Chromosomes today : proceedings of the third Oxford chromosome conference, September 22-25, 1970 / edited by C.D. Darlington and K.R. Lewis.

Contributors

Oxford Chromosome Conference 1970 : Oxford University)
Darlington, C. D. 1903-1981.
Lewis, Kenneth R.

Publication/Creation

London : Longman, [1972]

Persistent URL

https://wellcomecollection.org/works/uvefq2ug

License and attribution

You have permission to make copies of this work under a Creative Commons, Attribution, Non-commercial license.

Non-commercial use includes private study, academic research, teaching, and other activities that are not primarily intended for, or directed towards, commercial advantage or private monetary compensation. See the Legal Code for further information.

Image source should be attributed as specified in the full catalogue record. If no source is given the image should be attributed to Wellcome Collection.

wellcome collection

Wellcome Collection
183 Euston Road
London NW1 2BE UK
T +44 (0)20 7611 8722
E library@wellcomecollection.org
https://wellcomecollection.org

CHROMOSOMES TODAY

VOLUME 3

EDITED BY

C. D. DARLINGTON & K. R. LEWIS

Chromosome studies are an important aspect of genetical research and the Oxford Chromosome Conferences were inaugurated in 1964 to facilitate discussion and communication between workers engaged in this area of enquiry.

The present volume gives an account of the proceedings of the third international conference in the series which was held at the Botany School and Magdalen College, Oxford, in 1970.

The three years since the second conference have seen the resolution of many old problems and the introduction of new materials and methods for the study of those that remain unresolved. Recent research has also taken cytogenetics into new areas of investigation and furthered the break-down of those barriers of theory and practice which separate biologists both from each other and from scientists in neighbouring disciplines.

This volume records research, on a range of organisms involving a variety of experimental techniques, designed to elucidate problems of herdity and variation at the level of the cell, the individual and the natural population.

The fine structure and molecular organization of the chromosome is considered in relation to its replicative and transcriptive functions. Investigations on DNA, RNA and protein synthesis are included which employ the techniques of cytochemistry, autoradiography and molecular hybridization. The chromosome is treated also as a mechanical entity and its movements during cell division are discussed in the context of both ultra structure and genotypic control. Electron miscroscopy and phase control observations of living cells using time lapse photography are among the methods used in these studies.

The immediate consequences of spontaneous and induced chromosome aberrations are described and the evolutionary exploitation of chromosome mutations is considered in plant and animal populations. Autosomal polymorphism, B chromosomes and sex chromosome mechanisms are considered in this connection.

C. D. Darlington, D.Sc., F.R.S. is a fellow of Magdalen College and recently retired as Sherardian Professor of Botany at Oxford.

K. R. Lewis, M.A., Ph.D., is a University Lecturer at the Botany School, Oxford.

QH 605
DAR

000783

IMPERIAL

CANCER RESEARCH

FUND

LIBRARY

Author: DARLINGTON (C.D.) & LEWIS (K.R.) eds.

Title: Chromosomes today.

Acc. No.	Class Mark	Date	Volume
000783	QH 605	1972	3

CHROMOSOMES TODAY

VOLUME THREE

A Supplement to *Heredity*

CHROMOSOMES TODAY

VOLUME THREE

Proceedings of the Third Oxford Chromosome Conference
September 22-25, 1970

Edited by

C. D. DARLINGTON and K. R. LEWIS

Botany School, Oxford

LONGMAN GROUP LIMITED
JOURNALS DIVISION
LONDON

Longman Group Limited
Journals Division
5 Bentinck Street
London

IMPERIAL CANCER
LINCOLN'S INN
FIELDS
LIBRARY
RESEARCH FUND

QH605
DAR

First printed 1972

1560650

WELLCOME INSTITUTE
LIBRARY
Coll. welMOmec
Call No.
QH

© 1972 The Authors

Printed in Great Britain by
T. and A. Constable Ltd., Edinburgh

CONTENTS

PAGE

Photograph of Conference Members facing page vii

Names and addresses of Conference Members vii

OPENING ADDRESS: The future of the chromosomes. C. D. DARLINGTON 1

I. CHROMOSOME STRUCTURE AND SYNTHESIS

Ultrastructure of the induced lampbrush stage in polytene chromosomes. VEIKKO SORSA 5

Constitutive heterochromatin in *Drosophila melanogaster*. C. BARIGOZZI and C. HALFER 8

Differential DNA replication of the Y chromosome in spermatogonia of the Indian house shrew, *Suncus murinus*. S. R. V. RAO, S. G. A. RAO, V. K. SHARMA and V. C. SHAH 12

Molecular and structural aspects of the endomitotic chromosome cycle in angiosperms. W. NAGL 17

The relationship between nuclear DNA amount and the duration of the mitotic cycle. G. M. EVANS, H. REES, C. L. SNELL and S. SUN 24

DNA content of related species. D. P. FOX 32

The molecular structure of nucleohistone. J. F. PARDON and B. M. RICHARDS 38

Chromosome structure studied by nucleic acid hybridisation in cytological preparations. MARY LOU PARDUE and JOSEPH G. GALL 47

Regulation of RNA synthesis in the evaluation of bread wheat. H. K. JAIN and R. N. RAUT 53

Acidic protein and RNA in the initiation of puff formation. TH. K. H. HOLT 56

II. CHROMOSOME MECHANICS AND MUTATION

Influence of UV microbeam on spindle fine structure and anaphase chromosome movements. ANDREW BAJER 63

Anaphase behaviour of inversions in living crane-fly spermatocytes. ROLAND DIETZ 70

Mode of action of the pairing-limitation gene in wheat (*Triticum aestivum*, 6x). C. J. DRISCOLL, N. L. DARVEY and H. NEWTON BARBER 86

Genetic control of the post-synaptic phase of meiosis in ryegrass. B. S. AHLOOWALIA 93

Localisation of intraspecific variation in meiotic chromosome pairing. J. SYBENGA 104

Non-dividing cells in meristems. F. A. L. CLOWES 110

The action of 2-thiocytosine on the mitosis of human lymphocytes in culture. J. TIMSON and D. J. PRICE 118

Endosperm failure and incompatibility in crosses between *Triticum* and *Secale*. J. P. MOSS 124

Chromosomally marked populations of cells in human bone marrow. SYLVIA D. LAWLER 133

PAGE

Instability of the paracentric region and selective reduplication of chromosome 2 in man. M. Fraccaro, J. Lindsten, L. Tiepolo and N. Ricci 138
On the origin and nature of achromatic lesions. Jyoti P. Chaudhuri 147
The participation of chromosome arms in radiation induced chromosome type exchange. John R. K. Savage 152
The induction of chromosomal aberrations by sulphur mustard in marsupial lymphocytes. D. Scott and T. R. L. Bigger 162

III. CHROMOSOMES IN NATURAL POPULATIONS

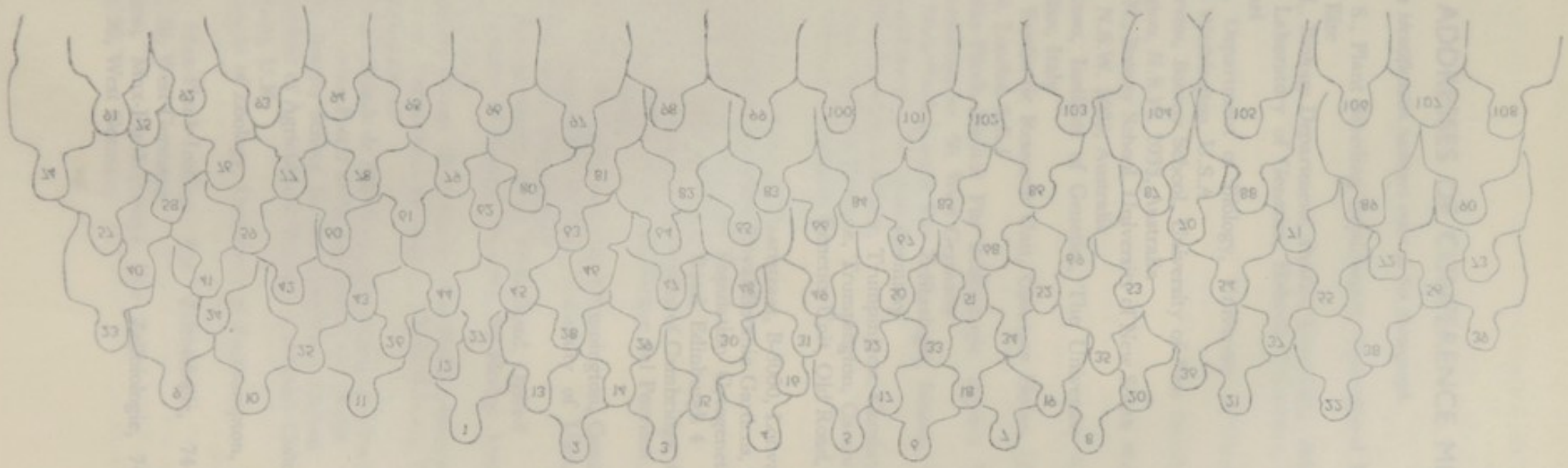
Cytogenetics of a group of morabine grasshoppers with XY and X_1X_2Y males. M. J. D. White and J. Cheney 177
The chromosomes of some chrysomelid Coleoptera: Diabroticites. Stanley G. Smith 197
The structure and role of B chromosomes in the mottled grasshopper. Godfrey Hewitt 208
Population cytogenetics of the Malayan house rat *Rattus rattus diardii* (Rodentia : Muridae). Yong Hoi-sen 223
Hybridisation in nature between two chromosome forms of spiny mice. Jacob Wahrman and Ruth Goitein 228
Cytology of some Indian Asclepiadaceae. Mira Jash and Archana Sharma 238
Polyploidy and chromosome size. A. K. Sharma 248
Meiosis and fertility in apogamously produced diploid plants of *Asplenium trichomanes*. J. Bouharmont 253
Cytogenetic patterns in *Amaranthus*. T. N. Khoshoo and Mohinder Pal 259
Interchanges and interpopulational gene exchange in *Clarkia speciosa*. William Bloom and Harland Lewis 268

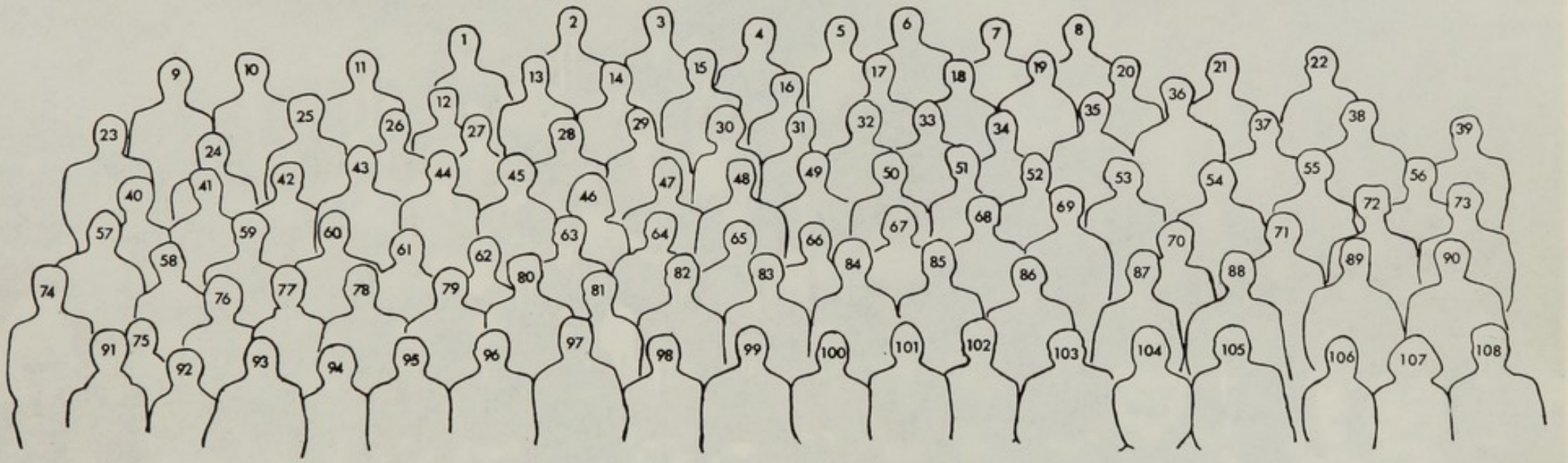
Abstracts of Papers 285

Abstracts of Demonstrations 299

Author and Reference Index 309

Species Index 317




1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108

NAMES AND ADDRESSES OF CONFERENCE MEMBERS

The identification numbers refer to the photograph

100 Ahloowalia, B. S., Plant Breeding Department, Agricultural Institute, Oak Park, Carlow, Eire

39 Ar-Rushdi, A. H., Biology Department, Pahlavi University, Shiraz, Iran

58 Atidia, Judith, Laboratory of Genetics, Hebrew University of Jerusalem, Jerusalem, Israel

7 Bajer, Andrew, Department of Biology, University of Oregon, Eugene, Oregon 97403, Washington, U.S.A.

105 Barber, H. Newton, Botany School, University of New South Wales, P.O. Box 1, Kensington, N.S.W. 2033, Australia

104 Barber, Mrs H. N., Botany School, University of New South Wales, P.O. Box 1, Kensington, N.S.W. 2033, Australia

65 Barigozzi, Claudio, Institute of Genetics, The University of Milan, Via Celoria 10, Milan, Italy

87 Barlow, Peter, Paediatric Research Unit, Cameron House, Guy's Hospital Medical School, London S.E.1

95 Bauer, Hans, Max-Plank Institut Für Zellbiologie, Institut Tübingen, 74 Tübingen, Melanchthonstr. 36, West Germany

94 Bauer, Mrs H., Max-Plank-Institut Für Zellbiologie, Institut Tübingen, 74 Tübingen, Melanchthonstr. 36, West Germany

53 Bayliss, M. W., Plant Breeding Institute, Trumpington, Cambridge

73 Bennett, M. D., Plant Breeding Institute, Trumpington, Cambridge

Bobrow, Martin, M.R.C. Population Genetics Unit, Old Road, Headington, Oxford

82 Bouharmont, J., Institut Carnoy, 24 Vaartstraat, B-3000, Louvain, Belgium

84 Brandham, Peter, Jodrell Laboratory, Royal Botanic Gardens, Kew, Surrey

67 Buckton, K. E., M.R.C. Clinical and Population Cytogenetics Research Unit, Western General Hospital, Crewe Road, Edinburgh 4

