the extra chromosome will tend to go to the pole opposite to that to which its partner goes (co-orientation). In an interchange trisomic with a normal chromosome extra, in metaphase for several reasons this chromosome is associated more frequently with its normal homologue than with the partly homologous interchange chromosome, and consequently it will most frequently go to the opposite pole. This is the pole of the interchange set of chromosomes. In an interchange trisomic of rye, 133 gametes had the interchange genome, 84 without the extra chromosome (n=7) and 49 with the extra chromosome (n+1=8). There were 181 gametes with the normal genome, of which 163 did not have the extra chromosome; 18 did have it (n+1=8). Clearly the interchange complement had received the extra chromosome much more frequently than the normal complement. The shortage of interchange gametes is explained by the lower viability of the n+1 gametes and $2n$+1 embryo's. Deviations from alternate segregation may result in trisomy for other than the original extra chromosome. This is called *trisome shift*.

Deficiencies caused by adjacent orientation of the interchange complex (section 4.5.5) can partly be compensated by the extra chromosome. This may reduce semi-sterility in comparison to normal interchange heterozygotes. In maize, a reduction could be observed from 50% sterility to only 15%-30% sterility, depending on the interchange and the extra chromosome. Of course, really balanced types are not obtained this way. When the presence of an extra chromosome by itself leads to a reduction of fertility, as

very often is the case especially in animals and in man, an improvement of fertility is not expected.

Telocentric interchange trisomics give somewhat comparable effects, with limitations like those of the normal telocentric trisomics. Although important for the analysis of chromosome pairing systems, they will not be discussed further. Nor will the scarce *inversion trisomics* be discussed.

Multiple trisomics usually do not give unexpected complications. *Tetrasomics* have genetic consequences equal to those of autotetraploids that will be discussed in section 5.6.4. Here analysis of chromosome behaviour is simpler than in tetraploids, because only one chromosome is present four times. The genetic balance, however, is disturbed even more than in trisomics, and abnormalities may well occur. *Hypoploids* (monosomics, nullisomics) are infrequently viable in diploids. Some small translocation monosomics may have a reasonable chance to survive when the material lost is of minor importance or when corresponding duplications are present. Hypoploids are of considerable importance for polyploids: they will be considered in more detail in section 5.7.7.

Euploidy

5.4. Haploids

5.4.1. *Origin*

Parthenogenetic development of unfertilized eggs results in haploid progeny of diploid individuals. In most animals haploids have a greatly reduced viability, but in certain insects (Hymenoptera: honey bees, wasps, etc.) haploidy is part of the sex-determination system: unfertilized, haploid eggs develop into males, fertilized diploid eggs into females. It is possible in the experiment, to obtain haploid animals (especially in Amphibia) by irritation of unfertilized or just fertilized eggs. This can be done mechanically or chemically.

In plants fertilization of the secondary pole nucleus and subsequent endosperm formation usually is a prerequisite for haploid

embryo development. Haploids of plants are often reasonably viable although reduced in stature. Haploids from cross-breeders usually suffer seriously from the hemizygous expression of detrimental recessives.

The frequency of spontaneous haploid formation is under genetic control: selection is possible. Cold treatment and heat-shock have some effect in increasing the frequency of haploids in Datura and maize, as have pollination with pollen of not closely related species and delayed pollination. In an experiment by Smith in 1946, the natural haploid frequency in *Triticum monococcum* appeared to be approximately 1 in 2000. By pollination with pollen of other species this could be increased to 1 in 50. Combined with late pollination even up to 1 in 5. Then, however, the total amount of seed obtained was small. In some cases (interspecific hybridization of barley) it could be made probable that the haploids obtained were secondary haploids resulting from the loss of all chromosomes of one species from an originally diploid zygote. It is not clear if this is a generally occurring mechanism.

In potato (*Solanum tuberosum*), usually considered to be a natural autopolyploid, species crosses, especially those with *S.phureja*, have been used extensively to obtain (di-)haploids, since Hougas and others in 1958 had considerable success with this method. The di-haploids can be useful in potato breeding. Using dominant seedling factors in the father makes it possible to tell the unfertilized (haploid) from the fertilized (diploid) seedlings. The fertilized endosperm that often determines the phenotype of the seed, however, has the dominant character from the father. Chase, without applying any method to increase the haploid frequency, used paternal seedling markers to recognize spontaneous haploids in cross-bred maize. The ultimate purpose was to obtain completely homozygous diploids after doubling of the chromosome number with colchicine and to use these in hybrid breeding. Since only two to eight per cent of the haploids obtained could ultimately be developed into diploid lines, there was quite a strong selection on viability and favourable gene combinations. The initial results were not encouraging, probably because with inbreeding selection is on other factors, while early generation testing is impossible when using haploids. Later attempts were more successful.

During the first generations the homozygosity and consequently the phenotypic homogeneity of the doubled haploid lines is great, but even with careful selfing it decreases rather rapidly as a consequence of spontaneous mutations, until a level of heterozygosity is reached that is comparable with that of an old inbred line. Apparently there is a balance between mutation and selection for superior heterozygotes on the one hand and inbreeding on the other.

In Datura, tobacco, rice and a few more plants it has appeared possible to grow haploids from developing pollen grains by culturing the anthers. The best stage for the initiation of the culture is around the first pollen mitosis.

In many plant species, twins contain a high percentage of haploids: a haploid synergid cell has developed together with the normal zygote. In wheat, barley, lily, pepper (Capsicum) many haploids have been isolated from twins. Around 1950 Morgan succeeded in raising the percentage of twins in Capsicum by selection until it reached a level of several per cent in some lines. The frequency of haploids increased accordingly. In this crop, that is predominantly but certainly not completely self-fertilizing, the doubled haploids appeared to be a good source of genetically improved material. The selection for fitness-characters that took place in the haploid stage appeared to fit well into the genetic system of Capsicum.

Occasionally, pollination with irradiated pollen has resulted in the development of haploid embryos.

A haploid as such can have a good phenotype: Daker in 1967 showed that the successfull small cultivar "Klein Liebling" of Pelargonium was a haploid.

5.4.2. *Chromosome pairing. Genetic consequences*

Pairing in true haploids is incomplete but usually not absent. Partly it is non-homologous pairing, perhaps between heterochromatic segments. This leads to the formation of pseudo-bivalents that can be maintained into the (usually rather irregular) metaphase I and that can even orient and segregate at anaphase I. There is also pairing between homologous chromosome segments:

old duplications that have enough homology in their pairing mechanism to associate in absence of competition and even to form chiasmata. In pachytene of rye, Levan found almost complete pairing, apparently predominantly between non-homologous chromosome segments (compare partial non-homologous pairing in structural heterozygotes). In tomato, Pennisetum and several other species similar complete pachytene pairing has been reported. Crossing-over in duplications leads to secondary structural aberrations (section 4.3.3) that have indeed been found in the scarce progeny of haploids. Usually it is difficult to distinguish with certainty between homologous and non-homologous associations in haploid diakinesis. Some authors consider all associations as non-homologous, others believe that close parallel alignment of chromosomes (side-by-side=s-s association) and end-to-end (e-e association) are indications of homology, only end-to-side (e-s association) being non-homologous. Polar organization is possible without bivalents, but the presence of at least some bivalents seems to be stimulating. The univalent chromosomes are often distributed over the two poles in a more or less random manner. The mechanism is unknown. Some may remain somewhere inbetween the poles and may split later in anaphase, contributing one chromatid to each pole. Others remain behind. There is ample opportunity for the formation of iso-chromosomes and telocentric chromosomes. Table 5.6 gives examples of the distribution of the chromosomes over the two poles, which appears to approach randomness. Sometimes more or less equal numbers are more frequent, which is perhaps caused by a beginning orientation as a result of loose association.

When two clearly distinguishable anaphase groups are not formed, a restitution nucleus can arise, containing all chromosomes. This nucleus undergoes a "second" division producing two haploid spores that may form normal haploid gametes. In haploids of *Coffea arabica* diploid seeds are formed this way with a reasonable frequency but not enough to form the normal two seeds per fruit: the fruits that develop have only a single seed. Before it was known that these trees were haploids, they used to be distinguished as a separate variety monosperma: the one-seeded variety.

Table 5.6
Anaphase I chromosome distribution in PMCs of a haploid of Datura (Belling and Blakeslee, 1927) and of rye (Levan, 1942). In rye several cells had dividing univalents; the remaining chromosomes usually having 4-2 and 3-3 distribution; these are not included in the table.

Datura	11–1	10–2	9–3	8–4	7–5	6–6	total
observed	1	3	12	19	38	27	100
expected	1	3	11	24	39	23	100
Rye			7–0	6–1	5–2	4–3	total
observed			1	11	10	19	41

Some haploids spontaneously form diploid sectors that are normally fertile.

The terminology of haploids that arise in polyploids is confusing. Sometimes the term "monoploid" is used for individuals with the basis number x, the term haploid being reserved for all cases that have half the number of chromosomes of the parent. It is not necessary to make this distinction. The potato (*Solanum tuberosum*) for instance is usually considered a natural autotetraploid with $4n = 48$. An unfertilized egg, after reduction, results in a functional diploid with $2n = 24$. There is no reason to use the term haploid or dihaploid here. In allotetraploids the term dihaploid, perhaps allodihaploid, in allohexaploids the term trihaploid is more relevant. When the genomic make-up of the allopolyploid is unknown, it is better to simply use the term haploid.

For reviews of haploidy in plants see Kimber and Riley (1963) and Magoon and Khanna (1963).

5.5. Triploids

5.5.1. *Origin*

Triploids originate from hybridizing tetraploids and diploids or by fertilization of a chance unreduced gamete by a normally reduced gamete. In plants the probability that an unreduced pollen grain can compete with a majority of reduced pollen grains is negligible. An unreduced egg-cell, however, does not suffer competition. There are exceptions: Rhoades found in 1936 a few cases

of spontaneous triploidy in maize where apparently the pollen had contributed $2n$, probably after abnormal pollen mitosis. For fertilization of the secondary pole nucleus another pollen grain must have functioned. In animals unreduced gametes from both sexes can function. In a number of Amphibia triploids have been obtained spontaneously but also by crossing tetraploid females with diploid males. Tetraploid males appear to be infertile. In plants, generally the most successful cross also is tetraploid female × diploid male, but in some species the cross does not succeed either way. This variation appears to be under genetic control. Mutants may be found that do permit hybridization between different polyploidy levels in species that normally are sterile in such crosses. Phenotypically, triploids are usually very well developed, in plants as well as in animals. In sugarbeets they are generally preferred over diploids and tetraploids, but it is not always certain that this is indeed due to triploidy as such. These triploids are usually produced by crossing unrelated, perhaps somewhat inbred tetraploids and diploids, which introduces a heterosis factor that may be more important than the ploidy level.

5.5.2. *Chromosome pairing. Chiasmata*

In respect to meiotic behaviour triploids can be considered as multiple trisomics. The great majority of observations on triploids is on plants. In the comparatively few triploid animals studied so far, chromosome behaviour was quite comparable to that in plants. Again trivalents are formed, and bivalents with univalents, in frequencies that depend on the system of pairing and on the location and number of the chiąsmata. Again exchange is reduced locally because of partner exchange. There is no genetic unbalance, however, that might reduce exchange frequencies. On the contrary, Mather concluded that in triploids of maize the number of chiasmata was greater than in the corresponding diploids. He explained this by assuming that the large number of chromosomes gave a large biochemical capacity for the formation of chiasmata. The total chromosome length available for chiasmata, however, is not increased in a triploid: only two partners can pair and have chiasmata at any place. Therefore, per paired chromosome seg-

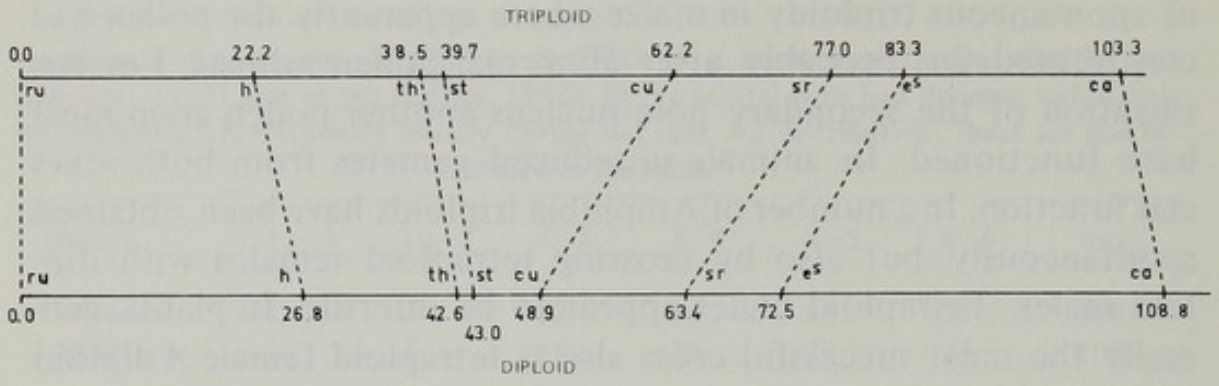


Fig.5.12. A comparison of crossing-over percentages (represented by distances in linear gene maps) in the third chromosome in diploid and triploid *Drosophila melanogaster* (According to Redfield, 1932.)

ment, more chiasmata are formed. Then, in diploids the limit to the number of chiasmata would be the biochemical system. In tetraploids the level of chiasmata per paired chromosome segment is equal again to that in the diploid. In rye and pearl millet similar relations could be found. This explanation of Mather corresponds with the explanation of interchromosome effects but remains speculative (section 2.8.4).

The greatly reduced fertility of triploids makes genetic research quite difficult. Yet Redfield (1932) succeeded in calculating several crossing-over percentages and bringing these into a map (compare chapter 6) in Drosophila triploids. Comparison with the diploid crossing-over percentages (fig.5.12) showed a reduction in some segments, accompanied by increases elsewhere in the chromosome.

5.5.3. *Anaphase I distribution*

The work by Satina and others in 1937 and 1938 on triploid Datura is probably the most complete cytogenic analysis of triploids ever done. It will be used here as an example.

Pollen mother cells and embryosac mother cells behaved differently and in neither of the two the distribution of univalents was random. Although in other cases it seemed that univalents, when included in one of the anaphase groups, were distributed more evenly than expected, in Datura groups of univalents seemed to move to one of the poles simultaneously: there was a greater

Table 5.7

Anaphase I chromosome distribution in PMCs of triploid Datura, and the chromosome numbers in pollen grains and progeny (Satina and Blakeslee, 1937). The extremes are more frequent than expected.

AI	12–24	13–23	14–22	15–21	16–20	17–19	18–18	total
% observed	0.8	4.5	8.5	14.5	22.9	30.8	18.0	1000
expected	0.05	0.6	3.2	10.7	24.2	38.7	22.6	

Pollen	12–24		13–23		14–22		15–21		16–20		17–19		18–18	total
% observed	2.6	1.2	4.0	2.6	7.2	3.8	11.0	5.0	16.4	9.2	16.0	10.8	11.2	500
expected	0.3	0.3	0.3	0.3	1.6	1.6	5.4	5.4	12.1	12.1	19.3	19.3	22.6	

Progeny of the cross $3n \times 2n$ (seed set 20%; germination 70%):	$2n$	$2n+1$	$2n+1+1$	$2n+1+1+1$	total
	58	138	79	10	285

average difference between the anaphase groups than expected (table 5.7). A similar irregular distribution may be realized when not the univalents together go to the same pole, but when a large number of trivalents have an identical orientation with two chromosomes to one pole, and the third to the other.

Only relatively few univalents were lost: on the average in the pollen mother cells in AI 0.05 chromosomes, in AII only 0.03 per cell. In the embryosac mother cells, however, these frequencies were higher: 1.1 and 1.3 respectively. The chromosome numbers in the spores are also shown in table 5.7. The normal haploid number of 12 was found in 2.6% of the microspores. These predominate in fertilization because of their better competitive ability. The severely aberrant types even do not develop. Because many deviating embryosacs degenerate and multiple trisomic embryos do not develop, there is a strong selection, as is also seen in table 5.7. It is clear that a triploid is a good source of trisomics. In most triploid plants, seed set is much lower than in this example of Datura and less trisomics are recovered. While in rye the cross $3n \times 2n$ yields predominantly trisomics with $2n+1$ and diploids with $2n$, and occasional double trisomics with $2n+1+1$, the cross $3n \times 4n$ gives predominantly progeny with chromosome numbers at about the tetraploid level: $4n-1$ and $4n-1-1$. Apparently, embryo sacs will be functional at the haploid level with one or two chromosomes in excess and at the diploid level with one or two lacking. Embryos, however, invariably abort at the triploid level, irrespective of whether they arise after fertilization of an approximately diploid female gamete by a haploid male gamete (triploid × diploid, which does not produce new triploids) or by fertilization of an approximately haploid female gamete by a diploid male gamete (triploid × tetraploid does not yield new triploids either). This is a matter of balance between embryo, endosperm and mother-plant, that plays a role also in diploid–tetraploid incompatibility. In some Amphibia, especially in salamanders, the cross $3n \times 2n$ does occasionally result in the production of a tetraploid: 3-4% unreduced gametes ($3n$) are formed that may be fertilized by haploid sperm.

There are naturally occurring triploid species of fish (carp), sala-

manders, toads and some insects, but all reproduce amictically and are always female.

The sterility of triploids is used in the production of seedless fruits. In Japan, triploid seedless watermelons are commercially available. The cross between tetraploid and diploid must be made by hand and parthenocarpic induction of the fruit, either naturally or with growth substances, must be possible. Most commercial bananas are natural, seedless triploids, reproduced vegetatively.

5.6. Higher polyploids

5.6.1. *Autopolyploids. Origin and phenotypic effects*

The origin of autopolyploids in *plants* has been considered in section 1.4.3. Nucleus and cell are larger than in the diploid, which will led to *gigas* (=giant) characteristics, provided the number of cells does not decrease proportionally. The relative dimensions of the organism change. In general, long and flat organs become shorter and thicker. Alteration in *surface–volume relationships* that result from increasing nuclear and cell size may cause problems in the diffusion of important substances and may thus have an unfavourable effect on development. Changes in *gene-dose* also lead to modifications in the biochemical relationships. When there is no strict regulation of the production or activation of enzymes, more alleles will result in more enzyme and activity will increase. For different genes, however, the relations may be different. While usually with increasing dose, activity increases also, this is not always the case. Seyffert (1959) for instance reported on a flower colour gene *f* in Silene, that conditions white flowers when fourfold recessive (nulliplex *ffff*). Simplex (*Ffff*) was dark red, duplex (*FFff*) medium red, triplex (*FFFf*) light red and quadruplex (*FFFF*) pink. Comparable is the action of a chlorophyll factor in Arabidopsis that according to Wricke increased chlorophyll content with increasing dose of one of the alleles up to triplex. With a fourfold dose, the chlorophyll content fell back again, Heterosis too can have a complex relation with gene-dose. In heterozygotes for the *Albina-7* factor in barley, heterosis is observed for plant

height and seed set. According to Ellerström and Hagberg (1967) heterosis in the tetraploid is strongest in *AAAa* and *AAaa*. These are equal and exceed *AAAA*. On the other hand, *Aaaa* is inferior to *AAAA*, while *aaaa* of course is lethal. It is clear that with such variable reactions of different genes, the original balance of the diploid is not maintained. In general, however, the disturbance caused by aneuploidy is stronger. With increasing level of polyploidy, the effect of aneuploidy gradually decreases. Fig.5.13B is a simplified and generalized graphical representation of the combined effects of polyploidy and aneuploidy on phenotypic development.

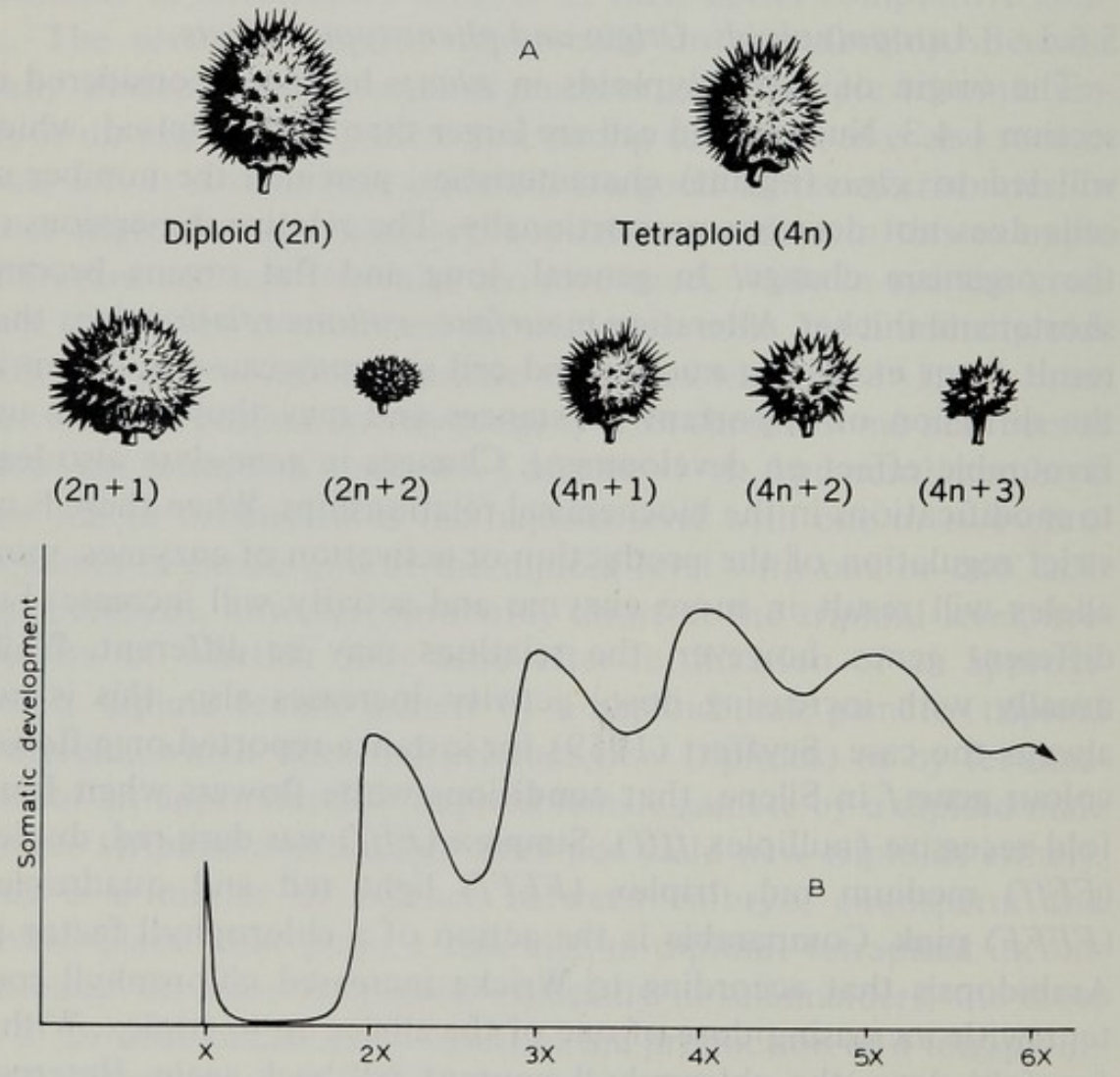


Fig.5.13. (A) Capsules of euploid and aneuploid *Datura stramonium* plants. (From: E.W. Sinnott, L.C. Dunn and T. Dobzhansky, Principles of genetics. After Blakeslee. Used with permission of McGraw-Hill Book Company.) (B) Generalized graph of the combined phenotypic effects of polyploidy and aneuploidy.