62 Buss, M. E., Department of Genetics, University of Cambridge, Cambridge

43 Castro-Sierra, Eduardo, Calle Felipe II, Parque del Porvenir, Bloque Y-4, 2º piso, No. 1, Sevilla, Spain

31 Chapman, Victor, Plant Breeding Institute, Trumpington, Cambridge

14 Chaudhuri, Jyoti, Cytogenetics Section, University of Ulm, D-79 Ulm (Donau) Parkstrasse 10/11, Deutschland

Clowes, F. A. L., Botany School, South Parks Road, Oxford

27 Cohen, Maimon, Division of Human Genetics, 86 Hodge Avenue, Buffalo, New York 14222, U.S.A.

10 Couzin, D. A., Gordon House, 27 Rubislaw Den North, Aberdeen, Scotland

55 Craig-Cameron, T. A., Department of Genetics, The University, P.O. Box 363, Edgbaston, Birmingham 15

33 Cunha, A. B. da, Universidade de São Paulo, Instituto de Biociencias, Departamento de Biologia, Caixa Postal 8105, São Paulo, SP, Brasil

90 Darlington, C. D., Botany School, South Parks Road, Oxford

99 Das, P. K., Department of Agricultural Botany, University College of Wales, Aberystwyth, Wales, U.K.

Dearn, J., Department of Zoology, University of Southampton, Hants., SO9 5NH

6 Dietz, Roland, Max-Plank-Institut Für Zellbiologie, 74 Tübingen, Melanchthonstr. 36, West Germany

5 Dietz, Mrs Isolde, Max-Plank-Institut Für Zellbiologie, 74 Tübingen, Melanchthonstr. 36, West Germany

Ellis, J. R., Department of Botany and Microbiology, University College, London
70 Evans, G. M., Department of Agricultural Botany, University College of Wales, Aberystwyth, Wales, U.K.
68 Faed, Michael, Cytogenetics Laboratory, The University, Dundee, Scotland
106 Fearn, Gillian, Botany School, South Parks Road, Oxford
32 Finch R. A., Plant Breeding Institute, Maris Lane, Cambridge, CB2 2LQ
30 Fischer, P., Institut für Krebsforschung der Universität Wien, 1090 Wien, Borschkegasse 8a, Vienna, Austria
Ford, C. E., M.R.C. Radiobiology Unit, Harwell, Didcot, Berkshire
25 Fox, D. P., Department of Genetics, Marischal College, Aberdeen, AB9 1AS
66 Fraccaro, Marco, Gruppo Euratom, Via Forlanini 14, 27100 Pavia, Italy
64 Fraccaro, Mrs M., Gruppo Euratom, Via Forlanini 14, 27100 Pavia, Italy
23 Freeman, M. G. St Clair, Zoology Department, Southampton University, Southampton
Godward, M. B. E., Department of Plant Biology and Microbiology, Queen Mary College, Mile End Road, London E.1
88 Grant, C. J., Botany Department, Bristol University, BS8 1UG
9 Hall, D. J., Department of Genetics, Marischal College, Aberdeen, AB9 1AS
42 Hansteen, Inger-Lise, Institutt For Generell Og Ekperimentell Patologi, Rikshospitalet, Oslo 1, Norway
45 Hartmann-Goldstein, I., Department of Genetics, The University, Sheffield, 10 2TN
19 Hauschteck-Jungen, E., Zoologisches Museum Der Universität Künstlergasse 16, 8006 Zürich, Schweiz.
20 Hauschteck-Jungen, Mr, Zoologisches Museum Der Universität Künstlergasse 16, 8006 Zürich, Schweiz.
63 Henderson, S. A., Genetics Department, Milton Road, Cambridge
1 Hewitt, Godfrey, School of Biological Sciences, University of East Anglia, Norwich, NOR 88C, U.K.
35 Holt, Thomas, Katholieue Universiteit Afd., Zoology II, Driehuizerweg 200, Nijmegen, The Netherlands
18 Hughes, D. T., Cytogenetics Laboratory, Institute of Child Health, University of London, 30 Guilford Street, London W.C.1
Hultén, M., Department of Clinical Genetics, Karolinska Hospital, Stockholm, Sweden
98 Jain, H. K., Genetics Division, Indian Agricultural Research Institute, New Delhi-12, India
91 Jaworska, H., Tornavagen 13, Lund, Sweden
4 John, Bernard, Zoology Department, University of Southampton, Southampton, Hants., SO9 5NH
3 Jones, G. H., Genetics Department, University of Birmingham, Birmingham 15
69 Jones, John K., Department of Agricultural Botany, The University, London Road, Reading, RG1 5AQ
108 Jones, Keith, Royal Botanic Gardens, Kew, Surrey
71 Jones, R. N., Department of Agricultural Botany, University College of Wales, Aberystwyth, Wales, U.K.
15 Khoshoo, T. N., National Botanic Gardens, Lucknow, India
Klinger, H. P., Department of Pathology, University of Oxford
61 Koopmans, Alie, Genetisch Instituut Kerklaan 30, Haren (Gr.), The Netherlands
96 La Cour, L. F., John Innes Institute, Colney Lane, Norwich
Land, John, Botany School, South Parks Road, Oxford
Lawler, Sylvia, Department of Clinical Research, Royal Marsden Hospital, London S.W.3
101 Lewis, Harlan, 14280 Sunset Blvd., Pacific Palisades, California 90272, U.S.A.

102 Lewis, Mrs H., 14280 Sunset Blvd., Pacific Palisades, California 90272, U.S.A.
74 Lewis, K. R., Botany School, South Parks Road, Oxford
36 Linden, A. G. J. M. van der, Department of Genetics, University of Nijmegen, Driehuizerweg 200, Nijmegen, The Netherlands
78 Marks, G. E., John Innes Institute, Colney Lane, Norwich
49 McLeish, John, John Innes Institute, Colney Lane, Norwich
107 Mirković, D., Jodrell Laboratory, Royal Botanic Gardens, Kew, Surrey
46 Mittwoch, Ursula, Galton Laboratory, University College, Gower Street, London W.C.1
Moore, D. M., Department of Botany, The University, London Road, Reading, RG1 5AQ
89 Moss, J. P., Department of Agricultural Botany, University of Reading, London Road, Reading, RG1 5AQ
60 Mu'azu, Sani, Genetics Section, Botany School, Downing Street, Cambridge, CB2 3EA
21 Nagl, Walter, Institute of Botany, The University, Rennweg 14, Vienna 1030, Austria
83 Newton, L. E., 256 Porters Avenue, Dagenham, Essex
57 Ortiz, Eugenio, Instituto de Genética y Anthropologia, Velázquez 144, Madrid 6, Spain
77 Pardue, Mary Lou, Department of Biology, Yale University, New Haven, Connecticut 06520, U.S.A.
86 Parker, J. S., Plant Biology Department, Queen Mary College, Mile End Road, London E.1
52 Paroda, R. S., Department of Agricultural Botany, University College of Wales, Aberystwyth, Wales, U.K.
44 Patau, Klaus, Genetics Building, University of Wisconsin, Madison, Wisconsin 53706, U.S.A.
Pearson, P. L., M.R.C. Population Genetics Unit, Old Road, Headington, Oxford
Pullen, Margaret, Department of Plant Biology and Microbiology, Queen Mary College, Mile End Road, London E.1
12 Rao, S. R. V., Department of Zoology, University of Delhi, Delhi 7, India
48 Ray-Chaudhuri, S. P., Department of Zoology, Banaras Hindu University, Varanasi 5, India
79 Rees, H., Department of Agricultural Botany, University College of Wales, Aberystwyth, Wales, U.K.
Renwick, J. H., Department of Genetics, University of Glasgow, Scotland
50 Richards, B., Searle Research Laboratories, Lane End Road, High Wycombe, Bucks.
8 Riley, Ralph, Plant Breeding Institute, Trumpington, Cambridge
26 Roper, J. A., Department of Genetics, The University, Sheffield, S10 2TN
Rowley, J. D., Department of Biochemistry, University of Oxford
92 Sansome, Eva, 3 Roydon Road, Diss, Norfolk
59 Sansome, F. W., 3 Roydon Road, Diss, Norfolk
29 Savage, John, M.R.C. Radiobiology Unit, Harwell, Didcot, Berkshire
41 Scott, D., M.R.C. Radiobiology Unit, Harwell, Didcot, Berkshire
75 Sharma, Archana, Department of Botany, University of Calcutta, 35 Ballygunj Circular Road, Calcutta 19, India
47 Sharma, A. K., Department of Botany, University of Calcutta, 35 Ballygunj Circular Road, Calcutta 19, India
24 Shaw, David, Department of Zoology, The University of Southampton, Southampton, Hants.
37 Smith, S. G., Box 490, Sault Ste Marie, Ontario, Canada
103 Smith-White, S., Department of Botany, University of Sidney, Sidney, N.S.W., Australia

51 Snoad, Brian, John Innes Institute, Colney Lane, Norwich, NOR 70F
38 Sorsa, Veikko, Department of Genetics, University of Helsinki, P.-Rautatiekatu 13, Helsinki 10, Finland
11 Southern, D. I., Botany Department, University of Manchester, Manchester, MI3 9PL
22 Sybenga, J., Department of Genetics, Gen. Foulkesweg 53, Wageningen, The Netherlands
Thomas, P. T., Welsh Plant Breeding Station, Plas Gogerddan, Aberystwyth, Cards.
13 Tiepolo, L., Gruppo Euratom, Via Forlanini 14, 27100 Pavia, Italy
72 Timmis, J. N., Department of Agricultural Botany, University College of Wales, Aberystwyth, Wales, U.K.
56 Timson, John, University Department of Medical Genetics, The Royal Infirmary, Manchester, MI3 9WL
40 Vardi, Aliza, Laboratory of Genetics, The Hebrew University of Jerusalem, Israel
97 Vosa, Canio, Botany School, South Parks Road, Oxford
Voss, Ruth, Laboratory of Genetics, The Hebrew University of Jerusalem, Israel
16 Waines, J. Giles, Botany School, South Parks Road, Oxford
28 Wahrman, Jacob, Laboratory of Genetics, The Hebrew University of Jerusalem, Israel
17 Walker, S., Cytogenetics Unit, Nuffield Wing, School of Medicine, University of Liverpool, Liverpool
76 Wargent, Judy, Department of Genetics, The University, Sheffield
2 Wedberg, Hale, Botany Department, San Diego State College, San Diego, California 92115, U.S.A.
54 Westerman, M., Genetics Department, The University, P.O. Box 363, Edgbaston, Birmingham 15
93 White, M. J. D., Department of Genetics, University of Melbourne, Parkville, Victoria 3052, Australia
80 Wolfe, Stephen, Department of Zoology, University of California, Davis, California 95616, U.S.A.
81 Wolfe, Mrs S., Department of Zoology, University of California, Davis, California 95616, U.S.A.
85 Woodell, S. R. J., Botany School, South Parks Road, Oxford
34 Yong, Hoi Sen, Unit of Genetics, School of Biological Sciences, University of Malaya, Kuala Lumpur, Malaysia

IMPERIAL CANCER
LINCOLN'S INN
FIELDS
LIBRARY
RESEARCH FUND

THE FUTURE OF THE CHROMOSOMES

C. D. DARLINGTON
Botany School, Oxford

In the last three years chromosome studies have answered a number of old questions. They have also raised a number of new ones. They have taken us into new fields and they have broken down old barriers of theory or practice that separated us from our neighbours. The scope of this new work (to which I give a few references) concerns discoveries and also the instruments of discovery. Both will I believe be well represented by what we are going to show one another.

At this meeting, we shall cover a range of organisms, of techniques and of experimental methods from whose efficient combination our future work will grow. We shall see the quantity and quality, the distribution, synthesis and interactions of DNA brought into relation with the activity of the cell and the vigour of the organism. We shall also see the connections of these properties with the differentiation of different kinds of chromatin, and of different kinds of chromosomes, A and B, as well as X and Y. And we shall see the different kinds of chromosomes, as wholes as well as in their parts, controlling heredity and variation. In all these things we shall, I have no doubt, try to keep in mind our genetic assumptions with their basis in physics and chemistry and their meaning in development and evolution.

We see that it is a big job; so big that we welcome the attempts that are made on all sides to connect the properties of chromosomes with physical models, with the processes of heredity and development and with the behaviour of animals or man. But in these attempts we face a difficulty. The chromosomes are a world of their own and we need to have our own view of them—a developing view. They are compelled to meet several obligations which are in conflict. They have to meet the momentary needs of the cell. They also have to meet the enduring needs of past and future life. And they have to create the discontinuity between individuals while they sustain the continuity between generations.

How do they do it? They do not, as some of our friends have imagined, obey or submit to the laws of heredity. Nor do they make the laws of heredity obey them—although they come near to doing so. Rather they show us the forms that heredity takes. They show us that heredity is the connectedness of life, the connectedness of which the chromosomes are the instruments and the materials. The view they give us of life is, therefore, as Weismann long ago suggested, and perhaps foresaw, different from what we may reach by other means. Looking at organisms or thinking of molecules offers us a view of temporary existence. It is a flattering and deceptive view. From it the permanence and connectedness of chromosomes can do something to emancipate us. For above the laws they offer us exceptions. And instead of the laws they offer us systems.

Let me give a couple of examples. It would be preposterous today to give any general account of variation or recombination in organisms except in terms of chromosomes. Variation may commit itself either to holding a species together or to breaking it apart. But it would be useless to discuss

how this happens except by seeing what the chromosomes can show us of the foundations of variation and of sterility and of the connections between them. For which purpose, of course, new techniques are continually being put in our hands. Again in future how can we expect to learn more except by reference to the chromosomes in their multiple capacities as the controllers of heredity, the indicators of cytoplasmic reciprocity and the foci of genetic systems?

In these circumstances we ought to ask ourselves how our studies should be connected with the rest of the framework of genetics. Is it our business to fill in the gaps left in the scheme presented to us by experimental breeding? Or must we reconsider large parts of the scheme? These are not, perhaps, the right questions to ask. In order to look at the future we must recall the past. We must go back to the beginning.

At its very beginning, indeed by the nature of living organisms, there were two macroscopic approaches to genetics: the analytical approach of Mendel and the integral approach of Galton. There is no inconsistency between these approaches. But there is an opposition between those who take them and who take their methods and ideas from them. There is an opposition between those who think in terms of genes and those who think in terms of organisms. From this opposition there has grown a gap in the general understanding of genetics and of biology. It is a gap which has rarely been noticed and has never been filled. But it is a gap which long ago split genetics into opposed parts which compete for our acceptance. Neither approach gives us a complete account of the differences between X and Y chromosomes. Or of the differences between the two sexes. Nor can we make the account complete by the direct study of the chromosomes, microscopic or molecular.

Mathematics also makes no bridge for it serves both sides and influences neither. What can we do? Among us also, in our studies of chromosomes, there is a divergence between the analytical and the integral approaches. But they are so close together that no opposition can develop. Our chromosomes require neither pedigrees nor ratios to prove their inheritance. Their continuity was authenticated by the half-century's work of our predecessors. Today, thanks to them, we may say that the chromosomes are self-authenticating. For this reason we can see certain things more clearly than others. We can see where the empty spaces lie in our understanding and how very large they are. Our corrections and interruptions and contradictions are therefore needed for the sound growth of biological understanding as a whole.

These are not problems which we need to pass on at once to our pupils. The need to pass something on must, however, be present to all of us. And with every new discovery the need becomes more obvious. There are scientists, we are told, who do not ask themselves or anyone else what effects their discoveries may have on the world in which they live. To people who have learnt to see connections as clearly as we do this cannot be possible. But to our colleagues this is less clear. I may, therefore, recall what happened in this respect when I came to this university.

When I first looked at chromosomes in 1923 it was not in one of our universities. That was indeed not the place where you were at that time likely to find them. After 30 years of looking at them, and after our knowledge of them, and of things connected with them, had been much advanced,

I saw that the universities had taken very little notice of what had been done. In general, they were (and at the higher levels, they certainly still would be) as willing to accept Lysenko's opinions of heredity as to accept ours. A dangerous situation for everybody. It was therefore in order to explain what we knew about chromosomes and heredity to those who could use that knowledge that in 1953 I thought it well to come to Oxford.

What did I find here? Every year I had been in the habit of sending a microscope slide to Oxford for the instruction of the Honours students in Zoology. It was always the same slide showing the control of crossing over at the first metaphase of meiosis. The material was the pollen mother cells of *Fritillaria meleagris*, a plant which roots and flowers on the banks of the Cherwell. The ideas that might have been conveyed by these chromosomes had not however taken root and when I arrived here this annual ceremony lapsed. Zoologists now graduated without having seen the chromosomes. For at that time no communication between sovereign departments was held to be practicable. It was only after 10 years' further discussion that botanists and zoologists in Oxford agreed to attend the same lectures and see together the same chromosomes of plants and of animals. Some day, no doubt after long delays and grievous misunderstandings, this obvious exchange may be extended to those who wish to study medicine or in other ways investigate the properties of man.

I hope we shall not have to wait too long. Because for me—why should I conceal it?—the study of chromosomes has been the means of lighting up the past history of man as well as of other animals. For others, I believe, it could be equally useful in revealing his and their future. But it will depend on you and on no one else to explain these things to those who are willing to listen.

References

BENNETT, M. D., AND REES, H. 1969. Induced and developmental variation in chromosomes of meristematic cells. *Chromosoma*, *27*, 226-244.

BODMER, W. F. 1970. The evolutionary significance of recombination in prokaryotes. *Symp. Soc. Gen. Microb.*, *20*, 279-294.

CASPERSSON, T. *et al.* 1969. Chemical differentiation with fluorescent alkylating agents in *Vicia faba* metaphase chromosomes. *Exp. Cell. Res.*, *58*, 128-140.

DARLINGTON, C. D. 1971. The evolution of polymorphic systems. *Ecological Genetics and Evolution* (ed. R. Creed). Blackwell, Oxford.

GIBSON, IAN, AND HEWITT, G. 1970. Isolation of DNA from B chromosomes in grasshoppers. *Nature*, *225*, 67-68.

HALFOR, C. *et al.* 1969. Timing of DNA replication of translocated Y chromosome sections in somatic cells of *Drosophila melanogaster*. *Chromosoma*, *27*, 395-408.

HARRIS, H. 1968. *Nucleus and Cytoplasm*. Oxford U.P.

JONES, R. N., AND REES, H. 1969. An anomalous variation due to B chromosomes in rye. *Heredity*, *24*, 265-271.

NAGL, W. 1970. Temperature-dependent functional structures in the polytene chromosomes of *Phaseolus*, etc. *J. Cell. Sci.*, *6*, 87-107.

NITSCH, J. P., AND NITSCH, C. 1969. Haploid plants from pollen grains. *Science*, *163*, 85-87.

NODA, SHOZO. 1968. Achiasmate bivalent formation by parallel pairing in PMCs of *Fritillaria amabilis*. *Bot. Mag. Tokyo*, *81*, 344-345.

PAVAN, C., AND DA CUNHA, A. B. 1969. Chromosomal activities in *Rhynchosciara* and other *Sciaridae*. *An. Rev. Gen.*, *3*, 425-450.

PEARSON, P. L. *et al.* 1970. Technique for identifying Y chromosomes in human interphase nuclei. *Nature*, *226*, 78-80.

SANDLER, L. *et al.* 1968. Mutants affecting meiosis in natural populations of *Drosophila melanogaster*. *Genetics*, *60*, 525-558.

SORSA, VEIKKO. 1970. Chromosome duplication and crossing over as illustrated by some recent chromosome models. *Ann. Ac. Sci. Fenn.*, *A:4*, 159.

SUNDERLAND, N., AND WICKS, F. M. 1969. Cultivation of haploid plants from tobacco pollen. *Nature*, *224*, 1227-1229.

TANAKA, R. 1969. Deheterochromatinisation of the chromosomes in *Spiranthes sinensis*. *Jap. J. Genet.*, *44*, 291-296.

VOSA, C. G. 1970. Heterochromatin recognition with fluorochromes. *Chromosoma*, *30*, 366-372.

YOSHIDA, M. C., AND EPHRUSSI, B. 1967. Hybrids between somatic mouse cells *in vitro*. *J. Cell. Phys.*, *69*, 33-39.

ULTRASTRUCTURE OF THE INDUCED LAMPBRUSH STAGE IN POLYTENE CHROMOSOMES

VEIKKO SORSA
Department of Genetics, Helsinki, Finland

1. Introduction

The banding pattern of salivary gland chromosomes provides clear evidence of a certain regularity in the linear organisation of the chromosome fibrils. The fine structure or organisation of an individual band is, however, very difficult to demonstrate even by means of the electron microscope. Occasionally, a distinct coiling in the band fibrils can be recognised (*e.g.* Sorsa and Sorsa, 1968*a*), and in very special cases uncoiling of an individual chromomere can be observed. This can be even more evident in electron micrographs taken from the puff regions in the salivary gland chromosomes (*e.g.* Sorsa, 1969*b*).

2. Results

For about 30 years it has been known that the normal organisation of the salivary gland chromosomes can be transformed into a lampbrush type by using a strong urea-alkali treatment (Calvin, Kodani and Goldschmidt, 1940; Kodani, 1941, 1942; Painter, 1941). An essential feature, which always appears in urea-alkali treated polytene chromosomes, is a clear central axis (*cf.* plate I, figs. 1-2). The preservation of the longitudinal fibrils following the removal of proteins and the complete uncoiling of the chromomeres points to a rather strong linear continuity of the axis, at least in certain parts of the salivary gland chromosomes.

After repeating the interesting works of Kodani and others, and extending them to the ultrastructural level (Sorsa *et al.*, 1970*a*, *b*; Sorsa and Sorsa, 1970), some experimental work was carried out in order to study the role of the urea and alkali components separately in the transition process of the salivary gland chromosomes. These experiments and their main results are presented schematically in fig. 1.

They indicate that saturated urea alone obviously causes a breaking of the banding pattern into individual chromomeres and, owing to this, a swelling of the whole chromosome. During the treatment, small chromomeres disappear completely through total uncoiling, but no signs of the formation of the central axis can be noticed (*cf.* plate I, figs. 3 and 4).

Sodium hydroxide, on the other hand, seems to have a specific effect on the uncoiling process of the chromomeres, and reveals a clear lampbrush image with a diffuse zone around the treated salivary gland chromosomes (plate II, fig. 5). The central axis with a large amount of a thin fibrillar network is also apparent during alkali treatment (plate II, fig. 6), but the longitudinal fibres in the core are not so clear as after the urea-alkali treatment (*cf.* plate I, figs. 1 and 2).