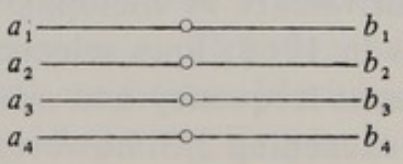
Clearly identifiable changes as a consequence of polyploidy (size of stomata, pollen grains, number of chloroplasts per cell) may sometimes facilitate the distinction between diploids and polyploids.

5.6.2. *Chromosome pairing. Chiasmata. Meiotic configurations. Fertility*

In pan-autopolyploids the affinity between the homologues is equal. With a single point of pairing initiation, only bivalents are formed as partner exchange is excluded. When there are two points of pairing initiation permitting partner exchange, each set of four homologues can form either two bivalents or one quadrivalent that includes all four chromosomes. The ratio between bivalent pairs and quadrivalents is 1:2 (table 5.8), on the condition that the points of pairing initiation are far enough apart to be

Table 5.8

Pairing configurations in an autotetraploid. Of each chromosome both arms are present four times. When pairing starts predominantly at the chromosome ends, each arm has three possibilities: a_1 pairs with a_2 (then a_3 pairs with a_4); a_1 pairs with a_3 (a_2 with a_4); a_1 pairs with a_4 (a_2 with a_3). The same is true for b. When partner exchange is free between the two points where pairing starts in each chromosome, there are a total of 9 combinations, all with the same probability of occurring. Of these, three give two bivalents (II+II) and six will give one quadrivalent (IV). When pairing initiates also in interstitial segments, more quadrivalents can be formed.

	Chromosomes: 		
	$b_1 - b_2$ $b_3 - b_4$	$b_1 - b_3$ $b_2 - b_4$	$b_1 - b_4$ $b_2 - b_3$
$a_1 - a_2$ $a_3 - a_4$	II + II	IV	IV
$a_1 - a_3$ $a_2 - a_4$	IV	II + II	IV
$a_1 - a_4$ $a_2 - a_3$	IV	IV	II + II

independent. Often, there are more points of pairing initiation with the possibility of partner exchange between them. Then more quadrivalents are formed. The restrictions on partner exchange are the same as mentioned with the trisomics (section 5.1.2): the number of multivalents will be limited even with a large number of points of pairing initiation. Here again, both pairing system and the number and location of the chiasmata determine which configurations will be observed at diakinesis and MI. For a quadrivalent to be observed at these stages, chiasmata must be present on both sides of the point of partner exchange. When this is not so, merely bivalents will be formed. Tetraploid teosinte ($4n$=40), for instance, has quadrivalents at pachytene, but only bivalents at later stages, as the chiasmata are localized on one side of the point of partner exchange. In autohexaploid Phleum ($6n$=42), where multivalents are not usually observed, the reason may be the same, or there may be a limitation in the number of points of pairing initiation: pachytene is not sufficiently accessible to decide between the two alternatives. In autotetraploid maize, usually more than 2/3 of the sets of four homologues occur as quadrivalents, so there must be more than two points of pairing initiation and more than one chiasma per pair of chromosomes. In rye, approximately 2/3 of the chromosomes are in quadrivalents suggesting that pairing initiation is restricted to the ends of the chromosomes. In rye, the frequency of chiasmata too has an effect on the number of quadrivalents at metaphase I. This was shown by Roseweir and Rees in 1962: lines selected for fertility appeared to have a higher chiasma-frequency and a higher quadrivalent frequency, the latter not exceeding 2/3, however. Thus, quadrivalent formation is determined genetically (responds to selection), but chiasma frequency is not the only factor involved. Swaminathan and Sulbha noted in 1959 that 19 generations of selection for fertility in autotetraploid *Brassica campestris* resulted in a decrease of the number of quadrivalents, although the chiasma frequency remained unaltered. In autotetraploid maize again, Gilles and Randolph reported in 1951 a decrease from an average of 8.47 to 7.46 quadrivalents per cell after 10 generations of selection for fertility, and in tetraploid barley, according to Bender and Gaul (1966) an improvement of

fertility is accompanied by a reduction of the quadrivalent frequency.

In all these cases, except in rye, quadrivalents are apparently unfavourable for fertility, as their frequency is reduced by selection. The reason is that irregular anaphase separation often follows quadrivalent formation and results in aneuploidy in the gametes. There are two causes for this irregular separation: (1) unequal orientation of the quadrivalents results in a 3:1 segregation of the four chromosomes; (2) reduction of chiasma frequency as a result of partner exchange and genetic unbalance, leads to relatively high univalent frequencies. The latter factor explains how fertility can increase with increasing chiasma frequency. This correlation between chiasma frequency and fertility has a second cause: both react positively on a general improvement of the genotype.

The exceptional case of rye needs further explanation: the combination of good fertility with frequent quadrivalent formation can be explained by the great regularity of orientation of rye quadrivalents. Although, unlike in interchange heterozygotes, the difference between alternate and adjacent orientation is not of importance for tetraploids, regular alternate orientation (especially in chain quadrivalents that are common with reduced chiasma frequencies) leads less frequently to a deviating distribution of the chromosomes.

The fertility of autotetraploids depends strongly on the frequency of aneuploids, but only when spores and zygotes have a reduced probability of normal development. This depends on background genotype and environmental conditions. Working with autotetraploid barley, Ising found in 1967 that F_1 plants with strong hybrid vigour had a better fertility than those with less or no hybrid vigour. The cause was not primarily an absence of aneuploids but a better development of the aneuploids. A tetraploid variety of rye that had only approximately 65% seed set in Sweden, had more than 90% seed set in California. Again not as a result of reduced formation of aneuploids, but as a result of the better opportunities for development of aneuploid gametes and embryos in the more favourable climate. Such an increase in fertility is entirely compensated in later generations by a strong de-

crease, as then the considerably reduced fertility of the many aneuploids strongly depresses the average of the population. In sugarbeets, where the productivity of the aneuploids often is considerably lower than that of euploids, the development of aneuploid seed is definitely undesired. In general, however, aneuploidy is not the only, often even not the main factor of reduced fertility of autopolyploids. Genetic unbalance frequently leads to deviations in one or more of the complex meiotic and postmeiotic processes, that can cause abnormal development or death of a certain percentage of the gametes or zygotes. Bosemark, in 1967, reported an instance of abnormal development of cell walls in pollen tetrads of autotetraploid sugarbeets, resulting in constriction and fragmentation of a large number of nuclei. Usually such extreme deviations are not observed, but disturbance of normal processes is common. Selection will gradually remove these abnormalities. From the work of Skiebe, for instance, in 1966 on tetraploid Primula, by Reinbergs et al. on tetraploid barley and by Gaul and coworkers (Bender and Gaul, 1966 and later) on induced mutations also in tetraploid barley, the importance of genotypic improvement for the fertility is apparent. Not only for fertility, but for all characters selection for adjustment to the tetraploid level is necessary. Only then full use can be made of the possibilities of polyploidy for plant breeding.

5.6.3. *Natural autopolyploids*

According to Müntzing, autopolyploids occur in nature in a relatively high frequency. Indeed, forms that were formerly considered to be diploids or allopolyploids are later often reported to be autopolyploids. Often this is due to the practical absence of multivalents (Phleum, teosinte) or the relatively low frequency of multivalents (*Lotus corniculatus*, *Medicago sativa*, *Anthoxanthum odoratum*) that concealed the autopolyploid nature of these species. In some natural autopolyploids multivalent frequencies are somewhat higher (the grass *Dactylis glomerata*, the potato *Solanum tuberosum*) or even rather high (*Hordeum bulbosum*). The less regular meiosis is, the more the emphasis is on vegetative reproduction. In autopolyploid forms of several insects generative

reproduction is entirely or mainly replaced by parthenogenesis as normal means of reproduction. One would expect, therefore, that one of the clearest examples of natural autopolyploidy in vertebrates, the South American frog *Odontophrynus americanus* ($4n$=44) would also reproduce parthenogenetically. According to Beçak et al. (1967), this is not the case. Here, in spite of a larger frequency of quadrivalents than bivalents, sexual reproduction proceeds normally and aneuploids are not observed. Nor is the sex differentiation disturbed. In relatives with higher degrees of polyploidy meiosis, however, is often irregular.

Several reasons have been advanced to explain the rarity of autopolyploid bisexual animals. One is that the event of an unreduced egg-cell being fertilized by an unreduced sperm is extremely improbable, while for reproduction the autopolyploid needs an autopolyploid mate, as otherwise sterility is bound to result. This makes the origin of a polyploid variety almost impossible. The second reason, that in an auto-(or allo-)polyploid the system of sex determination would break down, has been shown not to be valid. As soon as a simplex condition for the chromosome determining the heterogametic sex has been realized, the system can function normally. Female *ZZZW* mated with male *ZZZZ* in the silkworm (Bombyx) gives a normal 1:1 sex ratio (see Astaurov, 1969) and female XXXX crossed with male XXXY in the plant Melandrium does the same. The third reason is perhaps more important: one of the two sexes in spontaneous or artificial polyploids always seems to be sterile. This is not merely a consequence of abnormal chromosome segregation resulting in aneuploidy, but possibly mainly of developmental difficulties resulting from genetic unbalance.

Although scarce, polyploidy, perhaps also autopolyploidy has been detected in several sexually reproducing animals: earthworm, insects, shrimps, fishes, amphibia (see also Bungenberg de Jong, 1957). Many of these are probably secondary evolutionary products of parthenogenetic polyploids, that are far more common among animals. According to Astaurov (1969) who reported success with the same sequence of events in the laboratory with Bombyx, the process must have run as follows: From a diploid

bisexual species a diploid parthenogenetic form develops that is all-female. In this form parthenogenetic polyploid races arise that perhaps by incidental hybridization with related species produce revertants to bisexuality, but now at the polyploid level. This may also be the origin of the well-analysed tetraploid frog *Odontophrynus americanus* (see above) and other polyploid aneurans. It is well possible that in the early stages of evolutionary development of mammals, polyploidy has played a significant role (Ohno, 1970).

5.6.4. *Genetic consequences. Tetrasomy*

Even when the chromosomes are distributed regularly over the gametes, the presence of four instead of two homologues has its consequences for the genetic system. In the diploid there are three combinations of two alleles (*AA*, *Aa*, *aa*), one of which is heterozygous. In the trisomic and triploid there are four (*AAA*, *AAa*, *Aaa*, *aaa*), two of which are heterozygous. In the autotetraploid and tetrasomic there are five (*AAAA*: quadruplex; *AAAa*: triplex; *AAaa*: duplex; *Aaaa*: simplex; *aaaa*: nulliplex), and three of these are heterozygous and will show segregation in the progeny (ta-

Table 5.9
Gametic ratios in simplex, duplex and triplex of an autotetraploid, assuming 2-2 anaphase I distribution.

	Simplex	Duplex	Triplex
	1 *A* 2 *a* 3 *a* 4 *a*	1 *A* 2 *A* 3 *a* 4 *a*	1 *A* 2 *A* 3 *A* 4 *a*
Chromosome combinations, each pole receiving one set of two			
Pole a / Pole b			
1 and 2 / 3 and 4	*Aa* and *aa*	*AA* and *aa*	*AA* and *Aa*
1 and 3 / 2 and 4	*Aa* and *aa*	*Aa* and *Aa*	*AA* and *Aa*
1 and 4 / 2 and 3	*Aa* and *aa*	*Aa* and *Aa*	*Aa* and *AA*
Segregation	Simplex	Duplex	Triplex
AA : *Aa* : *aa*	0 : 3 : 3	1 : 4 : 1	3 : 3 : 0

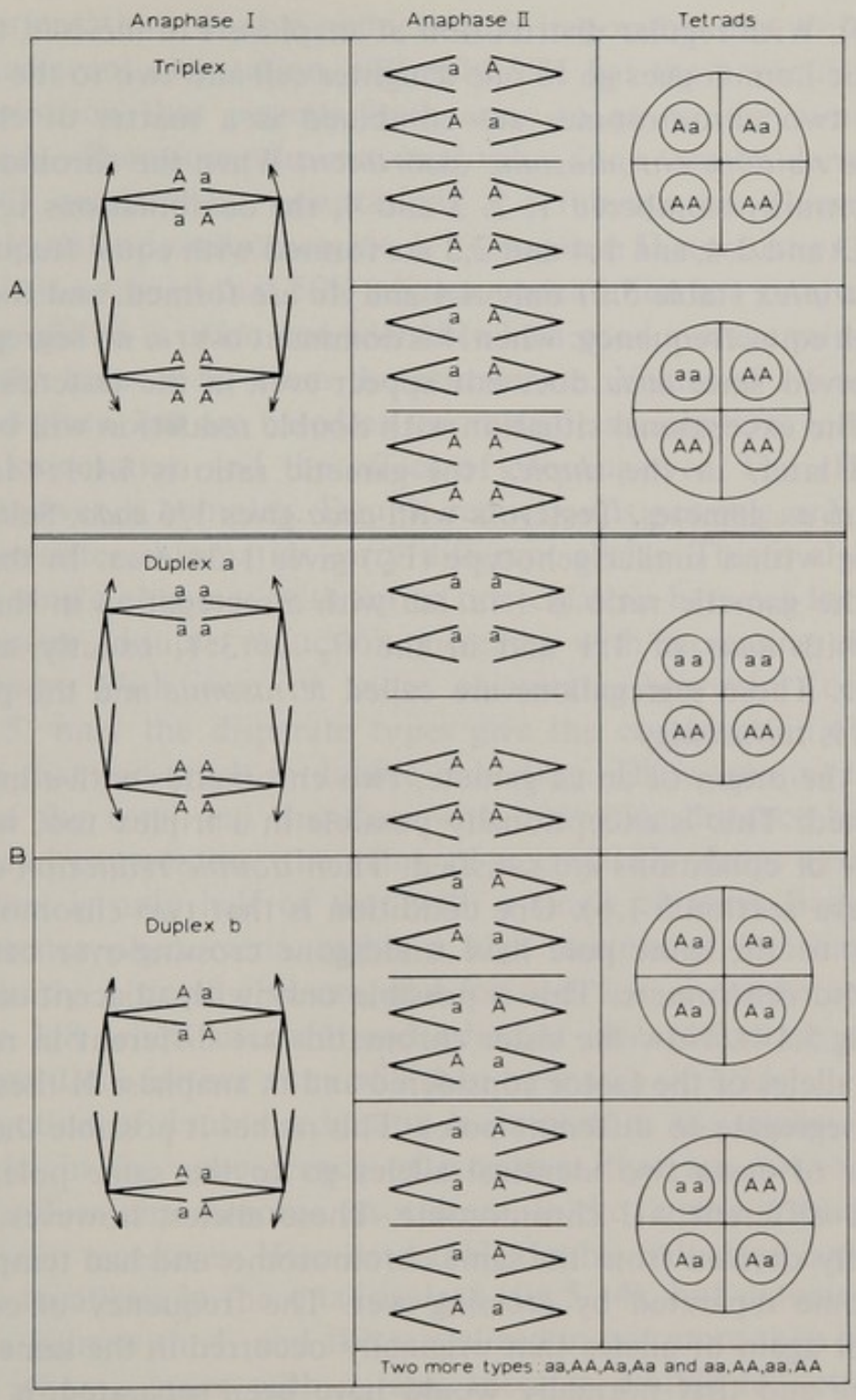


Fig.5.14. Double reduction in a tetrasomic (compare fig.5.7). (A) Triplex: the principle is the same as in a duplex-trisomic, and recessives are recovered only in one of the four tetrads. (B) Duplex: the system of pairing is important for the detection of double reduction: only when an *a* chromosome pairs with an *A* chromosome, double reduction can be detected, but this occurs simultaneously on two locations in the quadrivalent. The number of possible combinations is higher therefore than in A, and recessives can appear in two cells of the tetrad.

ble 5.9). With regular distribution at anaphase I of meiosis, two of the four homologues go to one daughter cell and two to the other. Which two chromosomes are combined is a matter of chance: there is *random chromosome assortment*. When the chromosomes are arbitrarily numbered 1, 2, 3 and 4, the combinations 1, 2 and 3, 4; 1,3 and 2,4, and 1,4 and 2,3 are formed with equal frequency. In the *triplex* (table 5.9) only *AA* and *Aa* are formed, and they occur with equal frequency: when *A* is dominant over *a*, no segregation is observed since *aaaa* does not appear even in the testcross with *aaaa*. The exceptional situation with double reduction will be considered later. In the *duplex* the gametic ratio is 1*AA*:4*Aa*:1*aa*, with 1/6 *aa* gametes. Testcross with *aaaa* gives 1/6 *aaaa*. Selfing or crossing with a similar genotype (F_2) gives 1/36 *aaaa*. In the simplex, the gametic ratio is 1*Aa*:1*aa* with a segregation in the testcross with *aaaa* of 1:1 and in the F_2 of 3:1, exactly as in a disomic. These segregations are called *tetrasomic* and the phenomenon is *tetrasomy*.

For the origin of an *aa* gamete, two chromatids with *a* must be combined. This is exceptionally possible in a triplex too, when a number of conditions are satisfied. Then *double reduction* occurs (compare section 5.1.6). One condition is that two chromosomes passing to the same pole have undergone crossing-over between locus and centromere. This is possible only with adjacent orientation (fig.5.14). Now the sister chromatids are different in respect to the alleles of the factor considered and in anaphase II these two alleles segregate to different poles. This makes it possible that in a number of cases two identical alleles go to the same pole, each from a different AII chromosome. These alleles, however, were originally derived from the same chromosome, and had temporarily become separated by crossing-over. The frequency of coming together again of alleles that originally occurred in the same chromosome and that normally would have been separated, is represented by α. This can happen to *aa* as well as *AA* alleles, but normally only *aa* gametes can be recognized, either in a testcross or in an F_2. Therefore, when considering a triplex, α equals four times the frequency of *aa* gametes: there are three possibilities of getting *AA* for one possibility of getting *aa*. With exclusively alter-

nate orientation, double reduction is impossible and $\alpha=0$. With 100% adjacent orientation, still only 50% has the particular locus in a position that permits both arms to go to the same pole (fig.5.14). Therefore, the *maximal* value for α can be derived as: 1/2 (MI quadrivalent orientation) × 1 (always one chiasma between locus and centromere) × 1/2 (anaphase II segregation) =1/4. Since it is assumed that 50% adjacent orientation is a good average a value of 1/8 is often considered to be a good average value for α when the locus is far from the centromere. Values of 1/6 and 1/7 are also given, but are based on less realistic assumptions in respect to MI orientation and the effect of crossing-over. The effect of crossing-over is complex. Double reduction is possible only when the two sister chromatids carry different alleles. This is always so with a single chiasma: with up to one chiasma between locus and centromere, double reduction increases with increasing chiasma frequency. With two or more chiasmata, however, (compare fig.2.15) only the disparate types give the combination of chromatids that can result in double reduction. These amount to only 50% of the total and therefore with increasing distance between locus and centromere, the value of α gradually drops again to a level that is only half of that with a single chiasma. Because of interference phenomena, rather a high number of loci have predominantly a single chiasma, and for such loci α can be approximately 1/8 when alternate and adjacent orientation are equally frequent. Loci further away will then have an α of 1/16.

Detection of double reduction is simplest in the triplex, where it results in the appeareance of recessives that are not expected with random chromosome assortment. In the duplex it leads to an increase in recessives. Here detectable double reduction is possible in two locations in the quadrivalent (fig.5.14), but detected only when *a* pairs with *A*, and this is realized in not more than half the cases: when *a* pairs with *a*, and there is double reduction, no deviation from chromosome segregation is observable. This introduces another variable: the four homologues do not necessarily form random pairs, i.e. there may be preference for association of specific chromosomes. If there is a preference for *a* to pair with *a* and *A* with *A*, apparent double reduction is reduced. But if *a* pairs

Table 5.10
Segregations with double reduction in autotetraploid Datura, triplex and duplex (Blakeslee et al., 1923). For *a* the deviation is significant also in the duplex: apparently the locus is relatively far from the centromere.