If an equal amount of sodium hydroxide is added after ten minutes pretreatment in saturated urea, the whole banding pattern of the salivary

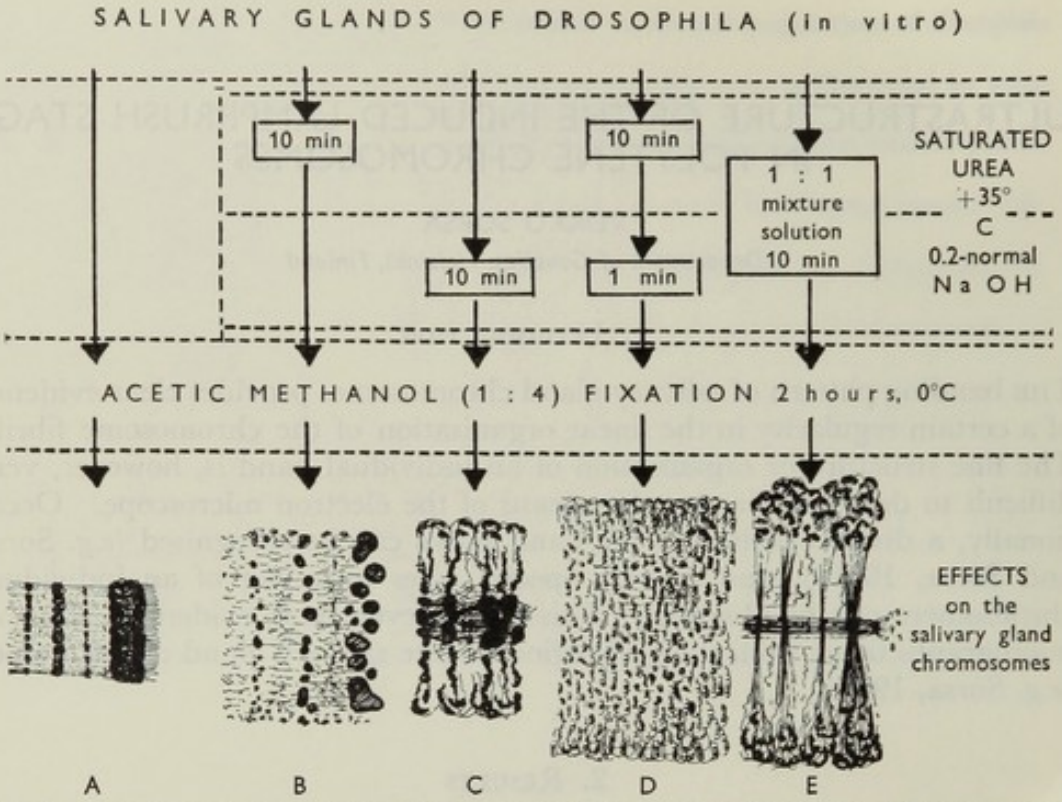


FIG. 1.—Schematic representation of the experiments and the effects revealed by the treatments on the salivary gland chromosomes of *Drosophila melanogaster*.

chromosomes is totally transformed into a fibrillar network (plate II, figs. 7 and 8). During this combined treatment, the polytene chromosomes are obviously at first dispersed into separate chromonemata, and only after this are the chromomeres of each chromonemata uncoiled as a result of the added hydroxide.

3. Summary

1. The effects of urea and sodium hydroxide, when used in *in vitro* experiments on salivary gland chromosomes of *Drosophila*, are strikingly different, probably because they remove different compounds from the chromosomes. Whether these are acidic proteins or histones or both, is still an open question.

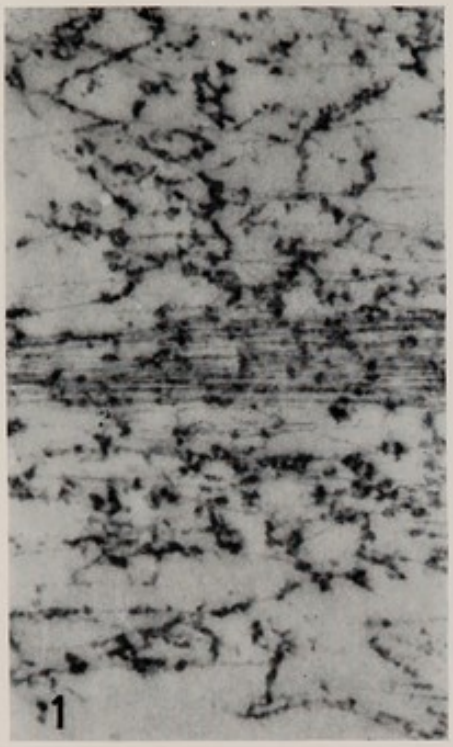
2. The hydroxide affects the formation of the distinct central axis in the polytene chromosomes. After treatment in a mixture of urea and alkali solutions, the central axis is revealed especially clearly. However, if a pretreatment in saturated urea is carried out separately, the axial core fails to appear.

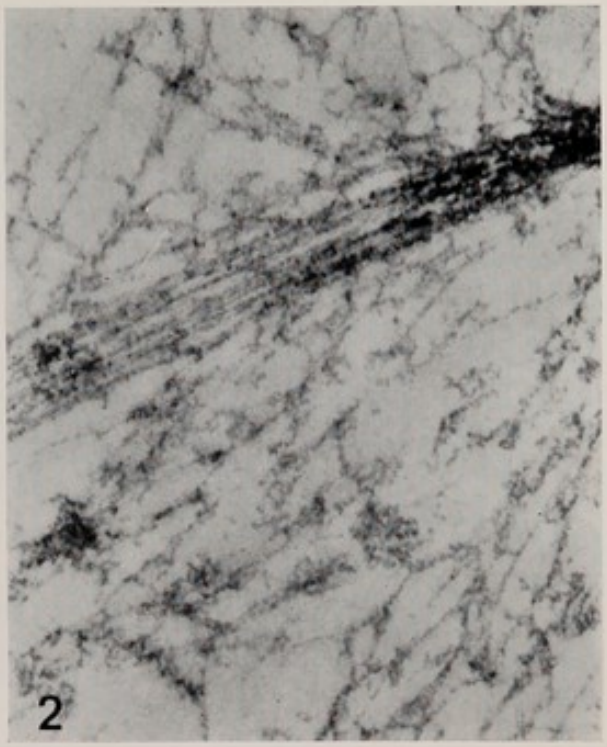
4. References

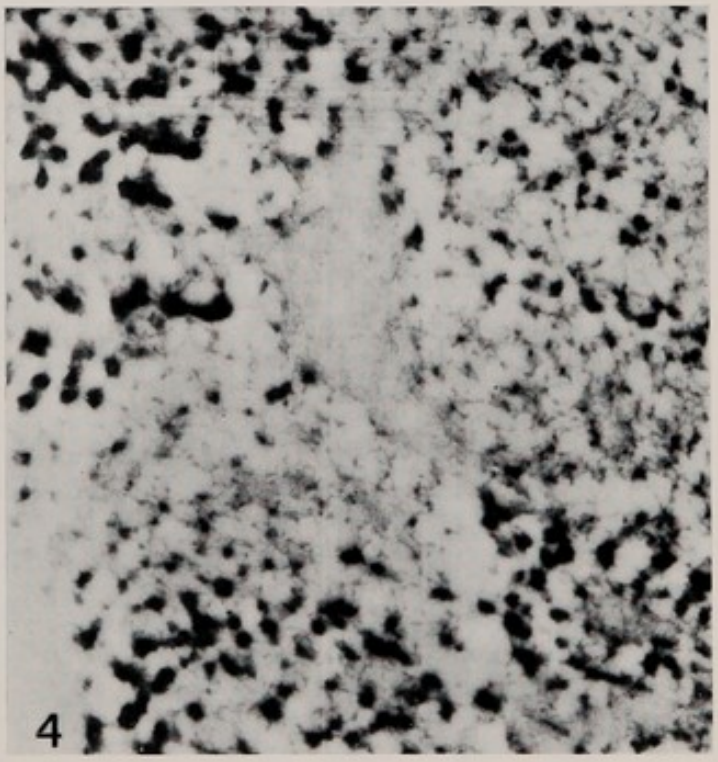
CALVIN, M., KODANI, M., AND GOLDSCHMIDT, R. 1940. Effects of certain chemical treatments on the morphology of salivary gland chromosomes and their interpretation. *Proc. Nat. Acad. Sci., U.S.A., 26,* 340-349.

KODANI, M. 1941. The structure of the salivary gland chromosomes of *Drosophila melanogaster*. I. The eu- and heterochromatin. *J. Hered., 32,* 146-156.

KODANI, M. 1942. The structure of the salivary gland chromosomes of *Drosophila melanogaster*.

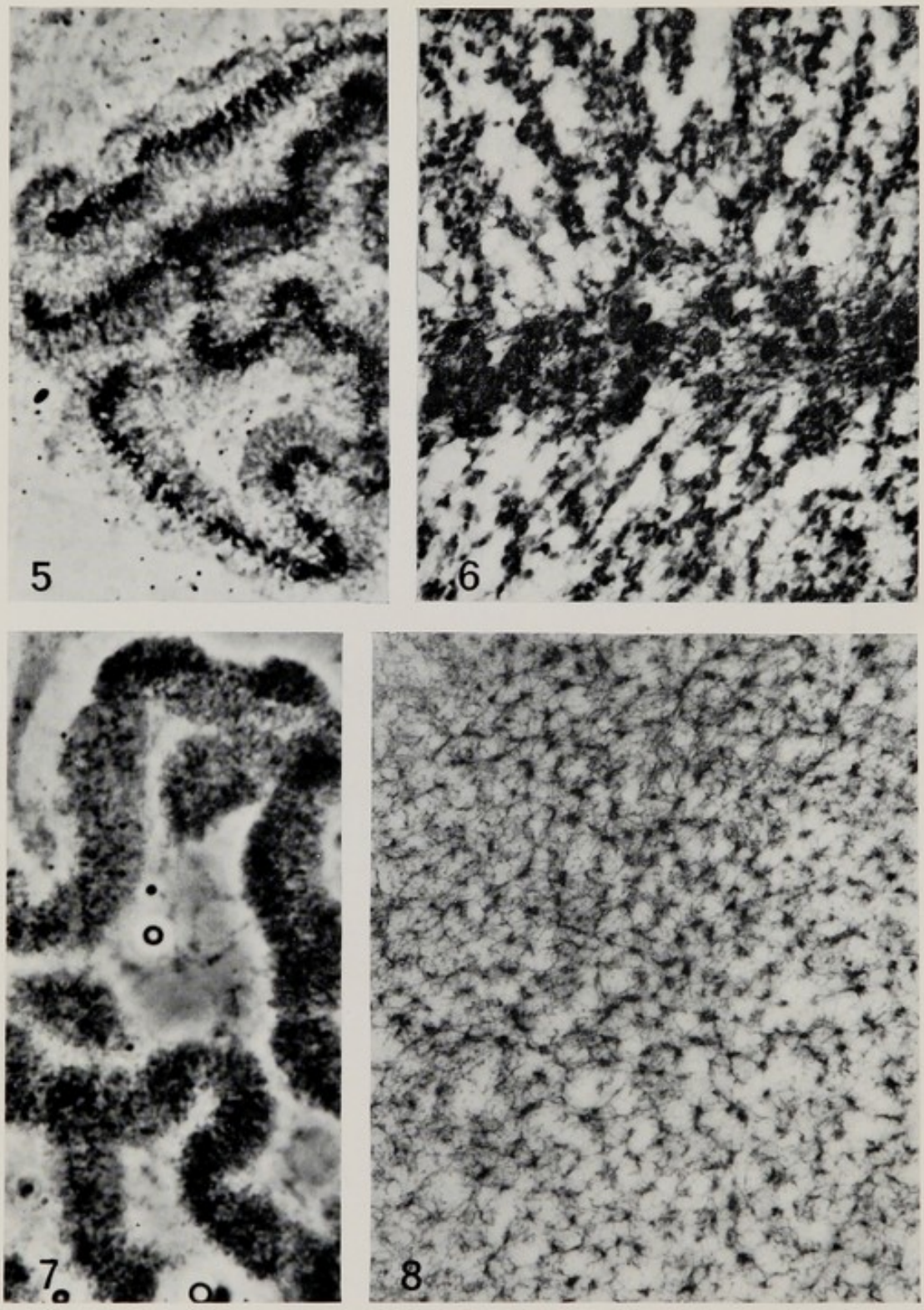






FIGS. 1 and 2.—Electron micrographs of the central axis in urea-alkali treated salivary gland chromosomes of *Drosophila*. Chromosomes fixed in acetic methanol and stained with 5 per cent. uranyl acetate (UA) (fig. 1), and 5 per cent. indium dichloride (fig. 2) in absolute methanol for three days after dehydration. Magnification c. × 20,000.

FIGS. 3 and 4.—Salivary gland chromosomes of *Drosophila* after urea treatment. Fig. 3. Photomicrograph, acetocarmine with acetic hematoxylin stain, magnification c. × 2500. Fig. 4. Electron micrograph, UA stain, magnification c. × 8000.



FIGS. 5 and 6.—Salivary gland chromosomes of *Drosophila* after treatment in sodium hydroxide. Fig. 5. Photomicrograph, magnification c. × 2000. Fig. 6. Electron micrograph, magnification c. × 30,000.

FIGS. 7 and 8.—Salivary gland chromosomes of *Drosophila* after treatment in saturated urea and sodium hydroxide. Fig. 7. Photomicrograph, magnification c. × 2000. Fig. 8. Electron micrograph, magnification c. × 30,000.

II. Effects of urea-alkali mixture and other chemicals on the structure of salivary gland chromosomes. *J. Hered.*, *33*, 115-133.

PAINTER, T. S. 1941. An experimental study of salivary chromosomes. *Cold Spring Harb. Symp. quant. Biol.*, *9*, 47-54.

SORSA, M. 1969. Ultrastructure of puffs in the proximal part of the chromosome 3R in *Drosophila melanogaster*. *Ann. Acad. Sci. fenn. A. IV*, *150*, 1-21.

SORSA, M., AND SORSA, V. 1968. Electron microscopic studies on band regions in Drosophila salivary chromosomes. *Ann. Acad. Sci. fenn. A IV*, *127*, 1-8.

SORSA, V., AND SORSA, M. 1970. Ultrastructure of induced transitions in the chromatin organization of Drosophila polytene chromosomes. *Chromosoma*, *61*, 346-355.