Parents	Dominant	Recessive	% recessive	Expected (chromosome segregation)
AAAa × *aaaa*	257	6	2.3	0.0
AAaa × *aaaa*	518	137	20.9	16.7
PPPp × *pppp*	160	1	0.6	0.0
PPpp × *pppp*	905	179	16.5	16.7

preferentially with A, double reduction will appear higher than it actually is. Hypoploidy is another reason for α to be apparently increased in triplex and duplex, when chromosomes with the dominant allele are lost. This is not of more than theoretical importance since the frequency of hypoploidy for specific chromosomes is usually too low to seriously affect the segregation ratios. Double reduction can be truly increased when adjacent orientation exceeds 50%, as is sometimes found with short and stiff chromosomes. Then theoretically α can approach 1/4.

An example of segregations with double reduction in triplex and duplex *Datura stramonium* is given in table 5.10.

5.6.5. *Crossing-over percentages*

The simplest way of calculating crossing-over percentages from recombination frequencies in autotetraploids is using the simplex with two factors in coupling phase, back-crossed by a nulliplex. Double reduction and change of partners between the two loci will not be considered. The classical example is that of de Winton and Haldane (1931) for *Primula sinensis*. S is a factor for length of the style (S: short; s: long) and G is a factor for repression of anthocyanin in the style and the ovary (G: green; g: red). The four chromosomes of the parents of the test cross can be represented as follows: $(SG)(sg)(sg)(sg)$ and $(sg)(sg)(sg)(sg)$. With a crossing-over percentage p, the following diploid gametes are expected on the

heterozygous parent, in the frequencies indicated:

$\frac{1}{2}(1-p)$	$(SG)(sg)$
$\frac{1}{2}p$	$(Sg)(sg)$
$\frac{1}{2}p$	$(sG)(sg)$
$\frac{1}{2}(1-p)$	$(sg)(sg)$

Combined with the $(sg)(sg)$ gametes of the test-cross parent, these gave the following phenotypic segregation:

SG	*Sg*	*sG*	*sg*	total
204	126	113	193	636

From this p is calculated as

$$p = \frac{126 + 113}{636} = 0.38.$$

In repulsion phase $[(Sg)(sG)(sg)(sg)]$ the situation is more complicated, as now the outcome depends on the choice of partners of the chromosomes. With random pairing, the *Sg* chromosome pairs with a *sg* chromosome (and consequently the *sG* chromosome with the other *sg* chromosome) in 2/3 of the cases. Then crossing-over does not lead to recognizable new types. The *Sg* chromosome pairs with the *sG* chromosome in 1/3 of the cases. Therefore, in 2/3 no recombination is observable even if crossing-over has occurred since the following four types of gametes are always formed when (Sg) pairs with (sg): $(Sg)(sG)$; $(Sg)(sg)$; $(sG)(sg)$; $(sg)(sg)$ each with a frequency of $2/3 \times 1/4 = 1/6$. In 1/3, *Sg* pairs with *sG* and this may lead to observable recombination. At the same time (sg) pairs with (sg) and each gamete gets a (sg) chromatid from a *sg* chromosome. With a crossing-over frequency of p, the gametic frequencies of table 5.11 are expected. The crossing-over frequencies for coupling and repulsion phase are in good agreement. In several comparable situations, including duplex segregation similar crossing-over frequencies were found. Comparison with diploid crossing-over frequencies for three factors in *Primula sinensis* again showed good agreement (table 5.12). In the diploid, there is a difference between male and female, which is apparently lost in the tetraploid. A comparison, of course, remains difficult as many irrelevant factors may have an effect.

Table 5.11

Gamete frequencies expressed in crossing-over percentages p in autotetraploid *Primula sinensis* (de Winton and Haldane, 1931). *S* long style, *s* short style; *G* green style and ovary, *g* red style and ovary. Repulsion phase, simplex with genotype *Sg, sG, sg, sg* test-crossed by fourfold recessive *sg, sg, sg, sg*.

Gametes		Frequency	Phenotype	Observed
Sg	*sG*	1/6	*SG*	126
SG	*sg*	1/6 p	*SG*	
Sg	*sg*	1/6 $(2-p)$	*Sg*	136
sG	*sg*	1/6 $(2-p)$	*sG*	146
sg	sg	1/6 $(1+p)$	*sg*	102

$$2 \times 1/6\,(1+p) = \frac{126+102}{510}\,;\; p = 0.34$$

Table 5.12

Recombination in diploid and tetraploid *Primula sinensis* (according to de Winton, compare Dawson, 1962). *S* and *G* as in table 5.11; *B*: magenta flowers, *b* red flowers.

Segment	Recombination grequencies			
	Diploid		Tetraploid	
	♀	♂	♀	♂
S – *G*	0.3329	0.4047	0.3758	0.3891
S – *B*	0.0735	0.1291	0.0801	0.0841
B – *G*	0.3115	0.3624	0.3518	0.3438

When double reduction is considered, the analysis becomes quite complicated. For this, the reader is referred to Fisher (1947), who, however, neglects some important complications.

5.6.6. *Inbreeding*

Table 5.13 shows the percentage of the population that will be homozygous (either *AAAA* or *aaaa*) in the generation indicated, when an autotetraploid is *self-fertilized*. Both simplex (which in this respect is equal to the triplex) and duplex are shown and for comparison a diploid is included. Random chromosome assortment is assumed; with double reduction, a slightly larger percent-

Table 5.13
The fraction of the population that after selfing is homozygous for one locus (either dominant or recessive), in the generation indicated, starting from the diploid heterozygote (*Aa*), the tetraploid simplex (*Aaaa*) and the tetraploid duplex (*AAaa*).

Generation of selfing	Diploid *Aa*	Tetraploid *Aaaa*	Tetraploid *AAaa*
1 (F_2)	0.5	0.25	0.05
2	0.75	0.38	0.194
3	0.875	0.493	0.326
4	0.938	0.558	0.438
5	0.968	0.648	0.531

age of homozygotes is expected. In a diploid population with *random mating*, starting from complete heterozygosity (all members of the population are *Aa*) and without selection, the next generation has a distribution of *AA*:*Aa*:*aa* types as in the F_2. This level of heterozygosity is maintained in all subsequent generations, but from the first to the second generation it was reduced from 100% to 50%. In a tetraploid, starting with a uniform duplex (*AAaa*) situation (the maximum level of heterozygosity), the first generation of random mating also results in a level of heterozygosity equal to that of the F_2, but this level is much higher than 50%. In the subsequent generations it decreases, but for a long time remains considerably higher than 50%. This is important in those cases where the level of heterozygosity is correlated with heterosis. In practice this is the case in hybrid varieties of cultivated plants. While for the maintenance of maximal heterozygosity in the diploid two homozygotes must be combined to produce a F_1 hybrid every time again, a tetraploid can maintain a very high level of heterozygosity during several generations of random mating. With the occurrence of multiple allelism (more than two alleles per locus, for instance a_1, a_2, a_3, a_4) maximal heterozygosity is even impossible in a tetraploid F_1 as at most two alleles can be combined: $a_1a_1a_2a_2$ or $a_2a_2a_3a_3$ etc. Only in later generations of random mating or planned crossing, the combination of more than two alleles can be realized, for instance $a_1a_2a_3a_3$ or even

$a_1a_2a_3a_4$. See also Demarly (1963). It is clear that also without multiple allelism, selection in autopolyploids proceeds slowly, while the stabilization of selected lines or populations, because of the continuing segregation of recessives, requires many more generations than in a diploid.

5.7. Allopolyploids

5.7.1. *Types*

In an allopolyploid the genomes of two or more different species are combined, each occurring twice. A normal, functional allopolyploid has no pairing between the genomes of the different parent species: it is restricted to the pairs of identical genomes. Like in a diploid, only bivalents are formed. Although the pairing-differentiation between the parent species must be complete for an allopolyploid to function properly, the difference in gene content may vary from very slight to considerable. A very high degree of genetic differentiation occurs in the cell hybrids that combine two mosquito genomes and two human genomes, as realized by Zepp et al. (1971). Somewhat less remote are the genomes in the hybrids of mouse and human cells (Ephrussi and Weiss, 1969). Even in cell culture, however, these allopolyploids are unstable: the human chromosomes are successively eliminated (see also section 6.3.2). Such hybrids are efficient tools in cytogenetic experimentation. Less extreme combinations often are more stable, but even when closely related species are combined, selection is usually necessary to yield well adapted types.

Allopolyploidy is one means of combining the genes from different species. The combination of the genotypes of two species into a new form can take place in two more ways: one is introgression, in which one species receives a number of genes from another species without loosing its general identity and without doubling of the chromosome number. The other is the origin of new species, separated from both parents by a sterility barrier, again without chromosome doubling. In plants allopolyploidy, directly leading to fertile forms separated from both parents by a sterility

barrier, has appeared to be the most efficient way of combining two existing species into a new one. In animals successful new allopolyploids are not known, but several polyploids in fishes, amphibia and insects are suspected to be allopolyploids, some with asexual reproduction. Besides genetic adaptation to the environment, a requirement for the success of allopolyploids is an effective restriction of the affinity between the chromosomes of the parent species. When there is only little difference in affinity between identical (homologous) chromosomes and those of the parent species (homoeologous chromosomes), the meiotic behaviour is similar to that in a hemi-autopolyploid. Then there is a mechanism for substituting the chromosomes of the different species for each other, and even multivalents may be formed. With even a slight tendency for inbreeding, there will be an increasing opportunity for all corresponding chromosomes to be of the same type: the autopolyploidy-character will predominate. Because of recombination, however, such a new functional autopolyploid will not resemble either of the parents, but will be of a new genotype.

When the difference in affinity between homologous and homoeologous chromosomes is large enough to make chromosomal substitutions infrequent, natural selection may effect a predominance of the allopolyploid character. The differentiation between the genomes is not necessarily equal for all chromosomes: some may have strong affinity to their homoeologues, others only a weak affinity. This may lead to *segmental allopolyploidy*: some chromosomes of the genome behave as in an autopolyploid, others as in an allopolyploid (Stebbins, 1947). When the autopolyploid character is not clearly expressed as a result of scarcity of multivalents, presence or absence of segmental allopolyploidy cannot be decided on cytological grounds. Only the occurrence of tetrasomic segregation for genes in some chromosomes and disomic segregation for genes in other chromosomes of the same genome, is a reliable criterion. It should be noted, however, that even with complete autopolyploidy some genes may show a 35:1 segregation and others a 3:1 segregation: the first is duplex, the latter simplex. Therefore, segregations are a reliable criterion only when the genotype of the polyploid to be tested is known. Convincing indications of segmental allopolyploidy are quite scarce.

Another combination of auto- and allopolyploidy is possible when more than four genomes are combined, as for instance in a hexaploid. Now four may be entirely homologous, the other two being sufficiently differentiated from the first four not to pair with them. When the four identical genomes are represented by A, the two others by B, this *auto-allopolyploid* can be represented by AAAABB. Transitions such as AAA′A′BB are cases where normally there is no pairing between A and A′, but where under certain conditions A-chromosomes can pair with A′-chromosomes. Such conditions may be: abnormal genetic or environmental situations or, more generally, when only one A and one A′ genome are present. Then there is no competition from complete homologues and related homoeologues may be able to associate with each other: the *polyhaploid* AA′B for instance, may have AA′ bivalents. An old example is that of Ljungdahl of 1924: the F_1 of *Papaver nudicaule* ($2n$=14) with *Papaver striatocarpum* ($2n$=10x=70) formed bivalents up to a maximum of 21 from the available 42 (=7+35) chromosomes. Seven of these bivalents arose from pairing between nudicaule and striatocarpum chromosomes, which is called *allosyndesis* since chromosomes of different species are involved. The remaining 14 bivalents were due to pairing between the striatocarpum chromosomes themselves: *autosyndesis*. The conclusion was that *P.striatocarpum* has one genome (C) in common with *P.nudicaule*, and further has four genomes four times each, but with some differentiation. The total of 70 chromosomes of *P.striatocarpum* may thus be represented as: AAA′A′ BBB′B′ CC. In perfect alloploids too, where no bivalents are formed in the polyhaploid, there are nevertheless clear differences in homology between the different genomes. Wheat, *Triticum aestivum*, for instance, is a good allohexaploid with $2n$=6x=42 and the genomes A, B and D, derived from *Triticum monococcum*, *Aegilops speltoides* (= *Triticum speltoide*) and *Aegilops squarrosa* (= *Triticum squarrosum*), respectively. The B and D genomes are much more closely related to each other than to the A genome (compare section 5.7.5), but do not form bivalents in the polyhaploid under normal conditions.

5.7.2. *Diploidization*

Pairing differentiation often goes parallel with genetic differentiation. Corresponding loci on homoeologous chromosomes may have different alleles, or even genes with a different function. How frequent this occurs depends on the difference between the parent-species and is increased continuously by mutation in the allopolyploid. For many genes duplication is not an advantage, and a slightly modified function of part of the loci may result in an improvement of the fitness of the organism. Because by such modifications the duplication character of the polyploid is gradually lost and converted into a situation resembling that in a diploid; the process is called *diploidization*. In a new allopolyploid, segregation of recessive mutations is usually not observed, as a result of the presence of homoeologous loci that have remained homozygous dominant: the allopolyploid seems to be very resistant against mutations. With proceeding diploidization, however, the number of duplicated loci decreases, and more recessive mutations will come to expression. Mutagenic treatment itself can promote diploidization: with repeated treatment, the frequency of visible mutations appears to increase. Between different allopolyploids there is a difference in degree of diploidization. Wheat (*Triticum aestivum*, $2n=6x=42$) is less far diploidized than oats (*Avena sativa*, $2n=6x=42$), which is apparent from the lower frequency of visible mutations in wheat. The difference in degree of diploidization is also demonstrated by a difference in *tolerance for aneuploidy*: more diploidized allopolyploids are less tolerant to aneuploidy, especially nullisomy. Further, the ability of *homoeologous chromosomes to substitute* for each other is (negatively) correlated to the degree of diploidization.

5.7.3. *Genetic regulation of chromosome pairing*

The fact that homoeologous chromosomes with such a close relationship that mutual substitution is possible to a considerable degree, still do not have any tendency to pair, was formerly explained by assuming that structural differences prevented pairing. When it appeared that only exceptionally a clear structural differentiation could be observed, it was assumed that *cryptic structural*

differences (very small translocations, inversions, duplications, deficiencies) were responsible for the differentiation. This view too was abandonned when it appeared that a rather simple genetic system could sometimes regulate chromosome pairing. In some cases, for instance bread wheat (*Triticum aestivum*), the organism best studied in this respect, a single major factor with a number of modifying factors can be distinguished. Such genetic systems, however, cannot operate without a basis of differentiation that resides inside the individual chromosomes and that is actually merely reinforced and made operational by an overall genetic system. The nature of this basic differentiation is not well understood. It is supposed that long distance attraction between chromosomes is usually realized by a limited number of rather specialized loci (zygomeres). In different (related) species during evolution different zygomeres would have assumed the dominant activity. In the hybrid, pairing is then still possible but not as efficient as in either of the parents. In the amphidiploid (=doubled hybrid, the new allopolyploid) multivalent formation is possible when the pairing differentiation is not complete, but there is a much stronger tendency for bivalents to be formed (between the two completely homologous chromosomes) than in an autopolyploid. Such incipient differentiation can be reinforced by the genetic background.

Simultaneously (1958) but independently, Sears and Okamoto in the United States and Riley and Chapman in England found that in hexaploid wheat the absence of chromosome 5 of the B-genome resulted in pairing between homoeologous chromosomes. Sears and Okamoto observed multivalents in nullisomic 5 B diploids, Riley and Chapman found bivalents and occasional trivalents in haploids that lacked the 5 B chromosome. Apparently this chromosome contains a dominant factor that reduces the tendency to pair to such an extent that the inefficient homoeologous pairing cannot be realized in its presence.

It appeared later that this factor is situated in the long arm of the 5B chromosome ($5B^L$), closer to the end than to the centromere. Riley and coworkers succeeded in inducing recessive mutations that partly suppressed the action of this factor.

The B genome of wheat presumably originates from *Aegilops speltoides*. The wild genome of *A. speltoides*, however, appears not to have this action. On the contrary, when wheat is hybridized with *A. speltoides*, the action of the (single) remaining wheat 5B chromosome is overruled by that of the wild 5B chromosome and pairing between homoeologues is observed. Thus the pairing factor on 5B has a definite activity that is not observed when the factor is absent or mutated, but it is recessive or hypostatic in respect to the wild type. This property of the wild *A. speltoides* genome can be used for the induction of homoeologous pairing and crossing-over between the chromosomes of wheat and those of other species (sections 5.7.5, 5.7.12).

According to Feldman et al. (1966) the $5B^L$ gene affects somatic pairing in premeiotic mitosis. With somatic pairing, homoeologues and homologues come to lie in each other's vicinity and when meiotic pairing starts, homoeologous associations get a chance to be realized. The $5B^L$ gene suppresses somatic pairing and the relatively inefficient attraction between homoeologues is insufficient to bring homoeologous chromosomes close enough together in the short time that is available for meiotic pairing.

A great variety of genetic factors has been described that affect pairing and crossing-over, some as modifiers of the 5B gene. See also Riley and Law (1965).

The genetic system that in wheat regulates the affinity between different genomes through a reinforcement of the chromosomal differentiation may occur in other allopolyploids also, but it has nowhere else been analysed on a comparable scale. Besides such a system and possibly acting simultaneously, undoubtedly other systems have developed.

5.7.4. *Allopolyploidization of autopolyploids*

In some respects the polysomic genetic system of autopolyploids has disadvantages in comparison to the disomic genetic system of allopolyploids. This may not only be important in natural situations, but also in plant breeding. Some consequences of the difference in genetic system have already been mentioned: lower fertility, slower reaction to selection, and a long period of stabi-

lization in autopolyploids, and gradual, although retarded loss of heterozygosity on inbreeding (compare table 5.13) On the other hand, autopolyploids have more flexibility in respect to recombination when outbreeding. Because of the advantages inherent to the disomic genetic system, it has been attempted to convert autopolyploids into functional allopolyploids by adjusting the chromosome pairing system. The introduction of a system of genetic reinforcement of differentiation, such as exists in wheat, of course is not effective as long as there is no pre-existing chromosomal differentiation. Nor is it of any use to prevent multivalent formation as such: the polysomic genetic system does not depend on multivalent formation but on the exchangeability of the homologous chromosomes that is based upon the random pairing between all homologues. This is possible with bivalents as well as with multivalents.

Very little is known about the small differences in affinity between homologous chromosomes that are probably generally present even within species and that can theoretically be used as a start for a chromosomal differentiation. More is known about the effect of heterozygous chromosomal rearrangements in polyploids. Their effect appears to be strongly dependent on the system of pairing of the chromosome involved. In 1961, Grell showed that inversions in triploid Drosophila reduced pairing between inversion and normal chromosomes when two normal chromosomes were present together with one inversion chromosome. With seven inversions the differentiation was complete. Similarly, Shaver in 1963 observed preferential pairing in tetraploid maize, duplex-heterozygous for an inversion; the two inverted chromosomes and the two normal chromosomes paired together more frequently than expected on basis of randomness. In tetraploid maize–teosinte hybrids the same inversion was quite effective in inducing preferential pairing, even more so than expected on the basis of an additive effect of the inversion and the reduced affinity between maize and teosinte chromosomes. It appears that in other organisms chromosomal rearrangements do not have clear differentiating effects. Walters and Gerstel, for instance, found no indication for preferential pairing between identical chromosomes in tetra-

ploid *Rhoeo discolor*, although each chromosome was involved in a translocation (section 4.5.9). The reason is that in such organisms the initiation of pairing is restricted to very small regions. As long as there is no rearrangement within this region, it operates equally well in attracting rearranged and unaltered chromosomes.

The best opportunities for changing the polysomic system into a disomic system probably are in a combination of special chromosomal rearrangements with selected affinity differences (Sybenga, 1969). Practical results have not yet been obtained.

5.7.5. *Genome analysis*

The purpose of genome analysis is the identification of the diploid species that have contributed their genome to the allopolyploid. The problem arises that neither the diploid ancestor nor the allopolyploid have necessarily remained unaltered since the origin of the allopolyploid. The latter has usually experienced a decrease of genetic duplication (diploidization). The first step in genome analysis is the selection, on the basis of morphology, histology, anatomy, biochemistry, of the species that are the best candidates. Anderson's (1949) method of "*extrapolated correlates*" can give quite good results on the basis of plant morphology and anatomy only. Biochemical methods can improve the exactness of the method. Johnson (1967) for instance, found that the proteins of the suggested parents of *Aegilops cilindrica (A. squarrosa* and *A. caudata)* in mixture gave the same electrophoretic pattern as those of *A. cilindrica* alone.

The next step is the analysis of *chromosomal homologies.* The allopolyploid to be analysed is crossed with the putative ancestor to see if the chromosomes of one or more of the genomes of the allopolyploid will pair with those of the parental species. This method has been in use since the early twenties. When there are several species that are eligible, the one with the most extensive chromosome association is considered the true ancestor. The test of the analysis is the artificial *resynthesis.* When synthetic and natural allopolyploid resemble each other closely and have a fertile F_1, it may be assumed that the analysis was correct.

Besides checking the analysis, artificial resynthesis has two more

purposes: (1) to increase the genetic variability of the old allopolyploid by hybridization with the artificial one, which can be of considerable importance for plant breeding; (2) to detect possible alterations in the ancestors and the allopolyploid since the formation of the latter.

An old example of genome analysis is that of tobacco, *Nicotania tabacum* ($2n=4x=48$) by Goodspeed and Clausen in 1928. On the basis of morphological information it was suggested that *N.sylvestris* ($2n=24$) and *N.tomentosa* ($2n=24$) might be the ancestors. The F_1 of *N.tabacum* with *N.sylvestris* had 12 bivalents and 12 univalents in meiosis. The F_1 with *N.tomentosa* also. It could be concluded that *N.tabacum* had one genome in common with both diploid species. This was not the same genome, since the F_1 of the two diploids had 24 univalents and only infrequently a bivalent. After doubling the chromosome number of the diploid hybrid, a plant was obtained that closely resembled *N.tabacum*. Although producing apparently fertile pollen, no seed was formed: the embryosac development was abnormal. Selection for fertility in genotypes that produced at least some seeds, led to improved fertility. It appeared later that the species *N.tomentosiformis*, related to *N.tomentosa*, did produce amphidiploids that were immediately fertile. Another species, *N.otophora*, however, did the same. Therefore one of the latter two species, combined with *N.sylvestris*, is the probable ancestor of tobacco. Cytological differences (translocations, inversions etc.) between the new amphidiploid and the established allopolyploid were not found. Genetic differences, however, were rather frequent: natural tobacco has already gone through an extensive process of genetic diploidization.

In wheats, Kihara started genome analysis. Three stages of polyploidy are distinguished: the A genome occurs in the diploid monococcum or "Einkorn" group. The A and B genomes together are found in the tetraploid "Emmer" group, which includes the hard wheats, *Triticum durum*, used for macaroni. The hexaploid group has the A, B and D genomes (D is derived from "Dinkel", spelt), to which, in addition to the spelt-wheat, the bread wheat, *T.aestivum*, belongs. The origin of the D-genome was analysed by McFad-

den and Sears. They crossed tetraploid wheat species with *Aegilops squarrosa* and doubled the number of chromosomes. A spelt-like form with 21 bivalents was produced that upon hybridization with normal hexaploid wheat gave fertile hybrids with good chromosome pairing. On the basis of Anderson's method of "extrapolated correlates", combined with karyotype analysis, *Aegilops speltoides* was the best candidate for the B-genome: the properties that did not occur in *T.monococcum* (the A-genome) could be found in only one section of the genus Aegilops and in this section *A.speltoides* has most of the characteristics of the tetraploid wheats. Although analysis of chromosomal homology and resynthesis confirmed the conclusion (Riley et al., 1958), some doubt remains.

Genome analysis has been carried out with success in several more natural allopolyploids, especially crop plants: cotton (Gossypium), several Brassicas, where sometimes the relationships between different species were elucidated. A well-known example of the possibly recent origin of a natural allopolyploid is the grass *Spartina townsendii* ($2n$=126), originated on the English coast as a tetraploid hybrid between *S.alternifolia* ($2n$=70) and *S.stricta* ($2n$=56), and so successful that it has spread over a wide area in a few decades.

In genome analysis the main interest is in close relationships that are expressed in good pairing and chiasma formation. When one is interested in the analysis of more remote relationships where asynapsis and desynapsis may result from reduced affinity and from genetic unbalance, a more refined analysis of bivalent formation is required. The purpose may then be to determine the number of chromosomes that in principle are capable of pairing. Gaul in 1959 developed a formula that makes it possible to estimate the number of chromosomes that in principle are able to pair, using the relation between chiasma frequency and number of bivalents:

$$p = \frac{X^2 + X - B}{(2X - B)C}.$$

Here X is the number of chiasmata, B the total number of paired chromosomes and C the number of cells analysed. With this

method Gaul could demonstrate that in the hybrid of *Triticum aestivum* ($2n$=42) and *Agropyron intermedium* ($2n$=42) there is a relationship of all three genomes of wheat with corresponding genomes of *Agropyron*, sufficiently close to make pairing in principle possible.

In order to study the relationship between the three genomes of wheat, Riley and Chapman (1966) used hybrids with *Aegilops speltoides*, that enabled homoeologous chromosomes to pair (compare section 5.7.3). Each of two homoeologous chromosomes were replaced by one telocentric and were recognizable as such. The frequency of pairing between the two homoeologous telocentrics varied depending on the genomes from which they were derived: a telocentric of the B genome paired reasonably well with its homoeologue of the D genome, but much less frequently with the homoeologue of the A genome. This appeared to be the rule and it could be concluded that at least in respect to pairing affinity the B and D genomes are closer related to each other than to the A genome.

Artificial allopolyploids (amphidiploids) without natural parallel have been produced already in the 1920s. The classical example is Raphanobrassica ($2n$=36), composed by Karpechenko by doubling the hybrid between *Raphanus sativus* ($2n$=18), the radish, with *Brassica oleracea* ($2n$=18), to which almost all varieties of cabbage belong. In the thirties, it was tried to combine the properties of wheat and rye into a new grain crop by synthesizing the amphidiploid between the two species. The resulting octoploid ($2n$=56) was named Triticale and contained two genomes of hexaploid wheat (*Triticum aestivum*, $2n$=42) and two of rye (*Secale cereale*, $2n$=14). Even after selection for the most fertile types, produced from specially selected parent lines, stability and fertility could not be improved satisfactorily. The hexaploid Triticale ($2n$=42), however, the amphidiploid between *Triticum durum* ($2n$=28) and other tetraploid wheats with rye, has been tried subsequently. Although the seed is not favourable for bread making, it has good qualities as a feed grain. Stability and fertility were significantly better than in octoploid Triticale, and it has some application in Hungary and in Canada.

5.7.6. *Genetic consequences. Deviating segregations*

Allopolyploids with a regular "diploid" meiosis have a completely disomic genetic system. Duplicate factors (polymery) are frequent, giving a 15:1 segregation when both are heterozygous; 3:1 when one is heterozygous and the other homozygous recessive, and no segregation when at least one is homozygous dominant. Auto-allopolyploids and segmental-allopolyploids have tetrasomic segregations in addition to disomic segregations. Incompletely stabilized amphidiploids may have *heterogenetic* association between homoeologues besides *homogenetic* association between homologues. In addition to disturbance of meiosis as a result of multivalent formation, this may lead to abnormal segregations, sometimes approaching those of tetrasomic inheritance. In synthetic amphidiploids of Gossypium and Nicotania that were expected to have disomic inheritance like the natural allopolyploids, Gerstel and others (1958) found several unexpected segregations, apparently a result of incomplete differentiation of the homoeologous chromosomes.

5.7.7. *Aneuploids in allopolyploids*

Trisomics are the aneuploids that are used most frequently for cytogenetic studies in diploids. There monosomics and nullisomics are viable only in exceptional cases. In allopolyploids, however, monosomics and even nullisomics may be reasonably viable and fertile, and offer special opportunities for chromosome manipulation and chromosome "engineering". They have had wide application in wheat (see for instance Sears, 1969). While often the monosomics, especially in allohexaploids show only a limited morphological deviation from normal, the nullisomics, as far as they are vital, generally have a characteristic phenotype. They usually are much less fertile than monosomics. Especially in wheat these hypoploids have been extensively studied and applied. All 21 have been isolated, for the first time by Sears in the variety Chinese Spring. In allotetraploid tobacco most monosomics could be isolated, but none of the nullisomics are viable.

Origin. There are several modes of origin for monosomics. (1) Spontaneous in a low frequency as a result of non-disjunction

Table 5.14
The average chromosome constitution in the progeny of wheat monosomics (Sears, 1954).

♀ \ ♂	$n = 21$ 96% (90 – 100)	$n - 1 = 20$ 4% (0 – 10)
$n = 21$ 25%	$2n = 42$ 24%	$2n - 1 = 41$ 1%
$n - 1 = 20$ 75%	$2n - 1 = 41$ 72%	$2n - 2 = 40$ 3%

during mitosis or meiosis. (2) Through non-disjunction induced by ionizing radiations and chemical treatment of somatic tissues. This leads to mosaics. (3) In meiosis of translocation heterozygotes. (4) As a result of asynapsis or desynapsis, determined genetically or caused by environmental extremes (drought, heat). Nullisomy for chromosome 3B in wheat results in appreciable asynapsis: this is the most important source of monosomics in wheat.

Nullisomics usually do not arise directly: they are obtained by self-fertilization of monosomics and are found together with disomics and monosomics (table 5.14). Although theoretically a 1:2:1 segregation might be expected, the actually observed ratios depend on the degree of univalent loss at meiosis, and on the relative competitiveness of the mono- and nullisomic gametes and di-, mono- and nullisomic zygotes and embryos. Loss of univalents increases the number of nullisomics. On the other hand, reduced vitality and especially the reduced competitive abilities of pollen lacking a chromosome reduces the chance of a nullisomic to be formed. This is demonstrated in table 5.14: only very few pollen grains lacking a chromosome will function (4% of the total). Since many univalents are lost and egg cells with a chromosome less are not seriously at a disadvantage, the combination of euploid pollen with hypoploid egg cells resulting in monosomic off-spring is the most frequent. The data of table 5.14 are averages: individual chromosomes can vary considerably.

A complication in the maintenance of monosomics is *univalent shift* (Person, 1956). Some monosomics may induce partial asyn-

apsis, leading to loss of chromosomes other than the original monosomic. If this happens to result in a monosomic zygote in which the originally monosomic chromosome by chance is present (24% according to table 5.14), then this plant will be classified as monosomic, but it concerns another chromosome than the original one. Univalent shift makes it necessary to make a regular check of the monosomic used.

Usually the single chromosome of a monosomic is univalent in meiosis. In some allopolyploids of which tobacco is an example, trivalents are formed as a result of homoeologous pairing. In wheat, trivalents are usually not found but in tobacco 25% of the pollen mother cells contain a trivalent when some chromosomes are monosomic. The frequency is lower for others. The loss of univalents, however, is greater in tobacco than in wheat. Since it is a tetraploid, the effect of monosomy is more pronounced and the different types are morphologically distinct.

Table 5.15
The female gamete composition of monosomic 5A of wheat, tested on 785 plants in the progeny of the cross mono 5A × normal (Sears, 1954).

Chromosome constitution	Number of progeny	%
n = 21	129	16.4
$n-1$ = 20	628	80.0
$n-1$ + telo = 21	16	3.6
$n-1$ + iso = 21	11	
n + telo = 22	1	
	785 = total	

Like in trisomics, deviant chromosomal types (telos, isos) arise as a result of centromere mis-division (fig.5.6). The two types of telocentric and the two types of iso-chromosome are formed with different frequencies for different chromosomes. An example is given in table 5.15.

5.7.8. *Identification of homoeologues*

Combination of nullisomy for one chromosome with tetrasomy

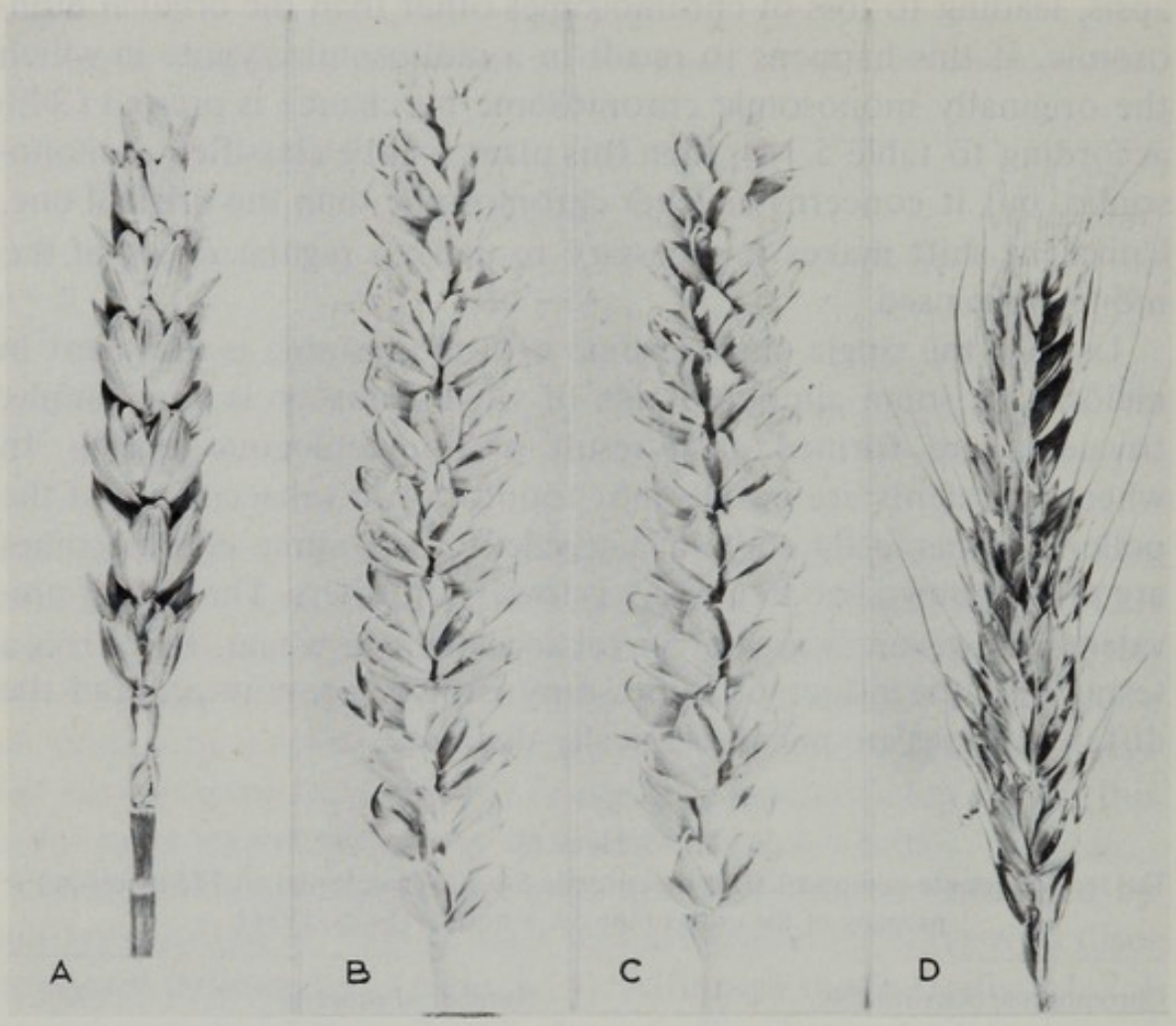


Fig.5.15. Nulli–tetra compensation in wheat. (A) nulli 2D; (D) tetra 2A; (B) normal wheat; (C) nulli 2D–tetra 2A. The close similarity between B and C demonstrates the close relationship between the chromosomes 2A and 2D.

for another will restore the normal chromosome number, but not the genetic balance and the normal phenotype will consequently not be recovered. In 1944 Sears observed that in wheat tetrasomy for specific chromosomes could to a great extent compensate for nullisomy for specific other chromosomes. Apparently homoeologous chromosomes were involved (fig.5.15). By making all possible combinations of nulli- and tetrasomics and selecting those where compensation occurred, Sears could classify all seven groups of 3 homoeologous chromosomes.

The next step was to find out which seven chromosomes together formed one genome. For this purpose, the hybrid between a monosomic and one of the ancestral diploid species was made.

The monosomic hybrids were analysed cytologically. If a bivalent less than the expected number (seven in wheat) was formed, the chromosome involved should correspond with a chromosome of this species. If the chromosome belonged to another genome, all seven bivalents should be present. By crossing the entire set of monosomics with two of the three parental species all chromosomes could be assigned to their genome.

A somewhat different method was used by Sears and Okamoto in 1957 in order to distinguish the chromosomes of the A genome from those of the B genome. Telocentric chromosomes were used. Plants with 20 chromosome pairs and a univalent telocentric of the A or of the B genome were crossed with the artificial amphidiploid of *T.aegilopoides* × *Aegilops squarrosa* (the genome composition of this amphidiploid is A′A′DD, as the genome of *T.aegilopoides* is closely related to the A genome of wheat). In the F_1 thus produced, the telocentric would give a heteromorphic bivalent if it had been derived from a chromosome of the A genome. It would remain univalent if it would be a B genome telocentric.

5.7.9. *Chromosome addition*

Addition of an extra chromosome derived from a different species is not restricted to allopolyploids but certainly it has been realized and used here more frequently than in diploids and autopolyploids. Originally the purpose was the introduction of characters that could not be introduced simply by hybridization. Positive results, however, have not been obtained with addition alone. It is presently used only as an intermediate step in other techniques for the transfer of chromosomal material from one species to another or for genetic and cytological studies. In order to effect addition, usually an amphidiploid (AABBCC) between the donor CC and the receptor AABB is made first and subsequently back-crossed to the receptor AABB with continuous selection for the presence of the chromosome that has to be transferred. Finally, one generation of selfing is necessary to establish disomy for the addition chromosome that has remained monosomic during all generations of back crossing. This last step may be difficult when transfer through the pollen is reduced.

5.7.10. *Chromosome substitution*

The substitution of one pair of chromosomes of an allopolyploid by a homologous or homoeologous pair from another variety of the same species, or from another species, respectively, has had more direct application in plant breeding and in experiments, than addition. Substitution lines are derived from addition lines by crossing the latter with the nullisomic or monosomic for the homo-(eo)logous chromosome. When using the monosomic, selection in the F_1 of plants that lack the chromosome to be substituted, is required. When substituting a pair of wheat chromosomes by a pair of rye chromosomes, one first crosses a rye-to-wheat addition line with a wheat monosomic to obtain plants with 42 chromosomes, 41 of wheat and one of rye. In meiosis, this gives 20 bivalents and two univalents: one the single rye chromosome and the other the monosomic wheat chromosome. Selfing results in progeny with only wheat chromosomes and other undesired types, but also in plants with 40 wheat and two rye chromosomes. Such substitution lines are usually not well balanced and have only a limited usefulness: they can be used to study homoeologies (by their capacity of compensation) or to study the specific effects of certain chromosomes. A few have had practical application.

Chromosome substitution between different varieties of the same species is presently of more direct interest. The technique of substitution is somewhat more complicated because the chromosome to be substituted and the substituting chromosome can pair, and exchange is possible but should be avoided. Therefore, the receptor variety must be monosomic for the chromosome that is to be replaced. If it is not, it can be made so by repeated back-crossing of a standard monosomic line to the receptor variety, selecting the monosomics in each back cross generation. When it is possible to use a *nullisomic*, the procedure of substitution is relatively simple. In wheat, this is possible for instance with chromosomes 7B and 7D, because of the reasonable fertility of the nullisomics. Now first the nullisomic is crossed with the donor variety: the F_1 is monosomic and the single chromosome is that of the donor. There is no homologue to recombine with. Next, a series of back-crosses is carried out with the nullisomic receptor, which is

the recurrent parent. The donor chromosome remains single in each generation. Occasional nullisomics are recognized and can be eliminated: they lack the donor chromosome. After about six generations of back-crossing the genotype of the receptor is considered to be completely restored and then the monosomic is selfed to obtain substitution plants.

Usually, nullisomics are not sufficiently fertile to be the recurrent parent. Then *monosomics* can be used, but this makes the procedure more tedious. Again in the first cross the (monosomic) receptor is the mother. The father is the donor of the substitution chromosome. In the monosomics of the F_1 the single chromosome necessarily is the paternal chromosome: the mother has made no contribution. These F_1 monosomics must often be selected cytologically since they are generally not morphologically sufficiently distinct. When it is certain that pollen lacking this chromosome cannot function, this F_1 can be used as the father in the next cross: the pollen then necessarily has the donor chromosome (there is no other). The mother is the recurrent monosomic receptor. The monosomics in this first back cross generation must again be selected: they contain only the donor chromosome. This procedure can be repeated until the receptor genotype is restored except for the single substitution chromosome. Selfing yields the disomic constitution.

Since it is clear from table 5.14 that a certain fraction of pollen lacking a chromosome can function, this procedure may not be very reliable. Monosomics isolated in the back-cross of the receptor monosomic with the F_1, may then occasionally be the result of a combination of normal egg cells with pollen lacking the chromosome. These monosomics do not have the donor chromosome but the (single) chromosome of the receptor and they are therefore useless. They cannot, however, be easily distinguished from the right types with the (single) chromosome derived from the donor. It is necessary, then, to self the F_1 monosomics in order to obtain disomic F_2 plants that have both donor chromosomes. These can be used as father since all their pollen contains the donor chromosome. The back-cross of these F_2 plants with the recurrent receptor yields monosomics with a single donor chromosome

and without doubt about its origin. These, however, must again be selfed to produce disomics that can be used as father. Thus each back-cross generation must be followed by a generation of selfing: the number of generations required is twice that necessary when the nullisomic can be used as recurrent parent. In addition, a cytological check on monosomy is necessary in each generation.

In all cases, a check on univalent shift is required after the last back-cross.

There is a third method, simpler than the second, but which requires the use of marked chromosomes: *telos* or *isos* in the recurrent parent. These are available for almost all chromosomes of wheat, but not for those of most other allopolyploids. The main advantages are that the fertility of the telocentric monosomics, although not always as good as that of normal monosomics, is better than that of nullisomics, since at least one of the arms is present. The F_1 between the mono-telo of the recurrent receptor and the donor consists of two groups: monosomics having a single normal chromosome of the father and others having the same normal chromosome of the father and in addition the telocentric chromosome of the mother. Only the first category is retained and used as pollen parent in the back-cross to the mono-telo receptor. In this back-cross again the normal monosomics with the desired donor chromosome can be distinguished from those with the telo, and are used for the next generation. There is no recombination between donor and receptor chromosome, since only those plants that lack the (marked) receptor chromosome are selected. The final check on univalent shift is simplified. This clearly is the most useful method of the three.

When translocations are present, complications arise that with some difficulty can be got around (see Burnham, 1962).

Substitution lines can be used directly in breeding programs. They also make it possible to study the contribution of specific chromosomes to the genotype, compared to a homologue of another genetic composition. With a complete series, all chromosomes can be compared. For instance, the contribution of different chromosomes in respect to disease resistance, earliness, awning, lodging, yield have been determined. At the same time, when

there are allelic differences between the two varieties, it is immediately clear whether or not the genes involved are located on the substituted chromosome. When such gene-localization is the main purpose, however, simpler methods are available (section 5.7.11).

A special application was reported by Law in 1966: by crossing a substitution wheat plant with a normal plant of the same variety, the progeny is heterozygous for only one chromosome. Heterosis and other quantitative genetic effects specific for this chromosome can then be analysed. Also, the role of crossing-over in this particular chromosome can be studied: all variation in the F_2 has its origin in recombination within this chromosome.