SORSA, V., PUSA, K., VIRRANKOSKI, V., AND SORSA, M. 1970*a*. Electron microscopy of an induced lampbrush stage of the polytene chromosomes. *Exptl Cell Res.*, *60*, 466-469.

SORSA, V., SORSA, M., VIRRANKOSKI, V., AND PUSA, K. 1970*b*. An electron microscopic study on alkali-urea treated salivary gland chromosomes of Drosophila. *Ann. Acad. Sci. fenn. A IV*, *166*, 1-10.

CONSTITUTIVE HETEROCHROMATIN IN *DROSOPHILA MELANOGASTER*

C. BARIGOZZI and C. HALFER
Istituto di Genetica, dell' Università, Milano, Italia

1. Introduction

Cell culture is now generally recognised as the most efficient way of obtaining preparations of mitotic chromosomes for high resolution observations. Until recently, however, this technique has been usefully used only in higher animals and invertebrates have given very poor results.

The techniques found by Horikawa and Fox (1964) permit the short term culture of embryonic *Drosophila* cells and they allow the utilisation of all the technical procedures routinely used for preparing mitotic stages in vertebrates (treatment with hypotonic solution and air drying). The analysis of the mitotic chromosomes of *Drosophila* which, hitherto, has been almost completely restricted to observations on neuroblasts, can thus be extended.

This paper discusses the problem of constitutive heterochromatin, whose existence in *Drosophila* has been denied by both Cooper (1959) and Brown (1966), but demonstrated in the guinea pig by Yunis and Yasmineh (1970). The evidence against the existence of entire chromosomes or sections of chromosomes characterised by both stable heteropycnosis and late DNA replication (called heterochromatic) in all the cells of the organism in contrast to facultative heterochromatin (heteropycnotic and late replicating in only some differentiated cells) rests upon comparative observations between embryonic and differentiated cells. Taking into consideration the description of Cooper (1959) of the Y chromosome in neuroblast metaphases of *D. melanogaster* without use of autoradiography, we made parallel observations on cells from 6-hour embryos cultured for 20 hours in a medium supplemented with tritiated thymidine for the last 4 hours. This procedure permitted the examination of cells which were in G_1, S or G_2 when the culture was started; during *in vitro* culture only one mitotic cycle can occur (Dolfini and Tiepolo, 1968). Technical data are not reported here (for reference see Halfer *et al.*, 1969).

2. Results

The observation of metaphase chromosomes of cultured embryonic cells, taken from the wild stock Varese have revealed four main facts: (i) heteropycnosis is very clear and allows the identification of all the heterochromatic components of the set, *i.e.* the Y chromosome in the male cells, a portion of the X and all centromeric sections of the large autosomes; (ii) all heteropycnotic parts replicate later than the euchromatic ones; (iii) the 4th pair of chromosomes shows such a strong incorporation of tritiated thymidine, simultaneously with the other heterochromatic parts, as to give the impression either that the heterochromatic component of the dot-like pair is consider-

able, or that the entire 4th chromosomes replicate later than the remaining euchromatin; (iv) the Y chromosome is composed of five blocks, which correspond entirely to those described by Cooper in the neuroblast.

We shall confine our comments to the last point, since the first and second have been already discussed elsewhere (1966, 1967, 1969) and the third does not permit us to reach a definite answer for the moment.

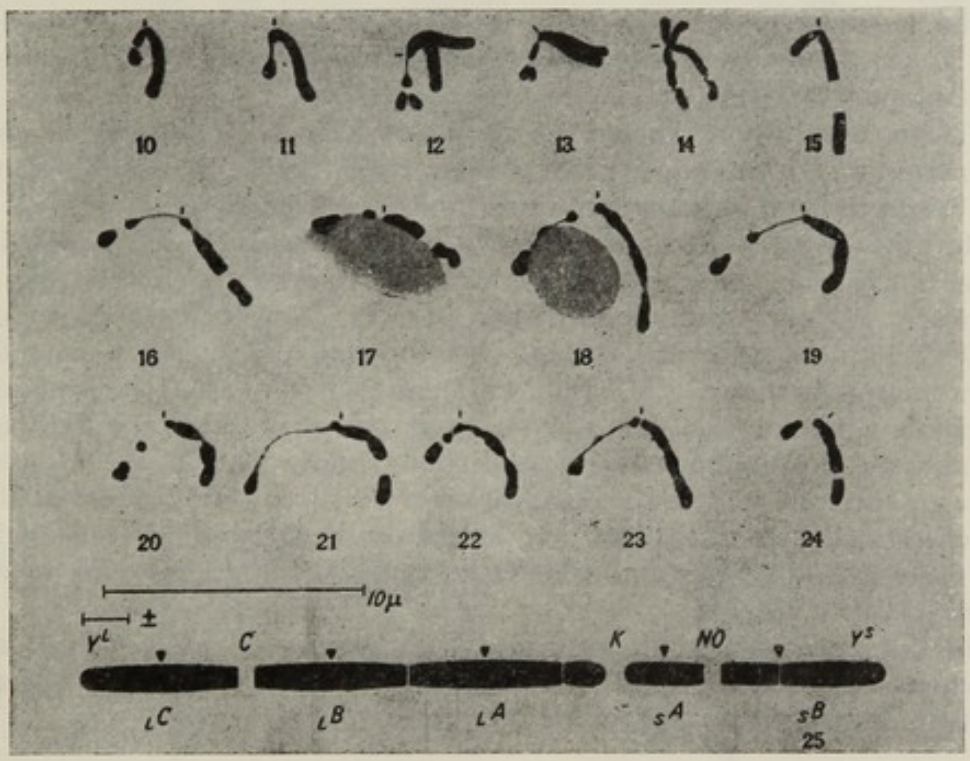


FIG. 1.—Different aspects of the Y chromosome in neuroblasts. Below, a diagram of the structure of the Y chromosome (from Cooper).

The five blocks described by Cooper are summarised in the scheme (fig. 1) which shows three blocks in the long arm, the first of which (LA) shows additionally a constriction near the free end. Between the second and the third block there is a rather broad secondary constriction. The short arm comprises two blocks: the nucleolar organiser is localised in the middle of the proximal block. Our observations completely confirm the description. Plate I, fig. 1 shows the five blocks in a prometaphase and fig. 2 also shows the main secondary constriction C. Fig. 3 clearly shows the two blocks of the short arm, while the three blocks of the long arm are packed, and reveal only two portions. In fig. 4 the high degree of condensation allows us to distinguish only the two arms. A similar variability of pattern is seen also in Cooper's illustrations.

The evidences obtained seem sufficient to demonstrate that both in embryonic (undifferentiated cells or those only at the beginning of differentiation) and in differentiated cells (neuroblasts) heterochromatin shows the same microscopic details. To these data can be added those kindly supplied by Dolfini, who made a cytological control of the established line K_c obtained and propagated by Echalier and Ohanessian (1969). In this case all the cells of a two-year-old line derived from 12-hour embryos show exactly the same distribution of hetero- and eu-chromatin as the embryonic

cells previously described, with the only difference that the structural analysis of the Y, which is here fragmented (the acentric fragment is probably translocated to the X), has not been pursued sufficiently to reveal the existence of blocks (plate I, fig. 5). Thus, in neuroblasts, embryonic cells and an established line from embryonic cells, both heteropycnosis and late DNA replication are present without exception.

The last point we wish to make, in order to emphasise the constance of the heteropycnotic pattern as inherent to heterochromatin, concerns the Y chromosome in stocks carrying a translocation affecting the Y chromosome.

Three cases of well-known translocations have been analysed in detail (Sterilizer, T(Y; 3) and T(Y; 2), Halfer *et al.*, 1969). A fourth involving the Y and the 2nd chromosome is still under investigation.

In all four cases the detectability of heterochromatin under the microscope is clear-cut, even when the translocated portion is shorter than the short arm of the Y chromosome (" Sterilizer "). Autoradiographic analysis of DNA replication reveals that the heterochromatin replicates later than euchromatin (Barigozzi *et al.*, 1966, 1967), and, in most cases, incorporation of tritiated thymidine occurs so quickly along the chromosome axis as to simulate a full synchrony in the whole body (plate II, fig. 6). However, there are rare cases where the distribution of the silver grains is uneven along the chromosome (plate II, fig. 7). When the Y chromosome is split into two portions and translocated (Halfer *et al.*, 1969, 1970), a clear asynchrony arises (plate II, fig. 8).

In this phenomenon the centromere plays no role, while a portion of the chromosome which is probably located in the 2nd block of the long arm seems to be critical for controlling the DNA replication time.

3. Concluding remarks

Our findings demonstrate that in *D. melanogaster* constitutive heterochromatin exists, distinguishable from euchromatin by its stable heteropycnosis and late DNA replication, in both undifferentiated and differentiated cells, even when embryonic cells have multiplied *in vitro* for two years, without any differentiation. Changes in replication time as they occur in the translocations studied, are never reflected by changes in heteropycnosis. This situation, as it can be studied at microscopical level, is doubtless the result of molecular conditions which we still ignore almost completely, as far as *Drosophila* is concerned. Recent work on the guinea pig (Yunis and Yasmineh, 1970) has shown that constitutive heterochromatin is characterised by satellite DNA, present in a heavy and in a light form. Whether this situation holds for *Drosophila* is still unclear, since satellite DNA in this species has not yet been detected (Ritossa, personal communication); therefore satellite DNA might not be an indispensable condition for heteropycnosis and late DNA replication.

Drosophila and guinea pig, however, can hardly be compared because in *Drosophila melanogaster* several activities of the Y chromosome have been discovered (fertility genes, effect on cell size, etc) while nothing is known about the genetic activity of the constitutive heterochromatin of the guinea pig.

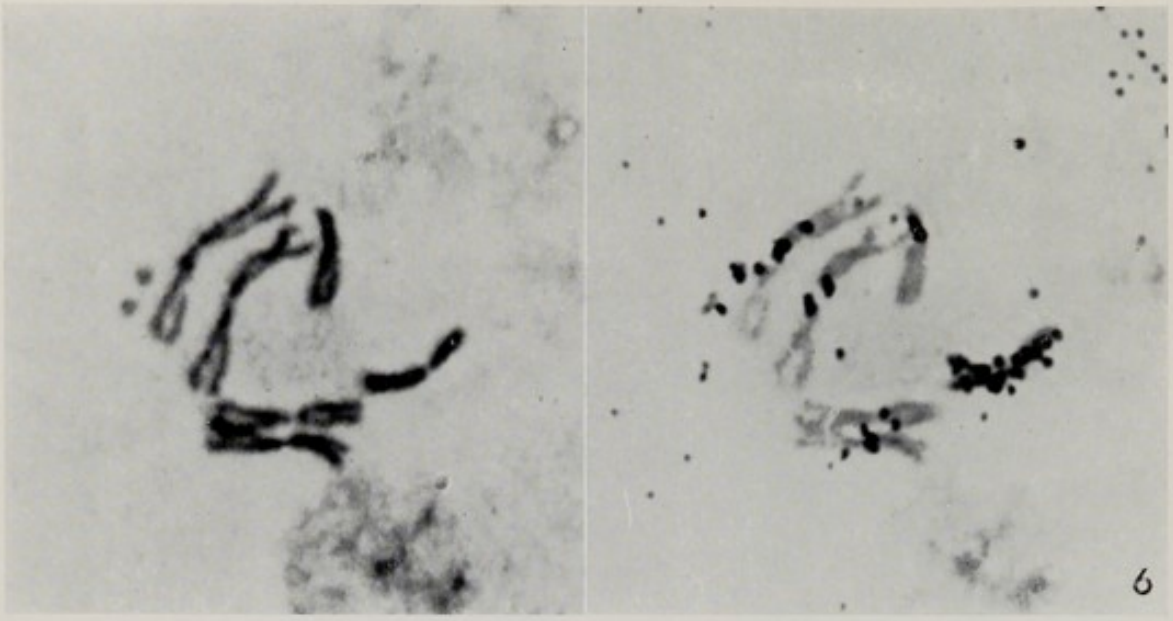
FIG. 1.—Metaphase of embryonic cell *in vitro*: the Y shows five chromatin blocks.