5.7.11. *Gene localization*

(1) Dominant factors, when absence of the locus results in the recessive phenotype, appear to be mutated to recessive in nullisomics for the chromosome in which they are situated. Wheat has three factors for red seed, one in each genome. In the variety Chinese Spring, the dominant allele is present on only one of the three loci. Nulli-3D is the only nullisomic with white seeds: this factor for red seed must therefore be located on 3D. When one or both of the other two factors have the dominant allele present, of course the method does not work (compare epistasy, polymery, section 2.5.2).

(2) Deviation of the 3:1 segregation in the F_2. A monosomic plant with a recessive factor is crossed with a normal plant with the dominant allele. The monosomics in the F_1 are selected and selfed. If the factor is not in the chromosome for which the recessive parent was monosomic, then a normal 3:1 segregation will be observed. If it is in this chromosome, the monosomic F_1 plant will only have the dominant allele of the normal parent. In the F_2 all monosomics and disomics will have the dominant allele, which is by far the majority (table 5.14), so there is a great deficit of recessives. When the deficiency phenotype does not correspond with the recessive phenotype, there are no recessives at all. An example is that of Unrau of 1950: red (dominant) and white (recessive) glumae in wheat. All monosomics except that for 1B gave a 3:1 segregation. In mono-1B, however, in the F_2 only 6.6%

of the plants had white glumae, and all of these were nullisomic. This method of gene localization has been applied for several genes for disease resistance in wheat.

Genes with an unusual expression in hemizygous condition may be assigned to their chromosomes simply by studying the monosomics.

Aneuploids in allopolyploid cell hybrids in culture have proven very efficient for gene localization because of the readily recognizable loss of specific characters (see Ephrussi and Weiss, 1969 and chapter 6).

5.7.12. *Introduction of genes from other species*

It is often desirable to introduce genes (for disease resistance for instance) from a wild species into a cultivated one. This can be realized by hybridization followed by back-crossing to the cultivated species under constant selection for the character involved. Frequently, however, pairing between the chromosomes of the two species is normally insufficient to permit recombination by crossing-over. Substitution of entire chromosomes is usually undesirable since this means the introduction of a whole series of foreign genes, most of which have a negative rather than a positive effect. Addition is not successful for similar reasons and also because only dominant genes are expressed. Two methods have been suggested that make use of artifically induced exchange between chromosomes, involving the substitution or addition of only a very small segment.

(1) Induced translocation between an alien addition chromosome with the desired gene and an arbitrary chromosome of the species into which this gene is to be introduced. The most useful is an intercalary translocation that introduces a small segment, not accompanied by the corresponding loss of a segment of the recipient species. The method is especially effective when a dominant character must be introduced. The recognition of a recessive gene in the intermediate steps is highly complicated.

(2) Exchange by crossing-over between homoeologues after removal of the pairing barrier. In wheat this may be realized in nulli-5B^{L} or after hybridization with *Aegilops speltoides*. Here

too, it is desirable to keep the segment exchanged as small as possible. When the locus is not close to the end of the chromosome, double crossing-over (on both sides of the locus) may be required, but it may be realized in two subsequent generations. Since homoeologous segments are exchanged, it is possible to transfer recessive alleles, but not without special precautions.

Both forms of gene transfer have actually been applied for introducing dominant disease resistance into wheat. The first method was originally used by Sears (1956) for transferring leaf rust (*Puccinia triticinae*) resistance from *Aegilops umbellulata* ($2n$=14) to wheat ($2n$=42). It has later been successful in several more instances. The second method was developed by Riley et al. in 1968 for bringing resistance for stripe rust (*Puccinia striiformis*) from *Aegilops comosa* ($2n$=14) into wheat. Both methods usually involve so many complications that easy application is not to be expected.

Chapter 6

GENETIC CHROMOSOME MAPS

6.1. Gene maps

6.1.1. *Three-point test*

The genetic factors all have their fixed place on the chromosomes. By locating this place on an idiogram, a gene map of the chromosome can be constructed that is comparable to a geographic map. Because of the one-dimensional structure of the chromosomes (compare section 2.10.1), this map is *one-dimensional* (*linear*). Each chromosome of the idiogram has its own map, that under normal conditions has no relation with that of any other chromosome of the genome.

Even without knowing the actual location of the genes on the chromosomes, it is possible to construct a linear gene-map, purely by genetic means. Already in 1911, T.H. Morgan formulated the principles, when he postulated that the crossing-over percentage between two factors is a function of the distance between those factors. The prototype of a gene-map is the graphical representation of a three-point test (section 2.8.1; table 2.4; fig.2.16). It not only gives the genetic distances between the three loci in map units (1% crossing-over = 1 map unit = 0.01 Morgan), but also the order: the largest distance found is that between the outer two loci.

6.1.2. *Mapping functions*

It has been indicated earlier that in a three-point test, the sum of the two smaller distances is larger than the largest distance and that this is due to double crossing-over (section 2.8.1). The shorter

the distance between two factors, the smaller the chance of double crossing-over, and the better the recombination percentage observed agrees with the crossing-over percentage. This means that with large distances the recombination percentage greatly underestimates the genetic distance. In order to make a good estimate of great map distances they must be broken down into smaller units that have a map distance proportional to the recombination percentage: *small distances are additive*, long distances are not. Since the frequency of double crossing-over is a function of the crossing-over percentage, it is possible to make a correction for double crossing-over. The formulations that have been designed to convert recombination frequences into map distances have been named *mapping functions*. The first attempt to construct an exact mapping function was made by Haldane in 1919: $y = \frac{1}{2}(1-e^{-2x})$ in which y is the recombination frequency observed and x the map-distance. It takes full account of double crossing-over, but does not consider interference: it is based on a random (Poisson) distribution of exchanges. Since interference causes a considerable reduction of double crossing-over, Haldane's correction is too strong. Therefore Kosambi in 1944 designed a partly empirical function that took account of an average value of interference such as is found in the middle of the chromosomes in many organisms: the Kosambi function is $y = \frac{1}{2}(\text{th } 2x)$ in which the hyperbolic tangent (tangens hyperbolicus) $= (e^{x}-e^{-x})/(e^{x}+e^{-x})$. It is not very effective for distances longer than about 35 map units. A third function was given by Owen (1950); it is somewhat more satisfactory for map distances over 35 units: $y = \frac{1}{2}(1-e^{-2x}\cos 2x)$. There further is the mapping function of Carter and Falconer of 1951 that is frequently used in human genetics $x = \frac{1}{4}(\text{th}^{-1}\, 2y + \text{tg}^{-1}\, 2y)$. For map distances smaller than 30 units the last three functions do not differ very much. For small distances x and y are practically equal. For very great distances where the recombination percentages approach 50, small variations in y correspond with such large variations in x, that a meaningful estimate of x is not possible. Thus mapping functions are important primarily in the intermediate range, but can never equal the precision of breaking down the segment into a large number of small segments. This, however, is possible only in exceptional cases.

Determining recombination in populations with or without definable mating systems requires a special approach. Man especially presents difficulties because the possibilities of experimentation are still very limited. There are several additional problems: low frequencies of classifiable genes (few families with segregation for a single factor, even fewer where segregation for several distinct factors can be studied); arbitrary combination of parents; small families; limited number of available generations. Partly these disadvantages are compensated by the large number of individuals and careful registration, especially of conscripts. Human genetics, therefore, was obliged to develop into a branch of genetics with special methodology. It has been possible, with variable success, to estimate recombination percentages for several linked genes. The *lod-score* method of Morton, partly based on sequence analysis, has been widely used, especially after the publication by Maynard-Smith et al. (1961) of tables that have greatly simplified its use. When only two generations are available, the method is reasonably effective for recombination percentages up to about 25%. For higher recombination percentages three generations of each segregating family are required. The recombination fraction in human genetics is often given as ϑ. For conversion into map-length, the mapping function of Carter and Falconer is commonly used. In spite of the fact that several linkages in man have been analysed, genetic mapping has been possible only on a very limited scale. Only for the X-chromosome something approaching a genetic map is available (see also Renwick, 1969).

6.1.3. *Intra-gene maps*

When different alleles are known for a factor, they usually are based on different mutations at different places *within* the same gene. In Drosophila, the lozenge (eye-shape) factor is known to have several alleles that have been studied in relation to each other, especially by Green. In a fly heterozygous for two alleles of the same gene, both causing an abnormal eye shape, no normal allele is present, and the mutant phenotype is observed. Now, unlike the situation where two identical alleles are combined, crossing-over can occur between the two mutated sites. This re-

sults in one chromosome with both mutant alleles and one with the two normal alleles. The latter can result in progeny with the normal phenotype. Such recombination between alleles has led to the concept of pseudo-allelism: two mutant genes between which recombination could occur were not considered to be truly allelic. This stand-point has now been abandoned. Especially since the work on micro-organisms has shown that a gene is much larger than the unit of recombination, it is accepted that recombination can occur between two mutant sites of a single gene. It can be argued, of course, that although the two mutated alleles of the gene as a whole are allelic, the sites of the mutations are not strictly allelic when recombination can occur between them, but this is a matter of definition. When the crossing-over percentages between different alleles are determined, it is possible to construct a *micro-map* for the gene. This has been done for the lozenge and a few more genes. With such mapping it is useful to be able to check crossing-over by concurrent recombination between outside marker genes: if such genes have not recombined simultaneously with the alleles within the locus studied, it is well possible that a mutation has taken place instead of a recombination. Mutation and recombination frequencies in such small segments are of the same order of magnitude.

In the case of the lozenge gene it appeared that there were only three points at which recombination occurred. It is well possible that in higher organisms in general exchange cannot take place on every place in the chromosome but is restricted to specific points. The sites of recombination may well be the beginning of the polaron in the model of Whitehouse (fig. 2.2).

6.1.4. *Viruses*

In principle the construction of genetic maps is carried out along similar lines in viruses and in higher organisms. The mapping functions mentioned above, however, are not generally used. Positive interference is infrequent but negative interference is common. There is a great variation in genetic behaviour between different viruses.

Recombination is possible only when more than one virus parti-

cle occurs in one cell. Unlike the situation with fertilization there is no system that combines one pair of two homologous chromosomes of different origin in a single cell: infection is a random process. Even when a bacterial colony is infected with equal numbers of two viral types, only a limited number of bacteria end up with an equal number of phage particles of the two types. If one wants to be sure that each cell has at least one particle of each type, the level of infection must be so high that each cell comes to contain a large number of virus particles. Then a large part of the crossing-over will be between identical particles, and there recombination is not detectable. Thus the maximum observable recombination is considerably lower than the actual crossing-over frequency, usually much less than 50%. For phage lambda it is 15%, for T4 it is 43%. The latter is quite high and may be related to the fact that recombination and replication occur simultaneously, so that in successive phage generations within a cell the possibilities for recombination are constantly renewed. A further complication is that each phage chromosome can exchange with several partners simultaneously. Therefore, a direct determination of crossing-over percentages and consequently of map distances from recombination frequencies is not possible. Several methods have been developed to overcome this problem.

When constructing the genetic map of phages, a special peculiarity is often encountered: there is no start and no end. A single phage chromosome is a segment from a long chain which is the manifold repetition of the same pattern. The segment released from the chain is larger than the basic segment and consequently the terminal segments are repetitions of each other (*terminal redundancy*). When constructing a map, it appears to take the shape of a ring (fig.6.1A). Note that in this map related functions are grouped together: early functions (replication of phage DNA, etc.) and late functions (head- and tail-protein synthesis) occupy special positions. Because of terminal redundancy, phage particles may be heterozygous for factors that are "diploid", i.e. occur in the repeated ends. In addition it seems to be possible that heterozygosity exists between the two strands of the DNA, probably as a result of recombination. Because of the large numbers of phages that can

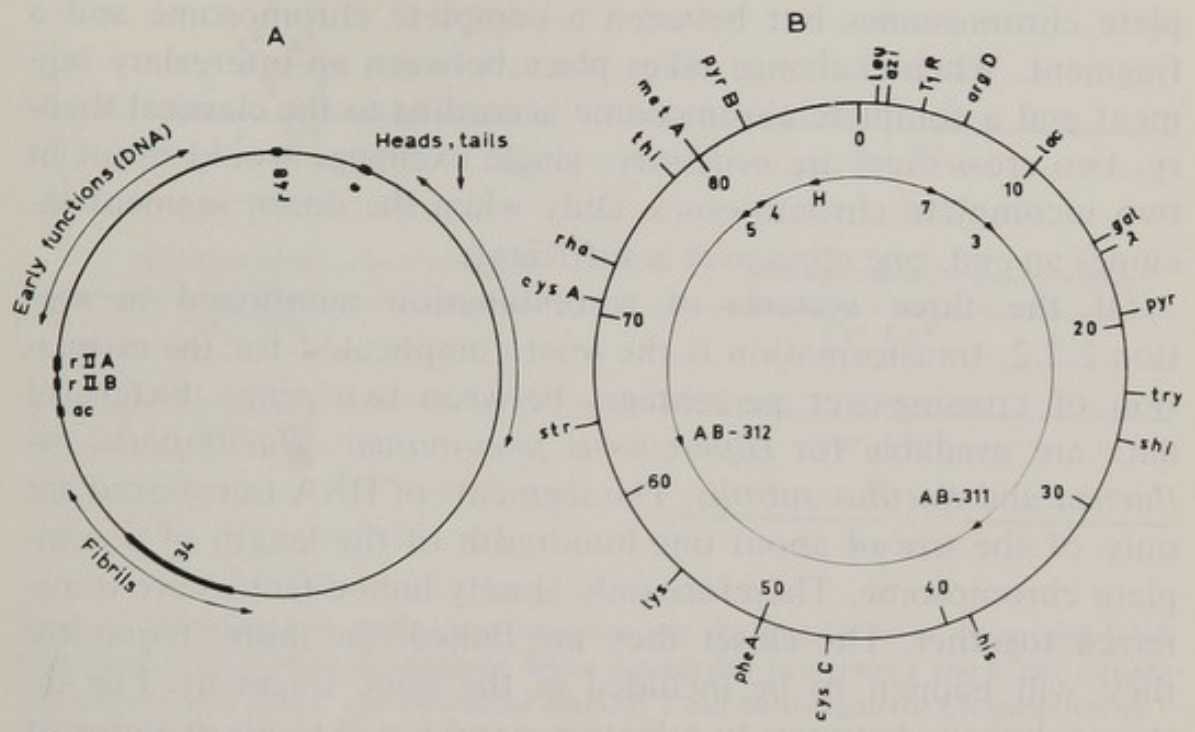


Fig.6.1. Circular genetic maps. (A) Bacteriophage T4. Factors coding for related functions are grouped together. The chromosome is not circular, but terminal redundancy results in a circular map. (B) The bacterium *Escherichia coli*. Map distances are based on the time (in minutes) required to carry the factors into the receptor cell (with conjugation). The zero point is arbitrary as different stocks have different initiation points. The factor codes are usually based on a functional deficiency induced by a mutation. Inner circle; initiation points and directions of entry for different donor stocks. The chromosome is circular, but is transferred linearly. The circular map results from combination of linear maps from different stocks with different initiation points.

be handled, very small distances between parts of the same cistron can be determined: from such fine analyses it became probable that the unit of recombination is the nucleotide. An example of a detailed analysis is that by Benzer of the *R*-locus of bacteriophage T4 of *Escherichia coli*, from which the concept of the cistron was developed (compare section 1.1.6).

6.1.5. *Bacteria*

In bacteria the genetic systems vary also, but less than in phages. Here pairing between more than two chromosomes or parts of chromosomes is the exception rather than the rule and in this respect bacteria resemble higher organisms. A complication, however, is that recombination is usually not between two com-

plete chromosomes but between a complete chromosome and a fragment. When exchange takes place between an intercalary segment and a complete chromosome according to the classical theory, two cross-overs are necessary: single exchange would result in two incomplete chromosomes. Only when the donor segment includes an end, one cross-over is sufficient.

Of the three systems of recombination mentioned in section 2.2.2, transformation is the least complicated for the estimation of crossing-over percentages between two genes. Extensive data are available for *Diplococcus pneumoniae*, *Haemophilis influenza* and *Bacillus subtilis*. The segments of DNA transferred are only of the size of about one hundredth of the length of a complete chromosome. Therefore only closely linked factors are transferred together. The closer they are linked, the more frequently they will happen to be included in the same fragment. For instance, linkage between two factors a and b will result in a greater frequency of simultaneous transformation of a^+ and b^+ to a and b by a DNA segment carrying a and b than expected when a and b are far apart. In the latter case of independent transformation, the frequency of simultaneous transformation would equal the product of the frequencies of transformation for a and b separately. These separate frequencies must be estimated in an independent experiment by mixing equal parts of a^+b and ab^+ DNA and to use this for transformation of a^+b^+ bacteria. Now, even when a and b are linked, only independent a^+b and ab^+ recombination chromosomes can be formed and their frequency can be determined. For very closely linked factors a somewhat related method is available (fig.6.2) that can also be used to make gene maps. Here an a^+b strain is used to which DNA isolated from an ab^+ strain is added. Another culture of the same a^+b strain receives DNA from an a^+b^+ strain. With close linkage between a and b, in the latter case transformation a^+b^+ will be observed more frequently than in the former: transformation to a^+b^+ will be realized also with exchange *outside* the segment a–b and this has a greater probability than exchange *between* a and b when they are close together. The relative frequencies of the two classes of transformation are a measure of crossing-over between a and b.

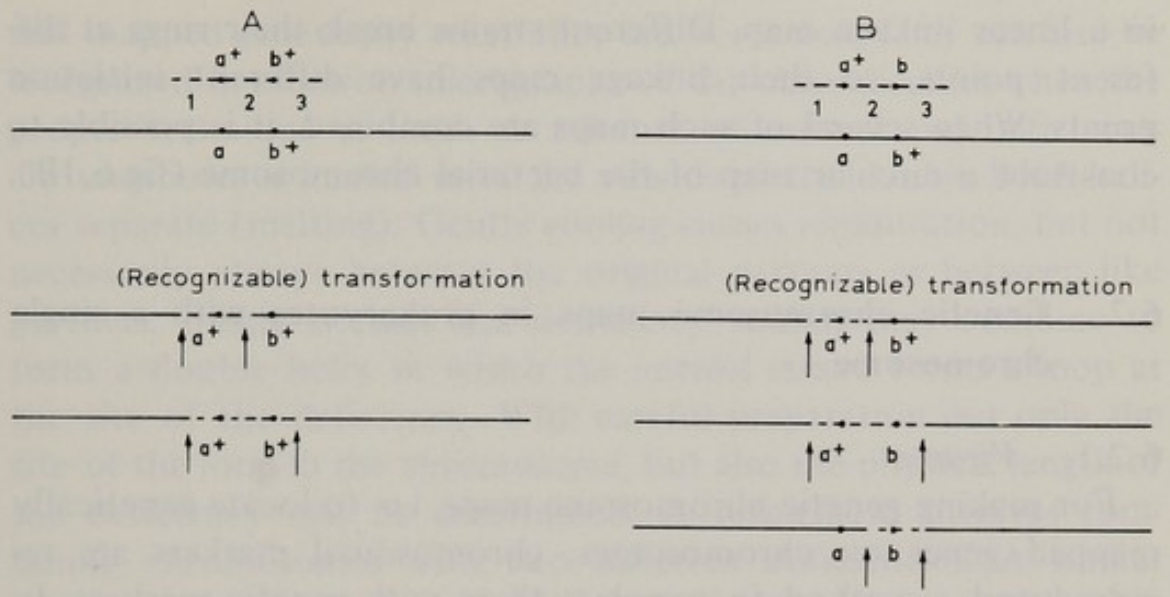


Fig.6.2. Recombination with transformation. For the transfer of an interstitial DNA segment two cross-overs are required. When considering two genes *a* and *b*, two outside segments (1 and 3) and one interstitial segment 2 can be recognized. Crossing-over in 1 and 2 transfers the gene *a*, crossing-over in 2 and 3 transfers *b* and crossing-over in 1 and 3 transfers both *a* and *b*. (A) With transforming DNA of a^+b^+ constitution and receptor ab^+, transformation of ab^+ to a^+b^+ is independent of the length of 2 and depends only on other factors. The frequency can be used as a standard in comparison with B where the transforming DNA is a^+b. Now transformation of ab^+ to a^+b^+ requires crossing-over in 2. The difference between the two cases is a measure of the genetic distance between *a* and *b*.

Quite a different system has been developed by Wollman and Jacob, especially for *Escherichia coli* strain K17. It is based on the possibility to interrupt conjugation by mechanical means at fixed intervals after initiation, and then to determine which factors have been brought over. Since normally recombination after conjugation is too infrequent for this method to be effective, special male strains with high recombination frequencies are used (*HFr* = high frequency recombination strains). Even then recombination is observed in only 10-40% of the chromosomes transferred. Just before conjugation the ring-shaped chromosome breaks at a specific point and here the transfer to the receptor cell starts. Complete transfer requires about 90 minutes. By varying the time made available for conjugation, and then tracing the genes that have been transferred, it is possible to determine exactly at which distance from the origin the different genes are located. This results

in a linear linkage map. Different strains break their rings at different points, so their linkage maps have different initiation points. When several of such maps are combined, it is possible to construct a circular map of the bacterial chromosome (fig.6.1B).

6.2. Genetic chromosome maps in prokaryotes with a single chromosome

6.2.1. *Viruses*

For making genetic chromosome maps, i.e. to locate genetically mapped genes on chromosomes, chromosomal markers are required and a method to correlate them with genetic markers. In viruses genetic markers and genetic maps are available but chromosomal markers are scarce. In principle it is possible to specifically stain the nucleotide bases with heavy metals and to make these visible in the electron microscope. The resolution is still very limited and the sequences found cannot readily be identified as segments of known genes. Local denaturation at A-T rich sites along the double-stranded DNA molecule is a possibility to detect chromosomal regions with concentrations of adenine and thymine, but again the possibility of correlating these regions with known genes is quite limited. A very valuable technique is *deletion mapping* (fig.6.3): deficiencies (deletions) can be used as genetic markers

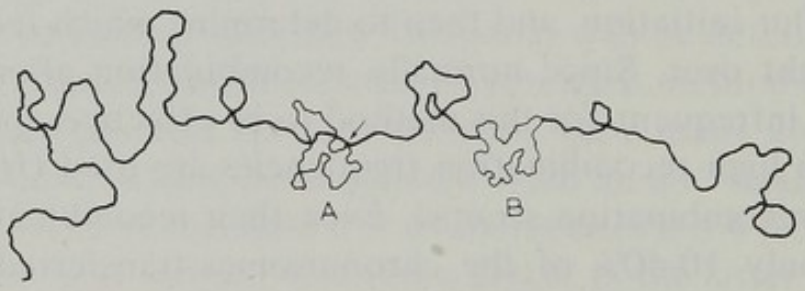


Fig.6.3. A heteroduplex bacteriophage lambda molecule formed by renaturation of a mixture of two different types of single stranded DNA. There is a deficiency at A in one of the strands, where the single normal strand forms a loop. The deficiency may be mapped genetically and is visible in the chromosome. In B, a heterozygous region shows two single stranded segments. This region too can be mapped on the chromosome and in the genetic map. (Drawn after an electron micrograph by Westmoreland et al., 1969.)

and mapped genetically when they are not lethal. They can also be recognized in electron micrographs of the chromosomes when the proper technique is used. A mixture of normal and deficiency chromosomes is heated until the strands of the double DNA helices separate (melting). Gentle cooling causes renaturation, but not necessarily always between the original partners or between like partners. Single normal and deficiency strands may combine to form a double helix in which the normal strand forms a loop at the site of the deficiency. With careful preparation not only the site of the loop in the chromosome, but also the physical length of the deficiency can be determined. A refinement involves combining chromosomes with two different deficiencies. A similar technique involves the combination of chromosomes with non-homologous segments at genetically established locations (fig.6.3).

6.2.2. *Bacteria*

The chromosomes of bacteria are larger, often circular and in general somewhat more difficult to work with. The techniques briefly mentioned for viruses have not been developed in similar detail in bacteria, but there are other possibilities. The circular chromosome has one point that can be marked by autoradiography: it is the point where DNA synthesis starts and where the two daughter chromosomes separate (fig.1.4). At or very near this point is the origin of the conjugation map and this makes it possible to correlate a genetic marker with a chromosomal marker: the beginning of a genetic chromosome map. The few more possibilities for correlating genetic markers with chromosomal markers will not be discussed.

In prokaryotes with a single chromosome, all genes are located in the same chromosome. Some RNA viruses of plants, the cowpea mosaic virus for instance, have two, perhaps occasionally even more components that infect the host independently. Such viruses have more than one chromosome. When the different chromosomes can be separated physically from a mixture and recombined with complementary chromosomes with mutant genes, it is possible in principle to distinguish in which of the two or more chromosomes the mutant genes are located. The genetics of these

organisms, however, is not yet sufficiently developed for such analyses.

6.3. The correlation of genes with chromosomes in eukaryotes

6.3.1. *Chromosomes and linkage groups*

Unlike most prokaryotes, eukaryotes with few exceptions have more than one chromosome per genome. Here the construction of a genetic chromosome map must be preceded by the determination of the chromosome in which the genes concerned are located.

Genes between which linkage is observed, form a *linkage group*. When one gene of a linkage groups has been found to be situated in a known chromosome, it may be assumed that all genes of the linkage group are in the same chromosome. This does not exclude the possibility that genes and linkage groups that at first appeared not to be linked, nevertheless are located in the same chromosome. They are simply so far apart that 50% recombination is observed between them. When the linkage groups are extended, it may be expected that genes are found that belong to both, and that connect the two groups.

6.3.2. *Haploidization*

In the fungus *Aspergillus nidulans* (n=8) it is possible to correlate genes with chromosomes by making use of a special process. Occasionally, anastomosis between non-sexual hyphae of genetically different strains produces cells with two genetically different types of nucleus: *heterokaryons*. In exceptional cases two different nuclei fuse and form a heterozygous diploid nucleus. Such diploid nuclei are not as stable as diploid nuclei in normally diploid organisms, and they may loose a chromosome. Once a chromosome has been lost, others usually follow rapidly until only one genome is left. This is called haploidization (compare section 2.3.1). Of course when by accident two homologous chromosomes disappear the cell is not capable of reproduction. The successive loss of chromosomes does not follow any system. What remains is a random combination of the chromosomes of the two

parent cells: there has been *somatic chromosome recombination.* When in different cases of haploidization two factors of the same parent remain together consistently, then it may be assumed that they are on the same chromosome. Usually haploidization in Aspergillus is preceded by somatic crossing-over, which occurs in a much lower frequency than at meiosis. This makes it possible to construct genetic maps that correspond with meiotic gene-maps but with greatly reduced distances per chromosome. Double crossing-over here is so infrequent that the distances are additive: mapping functions are not required.

In Aspergillus it has not yet appeared possible to recognize individual chromosomes cytologically. In another fungus, Neurospora, the situation is somewhat more favourable and chromosomal markers such as translocations have been used to correlate chromosomes with linkage groups (compare section 6.3.3). In Neurospora, however, parasexual mechanisms such as haploidization are unknown.

An important parallel with haploidization is found in somatic cell hybrids that are formed spontaneously when cell cultures of different genotypes are combined in one substrate, even when they do not belong to the same species. Spontaneous hybrids are scarce: approximately 100,000 cells are required of each parent to produce one hybrid cell. In order to isolate the rare hybrid cells from the parent cell lines, selective culture media are indispensable. They permit the hybrid cells to proliferate while the growth of the parent strains is inhibited. In a method devised by Littlefield in 1964 two mutant strains of mouse cells were used, one incapable of making the enzyme thymidine kinase, the other incapable of making the enzyme hypoxanthine-guanine-phosphoribosyl-transferase (HGPRT). When DNA synthesis is blocked by adding aminopterin to the culture medium, normal cells can synthesize DNA from nucleosides supplied by the medium. For this process the enzymes thymidine kinase and HGPRT are required: neither of the two mutants therefore can synthesize DNA in the presence of aminopterin, but their hybrid can. In a medium with aminopterin the hybrid cells grow, the parental strains do not. Half selective systems are more generally applicable since only

infrequently the right combination of mutants is available. They involve one normal strain of which only relatively few cells are mixed with a great majority of cells of a mutant strain. In a medium with aminopterin normal cells and hybrid cells proliferate but since both are low in number, they develop into separate colonies that can be distinguished.

After it had been detected that the Sendai strain of parainfluenza virus, even after UV inactivation, enhances hybridization by a factor of 100 to 1000, it became possible to isolate hybrid colonies without selective media, especially when the different types of cultures can be distinguished on the basis of morphological differences. Even then selective media may still be useful.

Loss of chromosomes is relatively infrequent in hybrids between cell strains derived from the same species. In cell hybrids from different species, however, the chromosomes of one of the two species usually are eliminated successively. In mouse–human hybrids elimination of the human chromosomes starts early in the development of the hybrid colonies and proceeds gradually. This elimination has been used to locate the gene for thymidine kinase on one of the human chromosomes. In a medium containing aminopterin, cell hybrids between thymidine kinase-deficient mouse cells and normal human cells will synthesize DNA as long as the human chromosome with the gene coding for the enzyme is present. When Weiss and Green in 1967 analysed several hybrid clones after 100 to 150 generations in a medium with aminopterin, two or three human chromosomes appeared to have remained. A small chromosome of the E group (compare fig.1.21) was always among them: it apparently carries the thymidine kinase gene. When these clones were transferred to a medium containing 5-bromo-deoxy-uridine, the presence of thymidine kinase was confirmed: all cells carrying this chromosome were killed by the action of the enzyme on this substance, but cells that in the process happened to have lost this particular human chromosome, survived. This and similar methods are very valuable for locating genes on chromosomes especially in those cases where formal genetic experimentation based on sexual reproduction is excluded or cumbersome. For a review see Ephrussi and Weiss (1969). Per-

haps even more promising are hybrids of human and Chinese hamster cells: the chromosomes of the Chinese hamster are less in number and better recognized, but most important, according to Kao and Puck (1970), the difference in growth rate between the parental species is greater, causing a more rapid elimination of the chromosomes of the slower (human) parent.

6.3.3. *Gene–chromosome correlation from abnormal segregations*

The simplest case is that of the *sex-linked factors.* In section 3.2.4 it was shown how it could be detected that a gene is located in the sex chromosomes. For genes in other chromosomes other methods are required.

Trisomics as duplex heterozygotes are the most generally applicable (section 5.1.5). Telocentric trisomics (section 5.2.2) can be used to locate genes in specific chromosome arms. In polyploids, *monosomics* are equally useful (section 5.7.11), where recessive alleles may come to expression as a result of hemizygosity, as well as *nullisomics,* where dominant alleles are conspicuous by their absence. Again, telocentrics enable a refinement in that they make it possible to locate a gene in a specific arm. When using *translocations* for correlating linkage groups with chromosomes, several methods are available, some of which have not been considered before:

(1) In translocation homozygotes, the composition of the linkage groups deviates from normal (section 4.5.8). When the chromosomes involved in the translocation are known, it is also known which two chromosomes contain the two changed linkage groups. For distinguishing in which of the two chromosomes each specific linkage group is located, it is necessary to repeat the test with another translocation, involving one of the two chromosomes of the first translocation, in combination with a third chromosome. When the linkage groups are reasonably large (contain several classifiable genes each) the translocation break-point can approximately be determined in the linkage groups. When this break-point can be recognized cytologically, for instance at pachytene in a heterozygote, a first step towards a genetic chromosome map is made.

(2) In a related method a translocation heterozygote is used. The

two linkage groups that segregate together must be located in the chromosomes involved in the translocation (section 4.5.8). Here again a second translocation is required to correlate the linkage groups with specific chromosomes. Now it is not possible to simply locate the translocation break-points in the linkage groups.
(3) When a recognizable alteration in chromosome shape accompanies the translocation, the three types: homozygote, heterozygote and normal can be distinguished by observation of the somatic chromosomes. When the segregation of these three types is not independent of the segregation of marker genes in one or more linkage groups, these linkage groups must be located on the chromosomes involved in the translocation. Now linkage between the translocation and the marker genes is an estimate of the genetic distance between the gene and the translocation break-points. There are a few complications in interpreting these linkages, but these will not be considered here. Of course, the genes must be close enough to the break-point to exhibit clear linkage: when the distance is large, the method fails. With this method it is not necessary that two linkage groups are available: even a single gene is enough. Again a second translocation is required to detect on which of the chromosomes the gene is located.
(4) When mitotic classification is not possible, the heterozygotes can still be recognized because of multivalent formation at meiosis or because of semi-sterility. Then the analysis is somewhat more complicated.

Inversions can be used in a similar way to detect whether or not known linkage groups are located on the chromosome involved. Often, inversions are less readily recognized, but they have the advantage that only one linkage group and one chromosome are involved.

6.4. The localization of genes within chromosomes

6.4.1. *Centromere mapping*

As soon as a cytologically recognizable point on a chromosome can be incorporated in a linkage analysis, a beginning of a genetic

chromosome map can be made. In the preceding section, a few possibilities have already been mentioned. Without using any abnormal situation a tetrad analysis (section 2.6.1) makes it possible to take up the centromere in a linkage analysis. Tetrad analysis, however, except in a number of fungi such as Neurospora (section 2.6.1) and much less perfectly in a few higher plants (e.g. *Salpiglossis variabilis* and a few genera of the Ericales and Juncales where the four cells of the tetrad remain together in the pollen) is possible only to a very limited extent. A variant of the tetrad analysis was described in 1966 by Rhoades and Dempsey: the elongate factor in maize inhibits the normal course of the second meiotic division in the embryosac mother cells. Diploid spores (half tetrads) are formed, and these have both chromatids of the same chromosome. When crossing-over has occurred between a locus to be tested and the centromere, the diploid spore is heterozygous. Crossing with a diploid results in triploid offspring from the aberrant diploid spores and diploid offspring from the normal haploid spores. The triploids are selected and tested on heterozygosity for the gene studied, by crossing to homozygous recessive diploids. The frequency of heterozygotes among the triploids is a direct measure of the frequency of crossing-over and therefore of the genetic distance between locus and centromere. It may be noted that another type of half-tetrad analysis can be carried out with the use of isochromosomes that are able to replace normal chromosomes, such as the attached X in Drosophila. The method will not be discussed.

More generally applicable for determining the genetic distance between a gene locus and the centromere are telocentric half-chromosomes (compare section 5.2.2). Especially in plants it is usually not very difficult to obtain telocentrics. There are basically three situations that permit centromere mapping: (1) A telocentric half-chromosome replaces an entire normal chromosome; the other arm is deficient. (2) A telocentric occurs in addition to a complete chromosome complement (trisomy). (3) Two telocentrics (one for each arm) replace a normal chromosome.

All three situations can be used in polyploids but because of the large deficiency involved in the first, only the last two are applica-

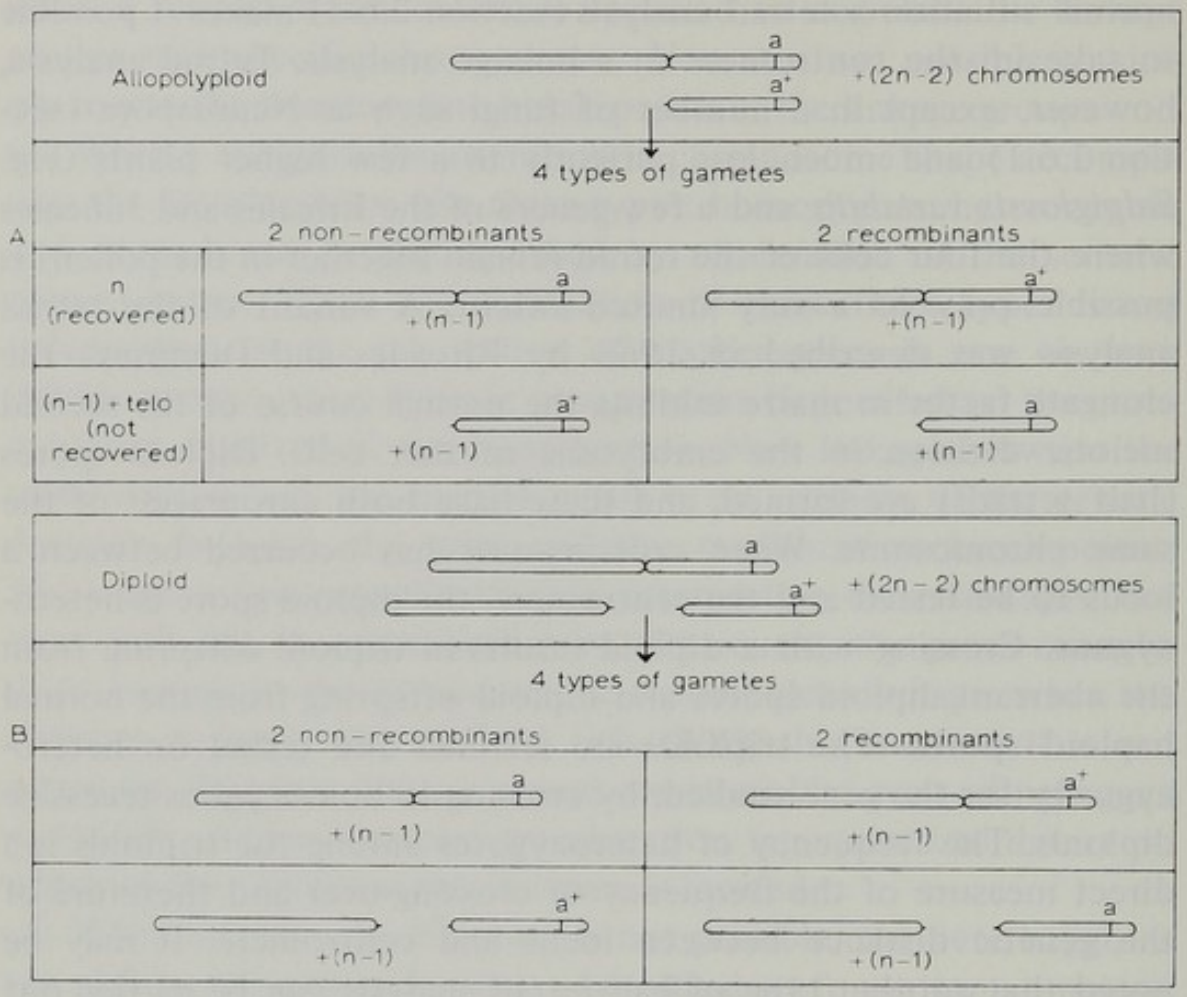


Fig.6.4. The use of telocentrics (derived from metacentric chromosomes) for determining the map distance between the centromere and a marker locus. (A) In an allopolyploid a telocentric replaces a normal chromosome. It carries the dominant allele of a marker gene (a^+). The normal homologue has the recessive allele (a). When used as the male parent in a testcross, pollen with the telo and the dominant allele cannot compete with pollen with the normal chromosome and the recessive allele. Only when the dominant allele by crossing-over has been transferred to the normal chromosome, it will be recovered. The frequency of recovery is a measure of crossing-over between locus and centromere. (B) The use of a telocentric substitution as a centromere marker. The heterozygote has one normal chromosome with the recessive allele a of a marker gene; its homologue consists of two telos, one of which carries the dominant allele a^+. In a testcross four gametic types are recovered, two non-recombinants: a normal chromosome with the recessive allele, and a telo-substitution with the dominant allele; two recombinants: a normal chromosome with the dominant allele, and a telo-substitution with the recessive allele. The frequency of recombinants is a measure of crossing-over between locus and centromere.

ble in diploids. Of course, when the normal chromosome is telocentric by nature, none of the three situations can be used for mapping the centromere: the telocentric condition serves as a centromere marker, that must be distinguishable from normal.

The first method (fig.6.4A) has been applied mainly in wheat and cotton (see Sears, 1969). Often use is made of the fact that most telocentrics are transmitted poorly through pollen in competition with the corresponding complete chromosome. Therefore, when the F_1 is the father, only pollen with the normal chromosome functions normally, and the allele in this chromosome is transferred to the progeny. With the locus close to the centromere, the allele in the telo is never recovered, but with the locus further away, crossing-over may transfer it to the normal chromosome. Consequently the frequency of recovery of the allele derived from the telo is a direct measure of crossing-over between locus and centromere. Several variants of this method, some requiring cytological classification of the telocentric, are possible.

An example of the second method (telocentric trisomic, fig.5.10), has been given earlier (section 5.2.2). It is especially convenient when the trisomic can be recognized phenotypically.

The third method (fig.6.4C) has as yet had little application. It cannot be used to determine in which arm a gene is located. Since no direct phenotypic effects of the double telo condition are expected, cytological classification is indispensable. The double telo chromosome simply functions as a centromere marker that is classified along with the two alleles of the genetic factor to be analysed. When two genetic markers are available, a three-point-test (section 2.8.1) can be set up to study the order of the three markers. This makes it possible to decide whether the two genes are in the same or in different arms of the chromosome at the same time giving the two genetic distances from the centromere.

As with all techniques that make use of structurally altered chromosomes, there is no certainty that the values observed correspond with those in the natural situation. It is probable that close to the centromere crossing-over is reduced in telocentric heterozygotes. More distally, crossing-over may be increased as a result of interference phenomena.

In principle there is a possibility to map the centromere in human chromosomes with the use of spontaneous trisomics. For the chromosome involved, a situation arises that is comparable to that with double reduction in primary trisomics (compare section 5.1.6 and fig.5.7). When the parent is heterozygous, non-disjunction causes both alleles to go to the same daughter nucleus: there are two chromosomes at the second division and in second anaphase each produces one chromatid for one pole and one for the other. Without crossing-over, the two resulting spores both have one chromosome with one allele and one with the other: they are heterozygous and carry an additional chromosome. With crossing-over between locus and centromere, however, after the first division the two chromosomes both have two different alleles. Now there are two possibilities for second anaphase: two identical alleles, one from each chromosome, go to the same pole, or two different alleles go to the same pole. The latter (50% of the cases) cannot be distinguished from non-crossovers. The first 50%, however, give two homozygous types, dominant and recessive: here crossing-over is observed. Since non-disjunction occurs after crossing-over, there is no effect of trisomy on crossing-over. On the other hand, it is conceivable, although not probable that certain cases of crossing-over enhance the possibility of non-disjunction. Trisomy as a result of non-disjunction is relatively frequent for the X-chromosome, and may result, for instance, in XXY Klinefelter males. There are many known genes on the X-chromosome (about 60), some of which occur with reasonable frequency, especially in some populations (*Xg*-blood group; glucose-6-phosphate-dehydrogenase deficiency (G6PD); red-green colour blindness (Deutan); haemophilia A).