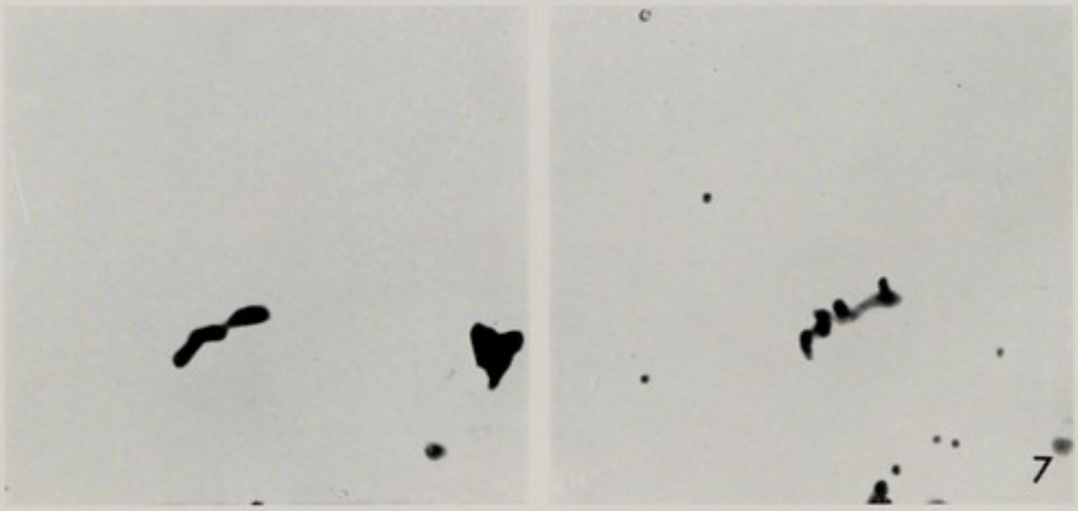
FIG. 2.—*idem*; the Y shows five blocks and the main constriction of the long arm. Heteropycnosis is particularly prominent.

FIG. 3.—*idem*; the Y shows four blocks.

FIG. 4.—*idem*; the Y (highly contracted) shows only the two arms.

FIG. 5.—Metaphase of a cell of an established cell line. The Y is heteropycnotic as is the heterochromatic portion of the X (Courtesy of Miss S. Dolfini).





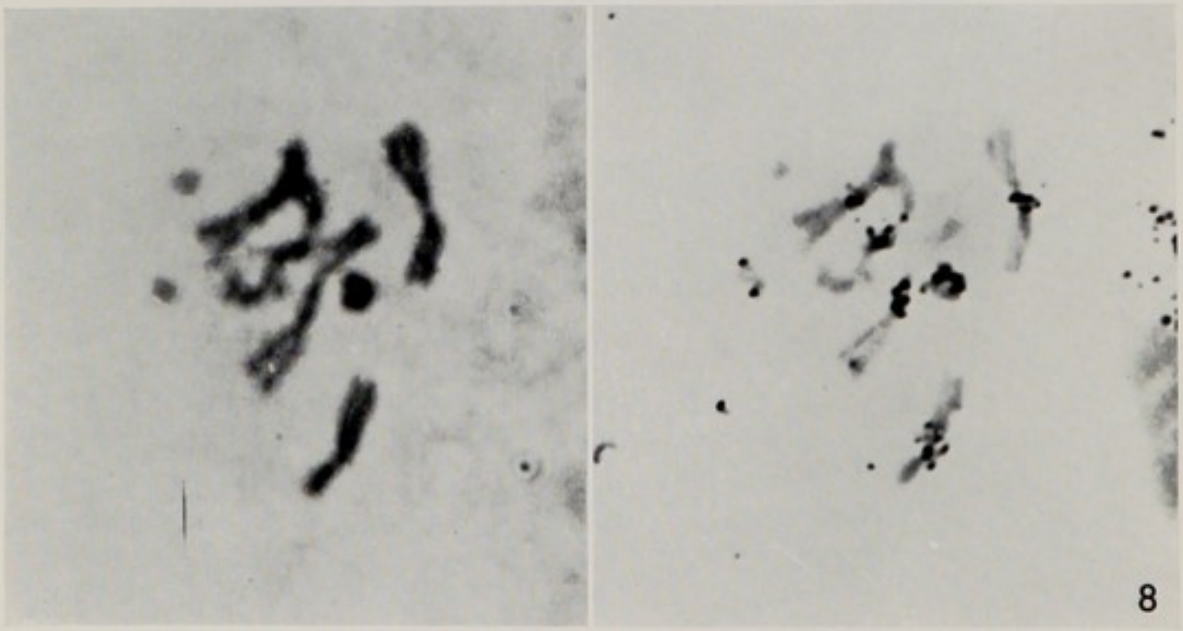


FIG. 6.—*Left*, metaphase of embryonic cell *in vitro*; *right*, the distribution pattern of silver grains in an autoradiograph, showing late DNA replication of all heterochromatic portions.

FIG. 7.—*Left*, Y chromosome (highly contracted) from a metaphase of an embryonic cell *in vitro*; *right*, the corresponding autoradiograph.

FIG. 8.—*Left*, metaphase of a "Sterilizer" cell *in vitro* showing the ring-shaped centric portion of the Y and an acentric portion translocated on the X (heterochromatic part); *right*, the corresponding autoradiograph showing asynchrony in replication between the two Y chromosome portions: the ring-shaped part is hot, while the acentric one (on the X) is cold (Halfer *et al.*, 1969).

4. Summary

1. Constitutive heterochromatin, distinguishable from euchromatin by its stable heteropycnosis and late DNA replication, has been demonstrated in differentiated and undifferentiated cells of *D. melanogaster*.

2. A change in replication time following translocation is not accompanied by changes in heteropycnosity.

Note Added in Proof

Recent investigations cast some doubt on the interpretation of the heterochromatic centric fragment as Y chromosome material. The heteropycnosis of the heterochromatin in established cell lines, however, is confirmed.

5. References

BARIGOZZI, C., DOLFINI, S., FRACCARO, M., REZZONICO RAIMONDI, G., AND TIEPOLO, L. 1966. *In vitro* study of the DNA replication patterns of somatic chromosomes of *Drosophila melanogaster*. *Exp. Cell. Res.*, *43*, 232-234.

BARIGOZZI, C., DOLFINI, S., FRACCARO, M., HALFER, C., REZZONICO RAIMONDI, G., AND TIEPOLO, L. 1967. A first approach to the somatic cell genetics of *Drosophila melanogaster*: studies on DNA replication. *Atti Ass. Gen. Ital.*, *12*, 291-300.

BROWN, S. W. 1966. Heterochromatin. *Sciences*, *151*, 417-425.

COOPER, K. W. 1959. Cytogenetics analysis of major heterochromatic elements (especially Xh and Y) in *Drosophila melanogaster* and the theory of " heterochromatin ". *Chromosoma*, *10*, 535-588.

DOLFINI, S., AND TIEPOLO, L. 1968. The cell cycle of embryonic cells of *Drosophila melanogaster* cultured " in vitro ". *Proc. 2nd Intern. Coll. Inv. Tissue Cult., Istituto Lombardo*, 182-188.

ECHALIER, G., AND OHANESSIAN, A. 1969. Isolement, en cultures in vitro, de lignées cellulaires diploïdes de *Drosophila melanogaster*. *C.R. Acad. Sci. Paris*, *268*, 1771-1773.

HALFER, C., TIEPOLO, L., BARIGOZZI, C., AND FRACCARO, M. 1969. Timing of DNA replication of translocated Y chromosome sections in somatic cells of *Drosophila melanogaster*. *Chromosoma*, *27*, 395-408.

HALFER, C., TIEPOLO, L., BARIGOZZI, C., AND FRACCARO, M. 1970. Sequenza temporale nella replica del DNA nel cromosoma Y di cellule somatiche di *D. melanogaster*. *Atti Ass. Genet. Ital.*, *15*, 111-129.

HORIKAWA, M., AND FOX, A. S. 1964. Culture of embryonic cells of *Drosophila melanogaster* in vitro. *Science*, *154*, 1437-1439.

YUNIS, J. J., AND YASMINEH, W. G. 1970. Satellite DNA in constitutive heterochromatin of the guinea pig. *Science*, *168*, 263-265.

DIFFERENTIAL DNA REPLICATION OF THE Y CHROMOSOME IN THE SPERMATOGONIA OF THE INDIAN HOUSE SHREW, *SUNCUS MURINUS**

S. R. V. RAO, S. G. A. RAO, V. K. SHARMA and V. C. SHAH
Department of Zoology, University of Delhi, Delhi-7, India

1. Introduction

The Y chromosome in somatic tissues of mammals (Galton and Holt, 1964, 1965; Gartler and Burt, 1964; Utakoji and Hsu, 1965; Rao *et al.*, 1968; Sharma *et al.*, 1970) including man (Schmid, 1963; Hsu *et al.*, 1964; Bianchi and de Bianchi, 1965) has been shown to synthesise DNA during late S phase. The only exception, as far as we are aware, is found in the somatic tissues of the Indian house shrew, *Suncus murinus*, where the Y synthesises DNA earlier than the rest of the autosomes (Rao *et al.*, 1970). On the other hand, there are conflicting reports of the behaviour of Y in the spermatogonial cells. Utakoji and Hsu (1965) reported in the Chinese hamster, an early replicating Y chromosome (differential pattern), whereas Fraccaro *et al.* (1969) have shown a late replicating Y (somatic pattern) in the same animal. The studies of Ghosal and Mukherjee (1966), and Mukherjee and Ghosal (1969) have shown the existence of both patterns of replication. Our studies on the DNA synthesis in the spermatogonia of *Suncus murinus* show the existence of two populations, one population showing early replication (somatic pattern) and the other late replication of long arm of Y chromosome.

2. Materials and methods

The animals were collected from the environs of Delhi. Ten male shrews weighing 40-60 gm. were given intraperitoneal injections of ^{3}H-thymidine (sp. act. 6·4 C/mM; Bhabha Atomic Research Centre, Bombay) at a concentration of 2 μc/gm. body weight. The animals were sacrified at 2-hour intervals, up to 20 hours. Colchicine was injected intraperitoneally 2 hours prior to each harvest. The spermatogonial as well as meiotic preparations were made as follows: the tubules were released in distilled water, kept for 10 minutes at room temperature and then transferred to acetic alcohol (1 : 3) fixative. The tubules were allowed to remain in the fixative for 1 hour, transferred to 45 per cent. acetic acid and thoroughly teased to release the cells. The cells were pelleted by centrifugation at 400 r.p.m. for 5 minutes, resuspended in acetic alcohol (1 : 3) and air dried preparations were made.

For autoradiography the slides were dipped in Kodak NTB-3 nuclear track emulsion, kept for 6-8 weeks in light tight plastic boxes and developed in Kodak D-19b developer for 3 minutes, fixed in acid fixer for 10 minutes and stained with buffered Giemsa at *p*H 5·75.

* This work was supported in part by grants from the Indian National Science Academy Atomic Energy Commission, India and Award No. 69.19 E-4 from the Population Council New York.

3. Results

The diploid chromosome number of 40, reported for somatic tissues (Sharma *et al.*, 1969), has been confirmed from the spermatogonial metaphases. The X and Y chromosomes are easily identifiable. The X chromosome is of duplicate type. In metaphases with well extended chromosomes one can find differences in the arm length of X. The Y chromosome, which is nearly two-thirds of the X in size, is the second largest submetacentric in the complement (Rao *et al.*, 1970).

During early prophase of meiosis, the sex bivalent lies within the sex vesicle but later resolves into X and Y chromosomes. A nucleolus is seen at the distal segment of the long arm of the Y chromosome (plate, fig. 1). At this stage a chiasmatic association can be seen between them (plate, figs. 1 and 2). This association persists till diplotene and diakinesis and seems to be confined to the short arms (plate, fig. 6, a-d). Terminalisation of the chiasma results in an end-to-end association (plate, fig. 3). The first meiotic metaphases show a chromosome number of $n = 20$ (plate, fig. 3). The first division is segregational for the sex chromosomes (plate, figs. 4 and 5).

Replication pattern

No labelled spermatogonial metaphases were observed in 0-2 hour and 2-4 hour samples. Later samples at 4-6 hours showed 3·4 per cent. of metaphases labelled. The long arm of Y chromosome comprising a nucleolar region was found to be heavily labelled (plate, fig. 7) in one-third of the labelled metaphases. The grain count data revealed that 18 per cent. of the total number of grains on the metaphase chromosomes were present on this segment. Samples at 6-8 hours (57·5 per cent. labelled metaphases) showed some unlabelled Y chromosomes (plate, fig. 8) and others heavily labelled in the long arm. An unlabelled Y chromosome was found in 36 per cent. while a heavily labelled Y was found in 5 per cent. of the total labelled metaphases. In 8-10 hour samples, 56 per cent. metaphases were labelled; 13 per cent. of these had a heavy label over the long arm of Y chromosome. In all these samples the DNA replication in the X chromosome and short arm of Y was similar to the autosomes. No unusual labelling pattern, generally found in organisms with a duplicate X chromosome, was observed during the late S period. In 10-12, 12-14 and 14-16 hour samples we have not observed any asynchrony in the Y chromosomes. This uniform labelling over the autosomes and sex chromosomes reflects the middle S period of the cells when ^{3}H-thymidine was available. A second wave of asynchrony of the Y chromosome was observed in 16-18 hour and 18-20 hour samples where the Y again appeared as unlabelled in 32·5 per cent. of the labelled metaphases (table 1). These metaphases showed single chromatid label. However, up to this time a heavily labelled single chromatid Y was not seen.