6.4.2. *Other cytological markers*

In sections 4.5.8 and 6.3.3 the possibility of including the translocation break points in a genetic analysis has been mentioned. When more cytologically recognizable points are available, a more accurate map can be constructed. In maize, strains with clearly recognizable heterochromatic knobs, especially at the chromosome ends, have been found and used in linkage analyses. Already

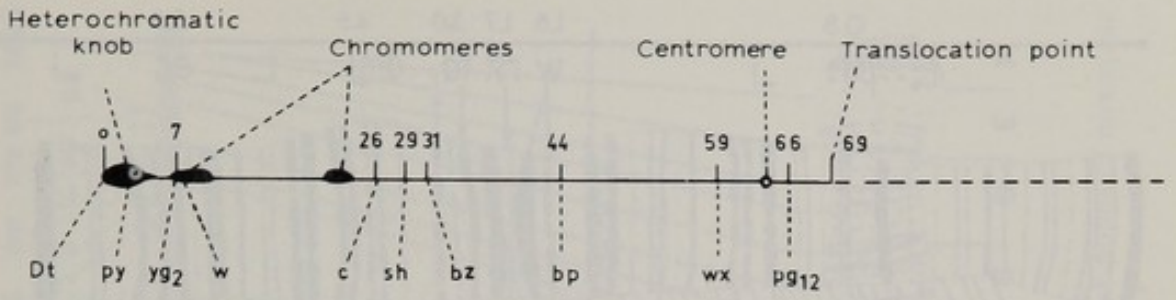


Fig.6.5. Genetic chromosome map of chromosome 9 of maize, including cytological markers: a heterochromatic knob at the end, two conspicuous chromomeres, the centromere and the break-point of an interchange with chromosome 8. (According to Creighton and McClintock, 1931, and others.)

in 1931, Creighton and McClintock (compare section 2.6.1, fig.2.13) constructed a genetic chromosome map of the short arm and part of the long arm of chromosome 9 in maize, using a 8-9 translocation, a knob and some conspicuous chromomeres in the short arm of one of the homologues, clearly visible at pachytene. Knobs and chromomeres behaved genetically like any gene but had to be classified in microscopic preparations of pachytene. The map is shown in fig.6.5.

Very efficient are deficiencies (compare section 6.2.1), especially when accurate chromomere analysis is possible, as in Drosophila and, to a lesser extent in maize and tomato. They have been used most extensively in Drosophila, where even very small deficiencies can be recognized in the salivary gland chromosomes. In section 4.2.2 an example has been mentioned of a series of deficiencies in the X-chromosome of *D.melanogaster*, from which the location of a number of genes could be deducted. Of course, only for a few loci very small deficiencies are available, permitting direct localization on a single band. But larger, overlapping deficiencies can be used in a series of consecutive experiments and can give very exact results. A locus that is shown to be included in two overlapping deficiencies, must be located in the overlapping segment. A locus that is included in one and not in the other deficiency, is not in the overlapping segment. By using a large number of deficiencies, Slizynska succeeded in 1938 in constructing a very accurate map of the white-notch segment of the X-chromosome of

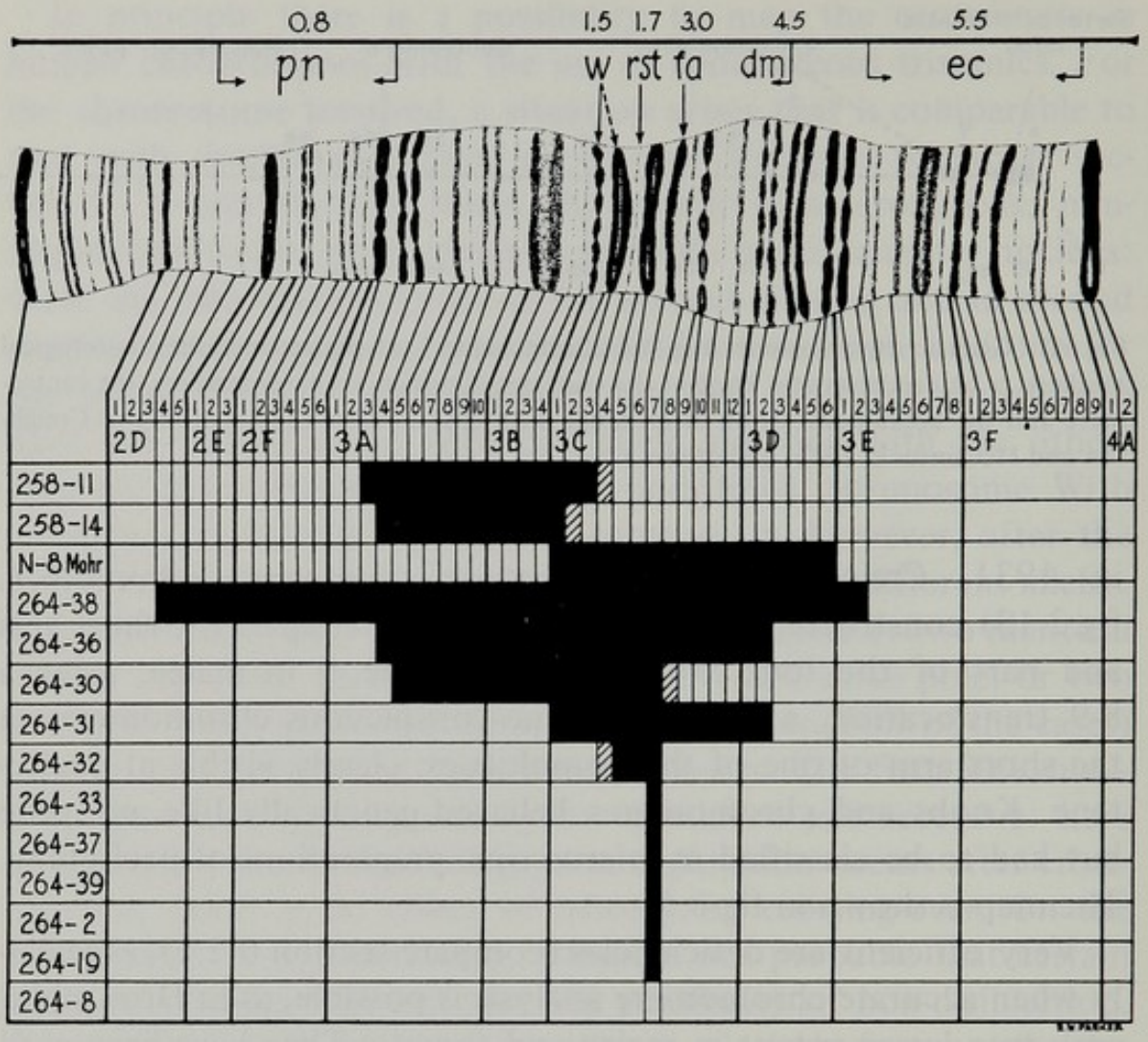


Fig.6.6. Deficiency mapping in Drosophila according to Slizynska, 1938.

D.melanogaster (fig.6.6). Later, other chromosomes have also been mapped with the use of deficiencies, often in combination with translocations, inversions and duplications. An example is given in fig.6.7. The relative distances on the genetic and the chromosome map do not always correspond as well as they do in the example. Sometimes the genes are relatively close together on the genetic map and widely spaced on the chromosome map in some segments, while in other segments the situation is reversed. This is due to differences in crossing-over frequency in different segments of the chromosomes (compare sections 2.8.1, 2.8.5): localization of crossing-over in certain segments is a frequently observed phenomenon.

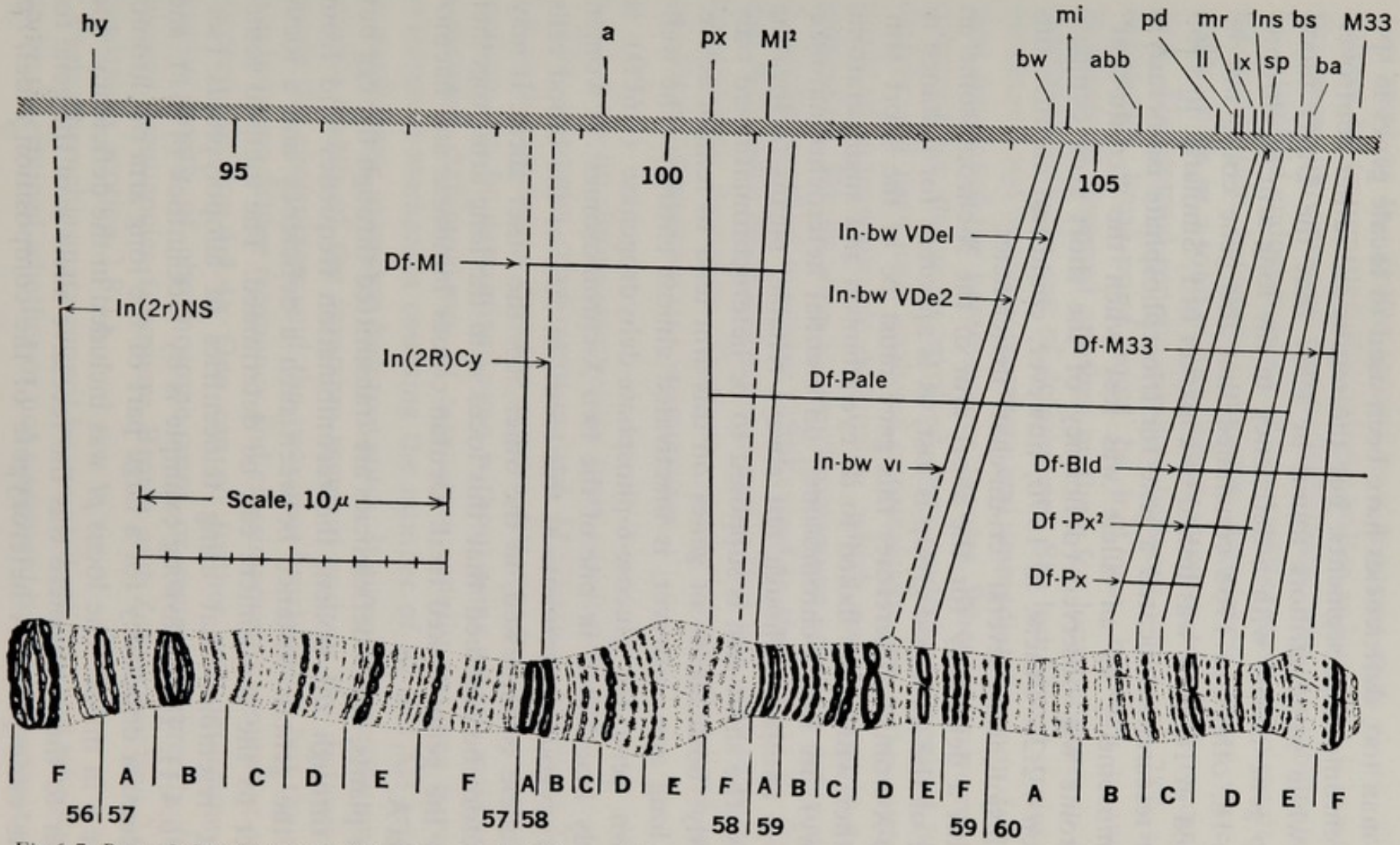


Fig.6.7. Part of the right arm of chromosome 2 of *Drosophila melanogaster* with the location of factors, break-points of inversions (In) and deficiencies (Df) shown in the salivary gland map. (Sinnot, Dunn and Dobzkansky, 1958, after Bridges.)

In man too, deficiencies have been used to locate genes in specific chromosome segments, but the results are rather controversial: while some authors found in 1967 that the Duffy blood group gene is lost with a deficiency in the long arm of chromosome 16, others in 1968 concluded that this gene could not be located in 16, as it was linked to a marker in 1. Similarly, in 1969 some researchers located a gene for triosephosphate isomerase in chromosome 5, as an allele was lost when the "cri-du-chat" syndrome was observed (deficiency of the short arm, compare section 4.2.2). Others, in 1970, however, could not confirm this conclusion, using several "cri-du-chat" deficiencies.

With a deficiency for the short arm of the X-chromosome an allele of the *Xg*-blood group is lost, as is apparent for instance in X-iso-X-woman. Therefore, this gene must be in the short arm, together with genes linked to it: eye-albinism and angiokeratoma (Fabry). In the X-chromosome, differential heterochromatinization is another, although not always reliable, means of locating genes: the short arm is supposed to be heterochromatinized only slightly: both alleles of genes on this arm tend to remain active. The long arm, however, is inactivated almost entirely. The well-known gene for glucose-6-phosphate-dehydrogenase (G6PD) is clearly inactivated in one of the two X-chromosomes: in women heterozygous for mutants at this locus, in 50% of the blood cells one allele is expressed, in the other 50% the other allele. It may therefore be assumed that this locus is in the long arm, together with the genes linked to it: Deutan colour blindness and haemophilia A.

In plants, when deficiencies are transmitted through the egg but not through the pollen, the recombination frequency and from this the genetic distance between such a deficiency and a locus closer to the centromere can be determined. The method somewhat resembles that using telocentrics in allopolyploids (section 6.4.1). The following example is by McClintock (1931), and concerns a deficiency for a large part of the long arm of chromosome 6 in maize. The locus *pl* was included in the deficiency, and closer to the centromere was the recessive allele *y* of the gene for white endosperm. A heterozygote of the composition *y* def/*Ypl*

was used as the pollen parent in a testcross with *yy* ++. Since *y* cannot be transmitted through the pollen as long as it is located in the same chromosome as the deficiency, non-recombinants give *yyY* triploid endosperm and all *yyy* (white) seeds must result from recombination between *y* and the deficiency. There were 101 white seeds in a total of 635, which amounts to 15.9% recombination. The normal genetic distance between *y* and *pl* is 28%, so the deficiency break-point must be half way between *y* and *pl*. McClintock designed another method for locating genes in chromosomes with the use of deficiencies. She hybridized strains with dominant and recessive markers and immediately upon fertilization irradiated the heterozygous zygotes. When recessive mutants occurred for the genes involved, they could be recognized immediately and then were analysed cytologically. When they were the result of a recognizable deficiency, which was checked in a test-generation, the location in the chromosome could be determined. A similar method was later (1968) used in the tomato by Khush and Rick, who irradiated normal pollen that was used to pollinate homozygous recessives. There are several more specialized methods for locating genes on chromosomes.

6.4.3. *Genetic chromosome maps from cytological data*

The example of Creighton and McClintock (sections 2.6.1, 6.4.2) demonstrates that the genetic distance between cytological markers can be determined even without using genetic markers. A somewhat different way of estimating genetic distance between cytological markers is counting the number of AI and AII bridges in paracentric inversion heterozygotes. It gives information on the genetic length of the inversion segment and on the distance between the centromere and the proximal break-point, but the relation between bridge frequency and crossing-over is somewhat complicated (section 4.4.4). One can go further and determine the genetic length of chromosome arms or segments directly from chiasma frequencies (compare Brown and Zohari, section 2.6.1; Henderson, section 2.8.1).

Even when chiasmata cannot be counted reliably and when only can be determined whether or not a segment has any chiasmata, an

approach is still possible. Assumptions are: (1) Independent formation of the first chiasma in the segment considered (compare section 2.8.1), i.e. there is no effect of interference on this chiasma, and (2) Poisson distribution of chiasmata disturbed only by interference. Since Poisson distributions are additive, chiasma localization is not in conflict with this assumption as long as it can be assumed that for each point of the chromosome the formation of a chiasma is independent of that in other points, except for interference. Then the segments without chiasmata (classifiable as such) amount to $e^{-2\mu}$, in which μ is the expected crossing-over frequency in absence of interference. The class with one or more chiasmata equals $m = (1-e^{-2\mu})$. This is twice the recombination frequency in Haldane's mapping function: $y = \frac{1}{2}(1-e^{-2x})$, that, similarly, does not take interference into account. In order to include interference, this function may be replaced by one of the more modern mapping functions, for instance that of Kosambi ($y = \frac{1}{2}\,th\,2x$), which is rather simple to apply. Then the frequency of segments with at least one chiasma, m, equals $th\,2x$. The corresponding genetic map length x can be read from tables of th. Application, of course, is possible only when the segments involved can be recognized individually or when the frequency of being associated with its homologue can be determined by other means. This is possible in translocation heterozygotes (section 4.5.3 and Sybenga, 1970), in double telocentrics where one chromosome is replaced by the corresponding to telocentrics (section 6.4.1), and even in telocentric trisomics (section 5.2.2). Using telocentric trisomics, the length of the short (nucleolar) arm of the satellite chromosome of rye was found to be 76 map units, the long arm 123 units. In another genotype, using translocations, the length of the short arm was 91 units, that of the long arm 124 units.

Chapter 7

DEVIANT CYTOGENETIC SYSTEMS

7.1. Apomixis

7.1.1. *Plants*

With apomixis, the normal sexual cycle is replaced by a system of asexual reproduction. Rather frequently, in lower organisms and in plants, several kinds of asexual reproduction occur *besides* a normal sexual cycle: vegetative reproduction of bacteria, fungi, protozoa; runners etc. of plants. This is not considered apomixis because the normal sexual cycle is not replaced but has retained an important share in the reproduction and maintains a significant level of recombination in the population. In true apomicts, incidental sexual reproduction may occur but it is of very limited significance for the genetic system, and, moreover, often does not lead to normal progeny because the chromosomal and genetic constitution has lost the proper adjustment to the process of sexual reproduction. Continuous asexual reproduction leads to an accumulation of usually recessive mutations and chromosomal aberrations that may give a (limited) basis for selection within the clone but that provide an ever increasing restriction on the possibilities of sexual reproduction.

Apomixis is determined genetically but it is also influenced by environmental factors. Polyploidy is found in many apomicts and sometimes it can enhance an already existing tendency towards apomixis. On the other hand, a number of polyploids would not have survived without apomixis.

In plants, several forms of apomixis are distinguished. The final cause is a deviation at some point in the development of the

sporophyte or gametophyte. A distinction is made between *agamospermy* involving seed formation, and *vegetative reproduction* without seeds, where reproduction takes place by means of bulbs, runners etc. Several forms of pseudo-vivipary belong to the same category: small plantlets develop in the place of flowers.

With agomospermy two types are distinguished:

(1) The alternation of generations is suspended: an embryo develops directly from one or more cells of a somatic tissue within the ovule: nucellus, integuments, chalaza. This is *adventitious embryony*. It is actually a form of vegetative reproduction leading to the formation of seeds. It is the most common type in several citrus species.

(2) The alternation of generations is maintained. There are two possibilities: (a) An embryosac is formed, not from the normal embryosac mother cell after meiosis, but directly from a diploid cell of surrounding tissue, approximately as in adventitious embryony. There is a true gametophyte, however, although it is diploid. Without fertilization one of the cells of the diploid embryosac, usually the "egg" develops into an embryo. This is *apospory*: a normal spore is not formed. (b) The proper embryosac mother cell with or without rudiments of meiosis, develops into a diploid embryosac, the egg nucleus of which develops into an embryo: *diploid parthenogenesis*. In several of the last two cases, especially in the case of parthenogenesis, fertilization of the secondary polar nucleus is required, followed by development of the endosperm that stimulates the division of the unfertilized diploid egg nucleus, or if division of the egg has started autonomously, is necessary for further development of the embryo: *pseudogamy*. If it is not the egg nucleus that develops into an embryo, but another cell (synergid, antipodal cell), there is *apogamy*. Sometimes a normal meiosis can be followed by parthenogenesis: *haploid parthenogenesis*, but in nature this is of no significance in plants. In the experiment, however, it is important as it is a major source of haploids. Occasionally, diploid parthenogenesis follows a normal meiosis but only after two haploid nuclei have fused to form a diploid embryosac. This may have genetic consequences.

The number of possible and observed variations is large. For

details see Rutishauser (1967), who also mentions the earlier, classical reviews by Battaglia (1963) and Gustafsson (1946, 1947, 1948).

7.1.2. *Animals*

In animals too apomixis occurs; in higher forms (usually not higher than insects and Crustaceae), almost exclusively as parthenogenesis. The entire order of Hymenoptera and many other insect families have haploid parthenogenesis as their sex determination system: the unfertilized, haploid eggs develop into males, the fertilized eggs into females (*arrhenotoky*). Diploid parthenogenesis (*thelotoky*) occurs also: unfertilized, unreduced eggs develop into females that are genetically identical to the mother. *Obligate parthenogenesis* without any possibility for sexual reproduction must be distinguished from *cyclical parthenogenesis*, where with the season diploid parthenogenesis alternates with sexual reproduction. Obligate parthenogenesis is often combined with polyploidy (compare section 5.6.3). It can revert to sexual reproduction, for instance after hybridization, and then lead to sexually reproducing polyploids (Astaurov, 1969).

Cyclic parthenogenesis permits an efficient adaptation to the environment but requires a special system of sex determination. In several species of aphids the fertilized eggs winter over and in spring each egg produces one wingless female (the fundatrix). This female produces parthenogenetically a number of winged daughters (emigrants) that migrate to other food plants and in their turn produce parthenogenetically numerous generations of female progeny. At the end of the season, the aphids start producing two types of oocyte. From one type arises, again parthenogenetically, a female with the normal diploid number of chromosomes ($2n=14$). She produces haploid eggs after a normal meiosis. In the second type of oocyte a rudimentary meiosis takes place: only the X-chromosomes pair and segregate. One of the two X-chromosomes goes to one pole and remains single. At the other pole the other X-chromosome and all the remaining chromosomes come together: $2n-1=13$. These egg cells develop (parthenogenetically) into XO males. The males have normal meiosis, but only the X-bearing

sperm (n=6+X) are functional. Fertilization of a normal n=6+X egg by such n=6+X sperm results exclusively in female eggs ($2n$=12+XX) that winter over and in spring again produce the wingless fundatrices. See further White (1954) and Swanson (1957).

7.2. Unusual segregations

In previous chapters deviating segregations resulting from anomalous behaviour of the gametes before fertilization have been mentioned briefly: differences in vitality (sections 2.5.1, 4.5.9), certation (section 2.5.2), incompatibility (sections 2.5.2, 3.2.1, 4.5.9). In addition, unusual segregations may be the consequence of unorthodox chromosome behaviour, of which a few cases have been mentioned also: sex-chromosomes, including some complex systems (section 3.2.4), B-chromosomes (section 3.2.7), translocation complexes (section 4.5.8). In the following a few more systematic cytogenetic causes of unusual segregations will be considered.