4. Discussion

In all mammals so far studied the Y chromosome has been shown to be late replicating in the somatic cells (Galton and Holt, 1964, 1965; Gartler and Burt, 1964; Utakoji and Hsu, 1965; Schmid, 1963; Hsu *et al.*,

TABLE 1

Labelling pattern

Time of harvest	Y	X	A	Percentage labelled metaphases	Total metaphases counted
4-6 h.	1. Distal segment of long arm heavy label	Light label on one of the arms and around the centromere, like autosomes	Light label	3·4	505
6-8 h.	1. No label on both arms	Light label around the centromere, like autosomes			
	2. Heavy label on distal segment of long arm	Light label on one arm and centromere like autosomes	Light label	57	178
8-10 h.	1. Heavy label on the distal segment of long arm	Light label around centromere and one arm, like autosomes	Light label	56	292
	2. No label	Light label around the centromere, like autosomes			
10-12 h. to 14-16 h.	Uniformly labelled along with autosomes		Medium to heavy	63	108
16-18 h.	No label	Light label around the centromere, like autosomes	Light label	75·5	219
	2. Uniform label as on autosomes	Uniform label as on autosomes	Medium to heavy		
18-20 h.	1. No label	Light label around the centromere, like autosomes	Light label		
	2. Uniform label as on autosomes	Uniform label as on autosomes	Medium to heavy	72·8	125

1964; Bianchi and de Bianchi, 1965; Rao *et al.*, 1968; Sharma *et al.*, 1970). In spermatogonial cells, however, a differential replication behaviour has been reported (Utakoji and Hsu, 1965; Mukherjee and Ghosal, 1969). Our studies on the house shrew show the late replicating long arm of the Y chromosome as heavily labelled in samples collected from very late S. Samples collected at slightly later hours (6-8 hours), still confined to the late S period, showed early replication of the entire Y chromosome. Thus the two types of replication pattern occurring at different times of late S represent the existence of at least two different populations of spermatogonial cells, differing in their G_2+P durations. The studies of Monesi (1962) in mouse also indicate the presence of more than one kind of spermatogonial population, having different durations of DNA synthetic and post-

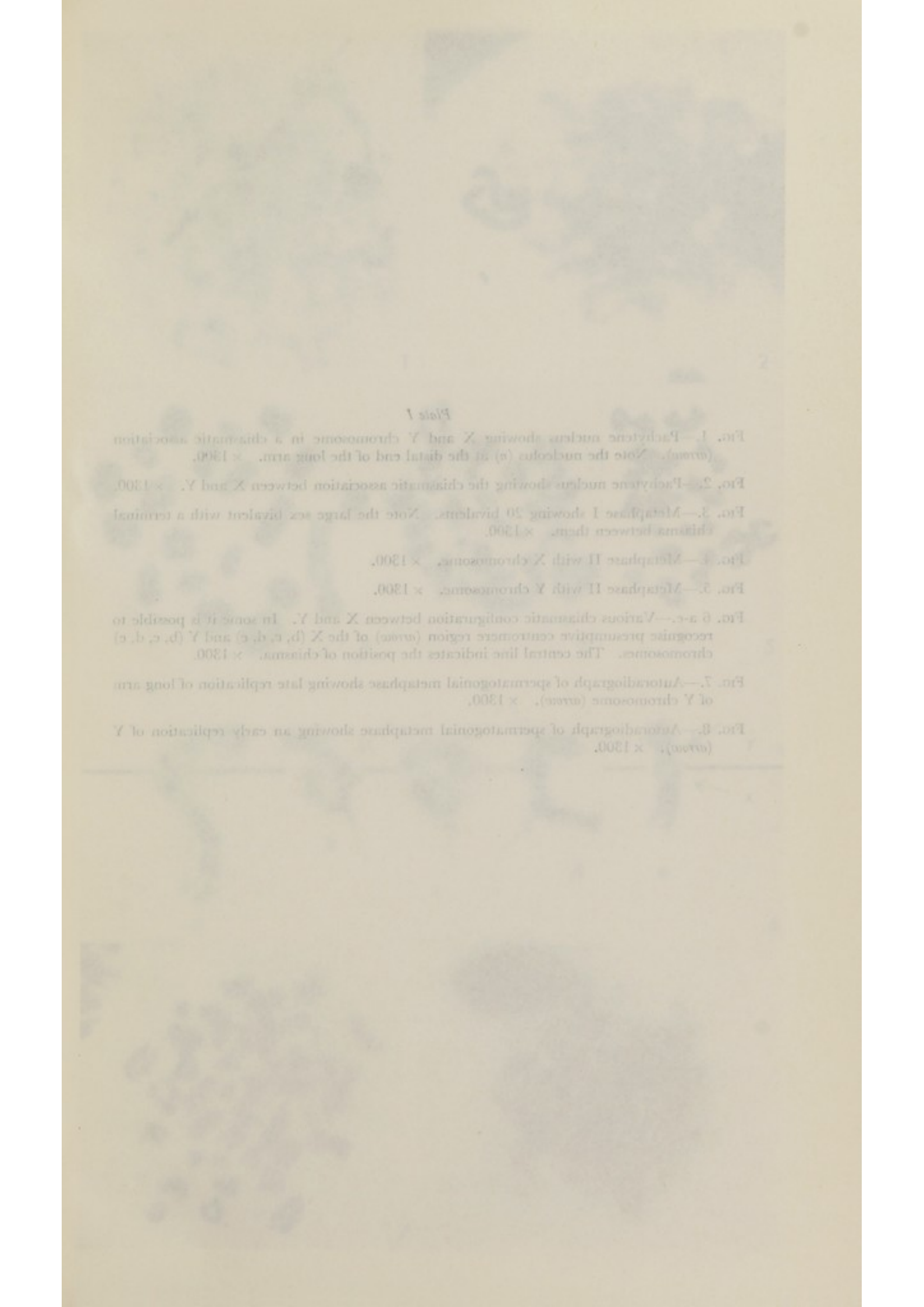


Plate I

FIG. 1.—Pachytene nucleus showing X and Y chromosome in a chiasmatic association (*arrow*). Note the nucleolus (*n*) at the distal end of the long arm. × 1300.

FIG. 2.—Pachytene nucleus showing the chiasmatic association between X and Y. × 1300.

FIG. 3.—Metaphase I showing 20 bivalents. Note the large sex bivalent with a terminal chiasma between them. × 1300.

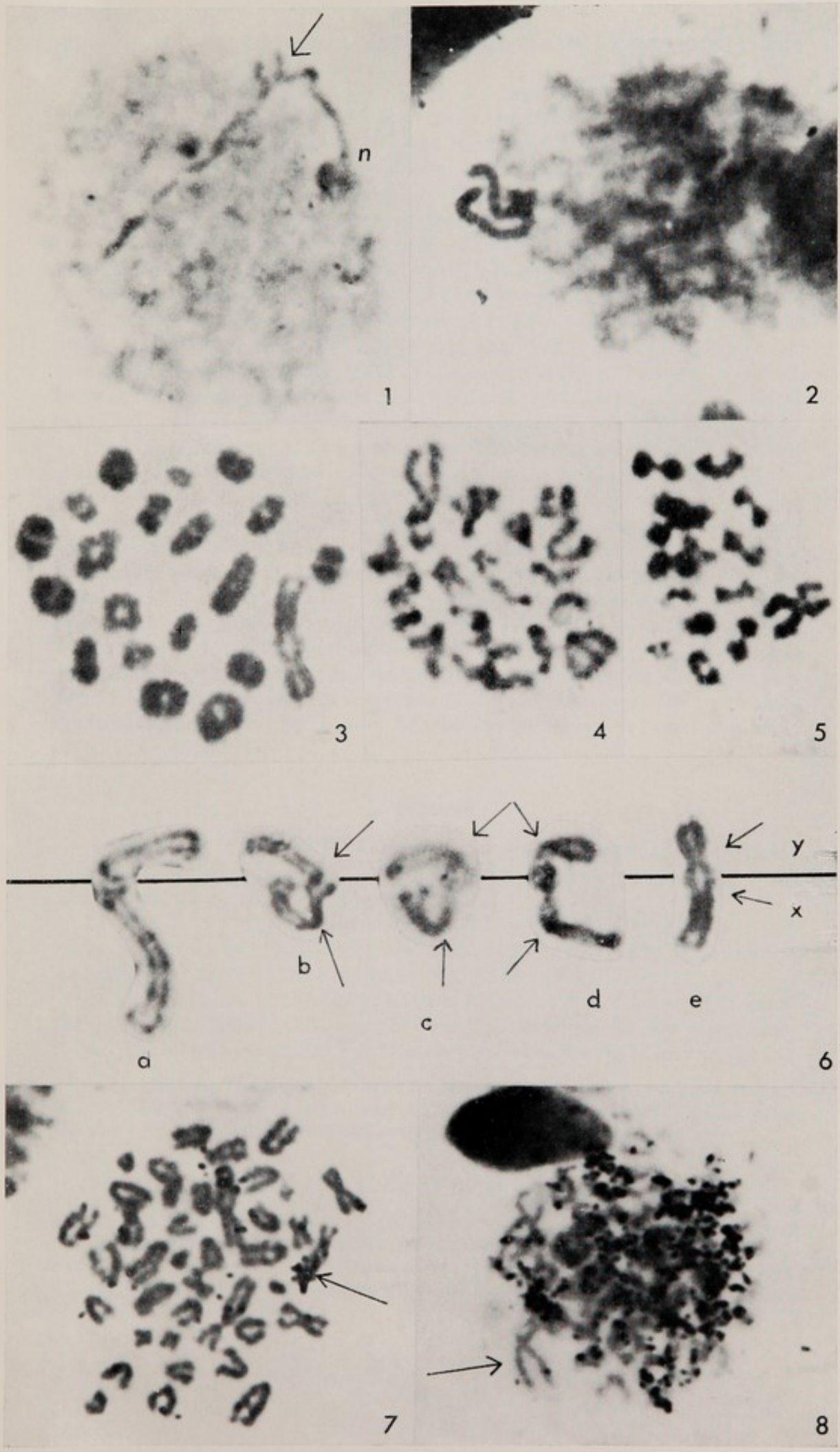
FIG. 4.—Metaphase II with X chromosome. × 1300.

FIG. 5.—Metaphase II with Y chromosome. × 1300.

FIG. 6 a-e.—Various chiasmatic configuration between X and Y. In some it is possible to recognise presumptive centromere region (*arrow*) of the X (b, c, d, e) and Y (b, c, d, e) chromosomes. The central line indicates the position of chiasma. × 1300.

FIG. 7.—Autoradiograph of spermatogonial metaphase showing late replication of long arm of Y chromosome (*arrow*). × 1300.

FIG. 8.—Autoradiograph of spermatogonial metaphase showing an early replication of Y (*arrow*). × 1300.


n
1
2
3
4
5
y
x
b
c
d
e
a
6
7
8

synthetic periods. A similar situation exists in the Chinese and golden hamster (Mukherjee and Ghosal, 1969).

Utakoji and Hsu (1965), who were the first to demonstrate an early replicating Y chromosome in the spermatogonial cells of the Chinese hamster, considered this reversal of the replication from late (somatic type) to early in spermatogonial cells as reflecting a change from genetic inactivity to genetic activity. Fraccaro *et al.* (1969), however, did not find any early replicating Y chromosome in the spermatogonia of the same animal. They reported a late replicating Y chromosome in the spermatogonial cells of the Chinese hamster. Our studies on the spermatogonia of the house shrew have clearly indicated the presence of both patterns. It is interesting that the Y chromosome in somatic tissues of *Suncus* is early replicating (Rao *et al.*, 1970). If, as assumed by Utakoji and Hsu (1965), early replication is indicative of genetic activity, the Y chromosome in *Suncus* seems to be active both in the somatic and certain populations of spermatogonial cells. However, the presence of a population of spermatogonial cells with a late replicating Y segment, comprising a nucleolus forming region, indicates that this segment probably remains inactive. This dual behavioural pattern of the Y poses an interesting problem as to its role in spermatogenesis.

On the basis of replicative behaviour we can comment on the homology between X and Y chromosomes. In somatic cells the Y chromosome is early replicating while the X chromosome finishes DNA synthesis along with the autosomes (Rao *et al.*, 1970). In the spermatogonial cells both the somatic pattern (early replication of Y) and differential pattern (late replicating long arm of Y) are seen. From a comparison of all these we can assume that the long arm of the Y is the differential segment and only the short arm can pair with either the short or long arm of the X. However, the cytological configurations (plate, fig. 6, *a-e*) favour the homology between the short arm of X and Y.

5. Summary

1. Terminal stages of DNA synthesis in the submetacentric duplicate type X and submetacentric Y chromosomes of *Suncus murinus* in the spermatogonia were studied autoradiographically.

2. The X chromosome synthesises DNA synchronously with the autosomes in spermatogonia. The Y replicates early in some cells and in others its long arm replicates later than the rest (differential pattern).

3. A reversal in the synthetic pattern occurs in the Y from early replication to late replication in a certain cell population of the spermatogonia.

4. The short arm of the Y is homologous with the short arm of the X.

Acknowledgments.—We wish to acknowledge the continued encouragement of Professor B. R. Seshachar, Head of the Department of Zoology, University of Delhi. Our thanks are also due to Mr E. A. Daniels for photography.