7.2.1. *Neocentric activity*

In some populations of maize an abnormal form of chromosome 10 occurs, that has an extra, almost entirely heterochromatic segment at the end of the long arm. In the same populations the normal form of chromosome 10 is also present, and the frequencies of the two forms vary with the population. During both meiotic divisions, but not in somatic mitoses, abnormal chromosome 10 exhibits at or near this segment localized kinetic activity (neocentric activity) similar to that of a normal centromere. It does not become apparent before first metaphase (fig.7.1A). Its strength increases until at anaphase I it dominates the activity of the original centromere. The original centromere may even be seen to reorient and start co-orientation with the neo-centromeres of the same chromosome (fig.7.1D). This, however, does not endanger anaphase separation, since the new centromere is the stronger of the two. The neo-centromere does not have an entirely terminal position (fig.7.1A, C) but occasionally it may give the

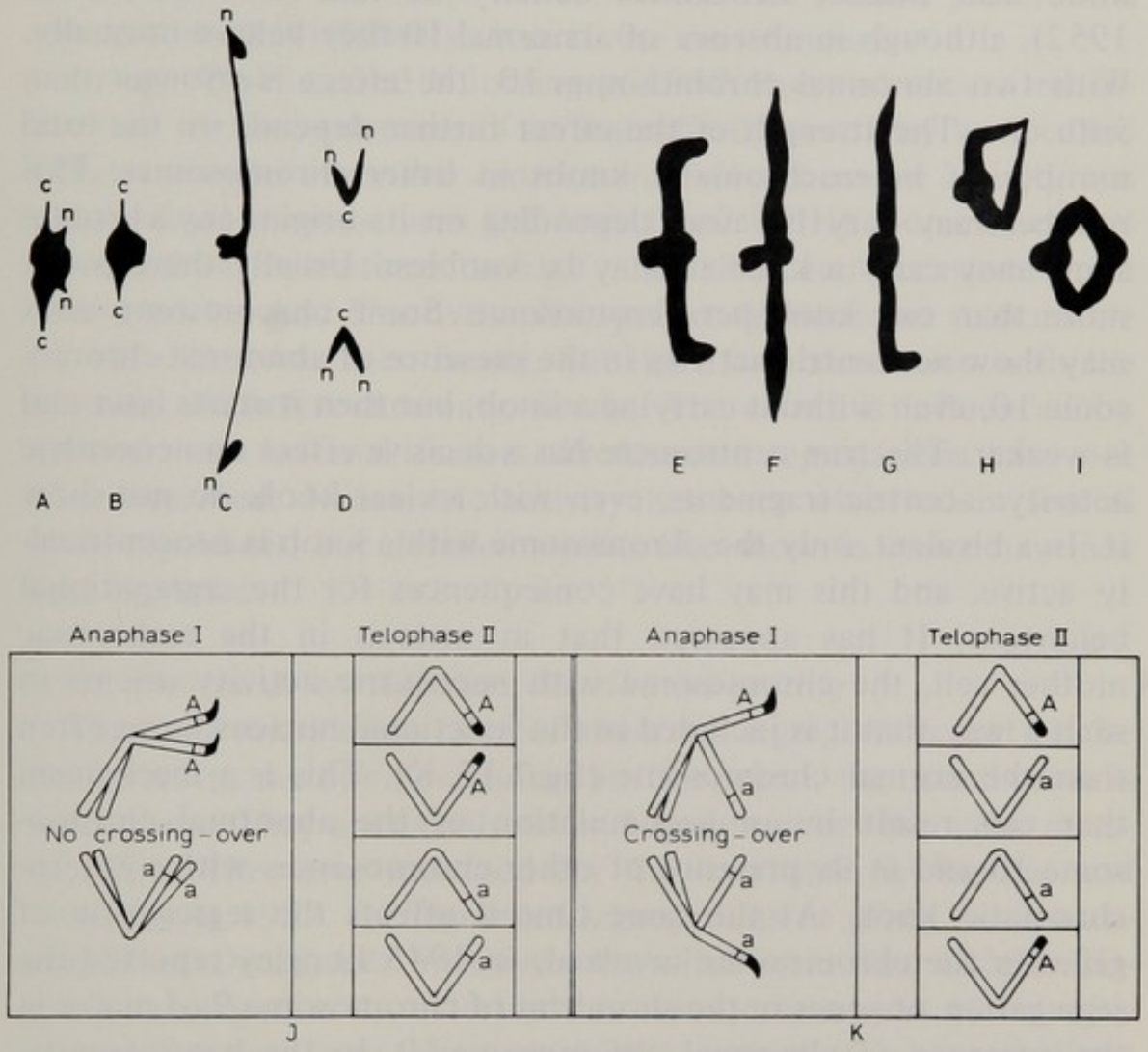


Fig.7.1. Neocentric activity in maize (A, C, D drawn after Rhoades, 1952) and rye (F, G, H). c, centromere; n, neocentromere. B, E, I normal bivalents. In maize, neocentromeres start activity during metaphase I and rapidly develop until they exceed the normal centromeres in strength (C). At anaphase (D) the centromeres may reorient against the neocentromeres. In rye, the neocentromeres are more terminal and weaker but may shift the position of a bivalent in respect to the equator (H). In maize, during EMC meiosis the neocentromere affects AII orientation by pulling the chromatid on which it is located to the extremity of the AI pole. When without crossing-over one chromosome carries the neocentromere and the other does not, the effect is negligible (J). When after crossing-over both chromosomes have one normal and one neocentric chromatid, the neocentric chromatids move to the extremes of the AI pole and are preferentially included in the terminal (functional) tetrad cell during AII (K).

impression that it does. When this segment on chromosome 10 is present, other chromosome arms with a distinct heterochromatic knob also exhibit neocentric activity at this knob (Rhoades, 1952), although in absence of abnormal 10 they behave normally. With two abnormal chromosomes 10, the effect is stronger than with one. The strength of the effect further depends on the total number of heterochromatic knobs in other chromosomes. This number may vary because, depending on its origin, any chromosome may carry a knob or may be knobless. Usually there is not more than one knob per chromosome. Some chromosome arms may show neocentric activity in the presence of abnormal chromosome 10, even without carrying a knob, but then it starts later and is weaker. The true centromere has a decisive effect on neocentric activity: acentric fragments, even with a clear knob, do not show it. In a bivalent, only the chromosome with a knob is neocentrically active, and this may have consequences for the segregational behaviour. It has appeared that in meiosis in the embryosac mother cell, the chromosome with neocentric activity orients in such a way that it is included in the functional nucleus more often than the normal chromosome (fig.7.1 J, K). This is a mechanism that can result in an accumulation of the abnormal chromosome 10 and in its presence of other chromosomes with a heterochromatic knob. At the same time it affects the segregation of genes in the chromosome involved. In 1945 Longley reported the segregation of genes in the short arm of chromosome 9 of maize in the presence of abnormal chromosome 10. In the heterozygote, one chromosome had the knob and the alleles *C*, *sh* and *wx* (compare fig.6.5). The homologue did not have the knob, and it had the alleles *c*, *Sh* and *Wx*. The locus of *C* is rather close to the knob, that of *Wx* is closer to the centromere. In the test-cross with *c*, *sh*, *wx* *C* appeared to be included in the functional tetrad cell together with the knob much more frequently than normal: instead of occurring in 50% of the gametes, it was found in 64%. The remaining 36% had *c*. This preferential segregation is due to frequent recombination between the centromere and the segment containing *C* and the knob (since recombination is necessary for functioning of the mechanism of preferential segregation,

fig.7.1K), but infrequent recombination between *C* and the knob. The factor *sh* behaved similarly as it also was rather far from the centromere. On the other hand, *wx* did not exhibit preferential segregation: it is relatively close to the centromere.

In rye, Prakken and Müntzing in 1942 observed neocentric activity in the chromosomes of several inbred lines. They were called T-chromosomes (terminal centromere), since here the activity was localized at or quite near the ends (fig.7.1 F,H). Sometimes one chromosome, sometimes two of a bivalent and occasionally all chromosomes had T-activity. No special chromosome responsible for the effect has been found, but there is a correlation with the presence of heterochromatic knobs. The effect is influenced by internal and external factors. Preferential segregations have not been observed. Neocentric activity has been observed in several more organisms, but detailed studies like those in maize have not been reported.

7.2.2. *Meiotic drive. Affinity. Distributive pairing*

There are many more cases of abnormal segregations that can be attributed to meiotic deviations, but for which the actual cause is not known. They are usually taken together under the name *meiotic drive*. The term was used for the first time by Sandler and Novitski in 1957, for disturbed segregations of genes in chromosome 2 of *Drosophila melanogaster*, when male flies with an SD-factor (segregation distorter) in this chromosome were mated with normal females. In extreme cases 26:1 instead of 1:1 segregations were found. The effect depends on several conditions. It has been ascribed to deviations in the structure of heterochromatic segments in the vicinity of the centromere, that may lead to breaks in the chromosome. Another explanation is preferential segregation of such segments to specific poles that are unable to organize functional cells.

A different phenomenon is that of linkage between factors in different chromosomes not involved in a reciprocal translocation: *affinity* (Wallace, 1961). Apparently, non-homologous chromosomes, derived from the same parent, tend to go to the same pole in anaphase I, resulting in pseudo-linkage of genes in different

chromosomes. The centromeres seem to play a decisive role. Affinity has been studied most thoroughly in mice, but there are reports (mostly not confirmed) of affinity in other species, including plants. The attraction between non-homologous chromomeres from the same parent seems to be restricted to cases where the genomes are of distinctly different origin: heterozygotes formed by hybridizing two different inbred strains or an inbred strain with a wild strain. Segregations of genes within inbred strains do not show affinity.

Again a different system is *distributive pairing* (Grell, 1964): non-homologous chromosomes of similar size sometimes have a tendency to associate. Although no crossing-over takes place, there is metaphase coorientation, leading to separation at anaphase: the two chromosomes are combined in one gamete less frequently than expected with random segregation. The phenomenon appears to be relatively common in Drosophila, but seems to be scarce in other organisms.

A special case of correlated coorientation without apparent physical connection was first described by Payne (1916) and by White (1954): in meiosis of mole cricket males (Gryllotalpa) there is a single metacentric X-chromosome in addition to 10 normal bivalents and one heteromorphic bivalent consisting of a large and a small chromosome. The large chromosome of the heteromorphic bivalent and the single X-chromosome always pass to the same pole and are included together in the same daughter nucleus. Artificial re-orientation of the heteromorphic bivalent (Camenzind and Nicklas, 1968) resulted in subsequent re-orientation of the X. The reversed did not occur: when the X was re-oriented, the heteromorphic bivalent did not follow, but the X returned to its original pole, to which the large chromosome of the bivalent went. This correlated orientation insures proper segregation, but the mechanism is not understood.

7.3. Other systems

7.3.1. *Chromosome elimination*

Incidental *somatic* elimination of chromosomes has been mentioned in the sections on haploidization in Aspergillus (sections 2.3.1, 6.3.2); haploidization in somatic cell hybrids (sections 5.7.1, 6.3.2); the origin of deficient cell lines with certain forms of cancer (section 4.2.2).

Systematic somatic elimination has been observed by Boveri in 1909 in the *diminution* divisions of *Ascaris megalocephala*. During the first cleavage division there are two (in some forms four) large polycentric chromosomes (compare section 3.2.8). At the second cleavage division one of the two dividing cells retains these chromosomes, but in the other the chromosome ends dissociate and are lost in the plasma. The remaining centre of the chromosome is split up into 50-70 small chromosomes, each with its own centromere. In all further divisions this situation is maintained, and these cells form the regular body cells. The cell with the large chromosomes divides also and at each division one of the two daughter cells undergoes the same process of diminution: always only a single cell with large chromosomes remains. From this cell a final series of normal divisions leads to the formation of the reproductive cells. The actual elimination only concerns the heterochromatic ends that apparently are not of importance for the somatic tissue.

In some species of Lepidoptera, elimination, probably involving specific chromosomes occurs systematically in later stages of development.

A relatively simple example of *meiotic* elimination has been mentioned in section 7.1.2: the formation of a single X-bivalent in arrhenotokous aphids leads to the elimination of one of the two X-chromosomes and this is the origin of X-O males. More complicated, but brought about by a similar mechanism is chromosome elimination in *Rosa canina*, the dog rose, and its relatives. It was described for the first time by Täckholm in 1922. The plants are pentaploid hybrids ($5x$=35). In PMC and EMC seven normal bivalents are formed, the remaining 21 chromosomes remaining uni-

valent. In the PMCs these univalents divide during the first division in such a way that seven chromosomes and 21 chromatids end up at each AI pole. In the second division, the chromatids cannot divide again and remain in the cytoplasm, where they degenerate. Microspores with seven, some with eight and nine chromosomes, are formed, but those with seven have by far the best chances of fertilization. In the EMCs also, the bivalents behave normally, but the 21 univalents move together to the pole that is nearest the micropyle, which is the place where the functional tetrad cell will develop into an embryosac. This pole therefore has the seven chromosomes of the bivalents and the 21 univalents. A normal second division follows, from which two cells with 28 chromosomes result, one of which develops into the embryosac. Fertilization with seven chromosome pollen reproduces the 35 chromosome type. Recombination is possible only between the seven bivalent-forming chromosomes. The remaining behave as in apomixis: they are transmitted from one generation to the other without change and variation occurs only as a result of mutation.

Even more complicated is chromosome elimination in some lower Diptera: especially the genus Sciara has developed peculiar systems. The male has a combination of mitotic and meiotic elimination of one of the genomes. In some way a distinction is made between the chromosomes of the father and those of the mother. In the male, the genome of the father is eliminated in a complicated way and only that of the mother is transmitted. In the next generation, however, this is the genome that is eliminated, because it had been derived from the father.

7.3.2. *Somatic segregation*

Somatic crossing-over and haploidization, from which somatic segregation results, have been mentioned above (sections 2.3.1, 6.3.2). *Somatic crossing-over* is observed occasionally in Drosophila, where somatic pairing is normal. It may lead to sectors in which recessive factors come to expression. Twin-spots in maize endosperm may be caused by breakage-fusion-bridge cycles (section 4.2.2) but also, like in Drosophila by certain forms of somatic recombination. In the crucifer Arabidopsis also, somatic recombi-

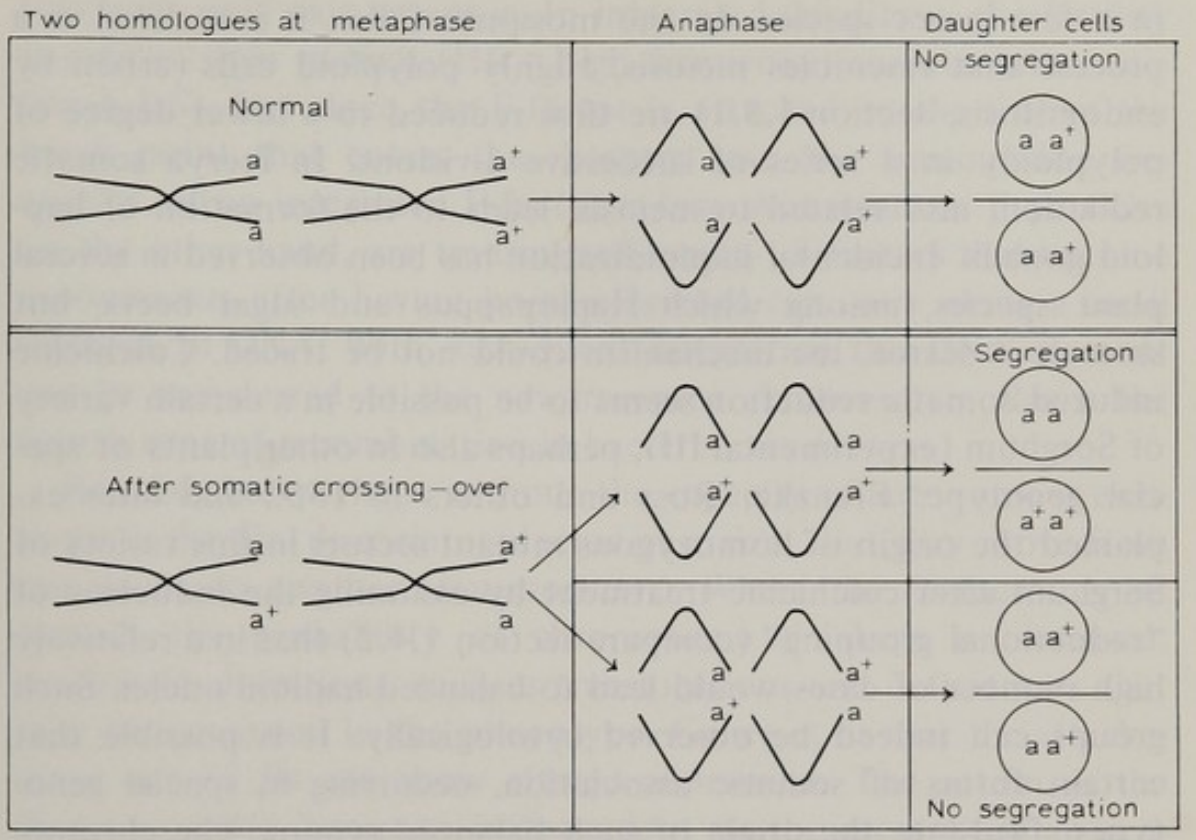


Fig.7.2. After somatic crossing-over the two cross-over chromosomes first remain together, and anaphase separation only separates the chromatids. These chromatids are different, and like with double reduction two recessive alleles can be combined, giving rise to a recognizable sector.

nation, probably caused by crossing-over, has been observed. Although the basis is probably some form of chromosome pairing, the process cannot be compared with meiosis, especially when there is no reduction in number. By *somatic crossing-over* two chromosomes with unequal chromatids are formed. In the following division (fig.7.2), in 50% of the cases two different alleles go to the same daughter nucleus and the original situation is restored. In the other 50% identical alleles go to the same pole, a situation comparable to anaphase II segregation with double reduction (section 5.1.6). Now, in one cell two recessive alleles are present and recognizable, marking the event of crossing-over. As long as a single gene is involved, the distinction with mutation is not easily made.

Somatic reduction occurs as a part of the reproductive system

in a few insect species. In the mosquito Culex it is realized in a process that resembles meiosis. Highly polyploid cells (arisen by endomitosis, section 1.3.1) are thus reduced to a lower degree of polyploidy in a series of successive divisions. In Icerya somatic reduction, also related to meiosis, leads to the formation of haploid gonads. Incidental haploidization has been observed in several plant species, among which Haplopappus and sugar beets, but since it is scarce, the mechanism could not be traced. Colchicine induced somatic reduction seems to be possible in a certain variety of Sorghum (experimental III), perhaps also in other plants of special genotype. Franzke, Ross and others in 1952 and later explained the origin of homozygous mutant sectors in this variety of Sorghum after colchicine treatment by assuming the induction of "reductional grouping" (compare section 1.4.2) that in a relatively high number of cases would lead to balanced haploid nuclei. Such groups can indeed be observed cytologically. It is possible that certain forms of somatic association, occurring in special genotypes, facilitate the origin of such balanced groups. The chromosomes in these haploid groups still have two chromatids, that are separated during the succeeding interphase, eventually leading to homozygous diploid nuclei. Recessive mutants, also induced by the colchicine treatment can then come to expression.

The opposite of reduction, *endomitosis* (section 1.3.1), leading to endopolyploidy, theoretically may also be considered a form of somatic segregation. Actually, any case, spontaneous or induced, of the origin of genetically different sectors in an organism (chimaera, mosaic) can be considered somatic segregation. This is not usually done.

7.3.3. *Episomes*

There is a group of cytoplasmic particles that may either function and reproduce *autonomously*, or *integrated* in a chromosome, at a specific site. When integrated these particles (episomes) follow the chromosomes in reproduction and transmission, but they may affect chromosome behaviour. A well-known example of this type of episome is the fertility (F) factor of the bacterium *Escherichia coli*. When the F-factor is present (the cell is F^+), the bacterium can function as a donor. When it is not present (the cell is F^-) it

functions only as a receptor. In integrated condition, the fertility is greater than normal (Hfr = high frequency recombination, section 6.1.5), but since the F-factor is attached to the side of the break point that enters the receptor last, the transmission frequency of an integrated F-factor is low. Autonomous F particles on the other hand, are transmitted readily. F-factors that become autonomous after having been integrated can carry along a small segment of DNA. With subsequent integration this DNA segment can be transferred to the new receptor chromosome: this is the fourth type of recombination in bacteria: *sexduction*.

Related, but perhaps without the capacity of autonomous existence is the *Ds*-(dissociator-)factor in maize, that may move from one locus to another (*transposition*). Where the *Ds*-factor is attached, the probability of chromosome breakage is increased, leading to deviations, such as translocations and deficiencies that may be expressed in the phenotype. Genes at or near the attachement site of *Ds* can show altered expression. The action of the *Ds*-factor itself, however, is dependent on the presence of an *activator*-factor *Ac* that also has an effect on the gene action of the locus to which it is attached; *Ac* too can be transposed. It is probable that heterochromatin plays a significant role. Several more or less related systems of episomes have been found in maize since McClintock discovered and analysed the *Ac-Ds* system of endosperm mosaics. There seems to be some correspondence between maize and bacterial episomes (Peterson, 1969).

7.4. Variation in chromosome mechanisms

Chromosome mechanisms, mitotic and meiotic, because of their dominant role in the determination of the gametic genotype, and consequently of that of each individual, but equally because of their importance for the evolutionary prospects of the species, occupy a central place in the genetic system of all organisms. In their turn, the chromosome mechanisms are part of the genotype and they have a large potential of variation. The complex of mechanisms that has developed into what is considered to be the stan-

dard system, is merely the most generally applicable variant. Besides this variant, apparently numerous more or less drastically deviating systems offer special advantages under special conditions. By mutation and hybridization, followed by recombination and selection, appreciable adaptations of existing systems are still very well possible, and these continue to give the genetic system opportunities to adjust itself to natural or artificial alterations in the requirements it has to meet.

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* Terms of an unspecific or general character (birds, heterozygons, insects, microorganisms, proteins, reproduction, etc.), and names and symbols of genes, except of man, have not been included.

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