6. References

BIANCHI, N. O., AND DE BIANCHI, M. S. A. 1965. DNA replication sequence of human chromosomes in blood cultures. *Chromosoma, 17*, 273-290.

FRACCARO, M., GUSTAVSSON, I., HULTEN, M., LINDSTEN, J., AND TIEPOLO, L. 1969. Late replicating Y chromosome in spermatogonia of the Chinese hamster (*Cricetulus griseus*), *Cytogenetics, 8*, 263-271.

GALTON, M., AND HOLT, S. F. 1964. DNA replication patterns of the sex chromosomes in somatic cells of Syrian hamster. *Cytogenetics*, *3*, 97-111.

GALTON, M., AND HOLT, S. F. 1965. Asynchronous replication of the mouse chromosomes. *Expt. Cell Res.*, *37*, 111-116.

GARTLER, S. M., AND BURT, B. 1964. Replication pattern of bovine sex chromosomes in cell culture. *Cytogenetics*, *3*, 135-142.

GHOSAL, S. K., AND MUKHERJEE, B. B. 1966. Abstract: Third International Congress of Human Genetics, Chicago.

HSU, T. C., SCHMID, W., AND STUBBLEFIELD, E. 1964. DNA replication sequences in higher animals. In *The Role of Chromosomes in Development* (ed. M. Locke), pp. 83-112. Academic Press, New York.

MONESI, V. 1962. Autoradiographic study of DNA synthesis and the cell cycle in spermatogonia and spermatocytes of the mouse testis using ^{3}H-thymidine. *J. Cell Biol.*, *14*, 1-18.

MUKHERJEE, B. B., AND GHOSAL, S. K. 1969. Replicative differentiation of mammalian sex chromosomes during spermatogenesis. *Exp. Cell. Res.*, *54*, 101-106.

RAO, S. R. V., SHAH, V. C., AND SESHADRI, C. 1968. Autoradiographic studies of the sex chromosomes of the Indian Gerbil, *Tatera indica cuverii* (Waterhouse) and its bearing on the Lyon Hypothesis. *Chromosoma, Berl.*, *23*, 309-316.

RAO, S. R. V., SHARMA, V. K., AND SHAH, V. C. 1970. DNA synthesis in the duplicate type sex chromosomes of the Indian house shrew, *Suncus murinus* (Insectivora). *Cytogenetics*, *9*, 384-395.

SCHMID, W. 1963. DNA replication patterns of human chromosomes. *Cytogenetics*, *2*, 175-193.

SHARMA, V. K., RAO, S. R. V., AND SHAH, V. C. 1969. Chromosomes of the house shrew, *Suncus murinus* (Insectivora). *Curr. Sci.*, *38*, 367-369.

SHARMA, V. K., RAO, S. R. V., AND SHAH, V. C. 1970. Autoradiographic studies on DNA synthesis in sex chromosomes of five striped squirrel, *Funambulus pennanti* (Wroughton). *Ind. J. Exp. Biol.*, *8*, 249-254.

UTAKOJI, T., AND HSU, T. C. 1965. DNA replication patterns in somatic and germ line cells of the male Chinese hamster. *Cytogenetics*, *4*, 295-315.

MOLECULAR AND STRUCTURAL ASPECTS OF THE ENDOMITOTIC CHROMOSOME CYCLE IN ANGIOSPERMS*

W. NAGL
Institute of Botany, The University, 1030 Vienna, Austria

1. Introduction

The endomitotic chromosome cycle is widespread in various tissues and organs of angiosperms, and results in a high percentage of endopolyploid nuclei in mature plants (reviews by Geitler, 1948, 1953; D'Amato, 1952, 1964; Tschermak-Woess, 1956, 1963, 1971; Torrey, 1965; Street, 1966; Wardlaw, 1968). The endomitotic cycle differs from the mitotic one in that mitosis is stopped during very early prophase, a stage which has been called "*Zerstäubung*" (dispersion stage) by Heitz (1929). This dispersion stage, during which the chromocentres are in the decondensed state, is the most striking feature of plant endomitosis (Geitler, 1948, 1953; Tschermak-Woess and Hasitschka, 1953; Tschermak-Woess, 1959; Nagl, 1965, 1968, 1970*c*, *d*). Because of the structural change, endomitosis is different from endoreduplication or interreduplication, but it is a proreduplication in the sense of Hsu and Moorhead (1956). The highest levels of endopolyploidy known in angiosperms are found in cells of the ovule (antipods, synergids, endosperm haustoria, suspensors), where 4096-ploidy (Nagl, 1962, 1970*a*) and even 32,768-ploidy can be reached (Erbrich, 1965).

2. Materials and methods

The studies on the endomitotic cycle presented in this paper were made in the root tip of *Allium* and the suspensor of *Phaseolus*. Endomitosis regularly succeeds mitosis, in the differentiating region of the root (Tschermak-Woess, 1956). The species *Allium carinatum* and *A. flavum* exhibit nuclei with several large chromocentres and allow, therefore, an easy detection of endomitotic events (Nagl, 1968, 1970*c*, *e*). The suspensor cell nuclei of *Phaseolus coccineus* and *Ph. vulgaris* are very suitable for studies of higher levels of endopolyploidy and of the formation of plant polytene chromosomes (Nagl, 1962, 1965, 1969, 1970*a*, *b*).

The methods of fixing, staining, preparation, volumetry, cytophotometric measurements of DNA (Feulgen) content, radioautography, and treatments by actinomycin D and histone f1 are published already (Nagl, 1967, 1968, 1970*c*, *d*).

3. Nuclear structure

As in the mitotic interphase, the nuclei of *Allium carinatum* and *A. flavum* exhibit small euchromatic elements and several distinct chromocentres in the G_1 stage of the endomitotic cycle. However, they display a denser euchromatic structure in form of enlarged and more intensely staining elements as well as increased masses of heterochromatin in G_2 (plate, fig. 1;

* Presented as a demonstration at the conference.

compare Nagl, 1968, 1970*c*, *d*, *e*). The relation between changing chromatin structure and interphase stage can be ascertained by volumetric, cytophotometric and radioautographic methods (Nagl, 1968, 1970*c*, *e*). The different nuclear structure in G_1 and G_2 makes it possible to distinguish nuclei with the same DNA content, with respect to their position in the endomitotic chromosome cycles (*e.g.* diploid nuclei in G_2 and tetraploid nuclei in G_1).

During endomitosis the chromocentres decondense (= dispersion stage; plate, fig. 2). As soon as the nuclei move on to stage G_1 of the following cycle, the chromocentres appear again, but in the shape of double-chromocentres. The euchromatic elements show the size and stainability of the G_1 state. Evidently the G_1 structure of the nuclei is restored without chromosome coiling and redistribution. It can be assumed that the structural change between the dispersion stage and G_1 is the morphological expression of chromatid separation. Therefore this section of the cycle might be called " stage of new organisation " (Nagl, 1968). Since the stages of dispersion and of new organisation are like those found in early mitotic prophase, endomitosis of angiosperms can be regarded as a short-cut of the chromosome cycle during early prophase (fig. 1).

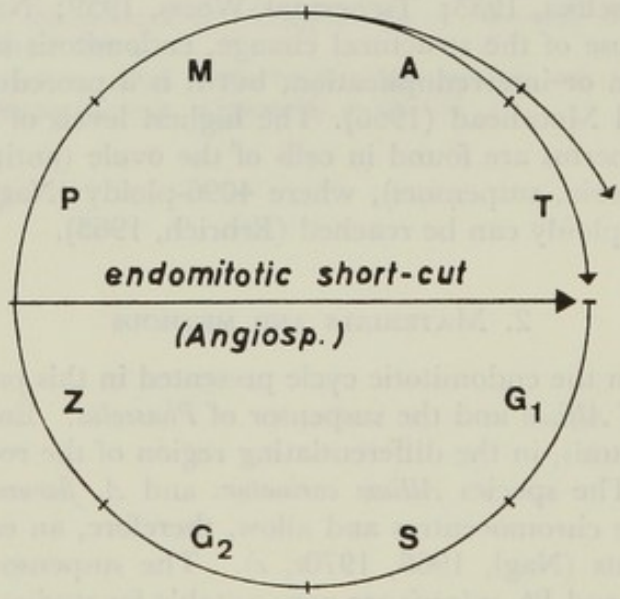


FIG. 1.—The endomitotic short-cut of the chromosome cycle in angiosperms. G_1, presynthetic gap; S, DNA replication period; G_2, post-synthetic gap; Z, dispersion stage (" *Zerstäubung* ") of very early prophase; P, prophase; M, metaphase; A, anaphase; T, telophase. Arrows indicate the direction of the cycles.

The failure of chromosome coiling and redistribution in the endomitotic cycle produces a striking interphase structure, polytene chromosomes, *i.e.* bundles of identical sister chromosomes (plate, fig. 3, compare Tschermak-Woess, 1963; Nagl, 1965, 1969, 1970*a*, *b*). If polyploidisation occurs by a different way, *e.g.* by colchicine treatment, polytene chromosomes cannot arise owing to the metaphasic condensation of the chromosomes and with it the far separation of the chromatids. Since endomitosis in *Heteroptera* shows prophasic chromosome condensation (Geitler, 1948, 1953), no giant chromosomes can be found in this group of animals. But they can be found in *Diptera*, because the nuclei of salivary gland cells (and others) grow without chromosome coiling (compare Beermann, 1962; Nagl, 1970*b*). However,

Description of Plate

Enlargement of all figures × 1200. Fixation: ethanol : acetic acid = 3 : 1; staining methods: 1, Feulgen; 2, aceto-carmine; 4-7, toluidine blue 0; 3, 6 and 7 = phase contrast.

1. Differentiating region of the root of *Allium fauxe*: Tetraploid nucleus in G_1 (above) and one in G_2 (below). Note different chromatin structure.

2. Differentiating region of the root of *Allium carinatum*: Octoploid nucleus (above), nuclei in the endomitotic dispersion stage (*arrow*), and diploid nuclei (below). Note the absence of chromocentres during endomitosis.

3. Mechanically stretched giant chromosome of the suspensor of *Phaseolus vulgaris* showing the polytenic structure (the polytene chromosome divides into several oligotene bundles, each consisting of some dozens of sister chromosomes).

4, 5. Diploid and octoploid nuclei of the root of *Allium carinatum* in the late S period. Note labelling of the chromocentres by ^{3}H-thymidine in the condensed state (10 min., 10 µCi/ml., exposure 7 days).

6, 7. Diploid and probably 16-ploid nuclei of the root of *Allium fauxe*, showing RNA synthesis in euchromatin, and failure of ^{3}H-uridine incorporation in the chromocentres (5 min., 10 µCi/ml., exposure 7 days).

Description of Plate

Enlargement of all figures × 1200. Fixation: ethanol : acetic acid = 3 : 1; staining methods: 1, Feulgen; 2, aceto-carmine; 4-7, toluidine blue 0; 3, 6 and 7 = phase contrast.

1. Differentiating region of the root of *Allium flavum*: Tetraploid nucleus in G_1 (*above*) and one in G_2 (*below*). Note different chromatin structure.

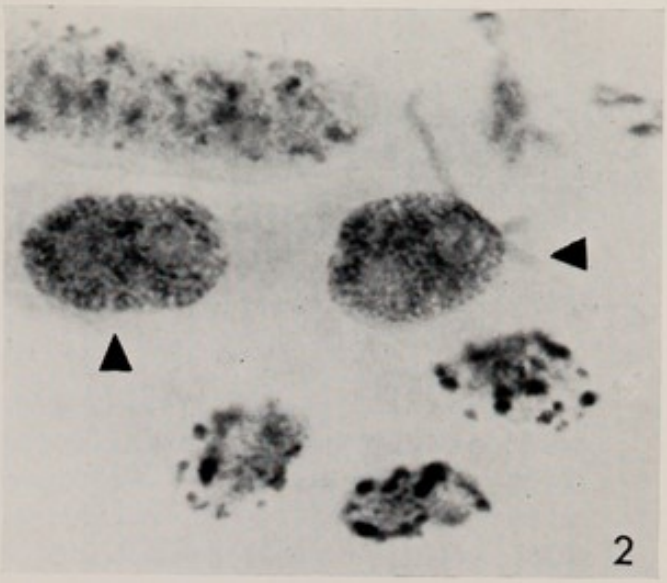
2. Differentiating region of the root of *Allium carinatum*: Octoploid nucleus (*above*), nuclei in the endomitotic dispersion stage (*arrows*), and diploid nuclei (*below*). Note the absence of chromocentres during endomitosis.

3. Mechanically stretched giant chromosome of the suspensor of *Phaseolus vulgaris* showing the polytenic structure (the polytene chromosome divides into several oligotene bundles, each consisting of some dozens of sister chromosomes).

4, 5. Diploid and octoploid nuclei of the root of *Allium carinatum* in the late S period. Note labelling of the chromocentres by ^{3}H-thymidine in the condensed state (10 min., 10 μCi/ml., exposure 7 days).

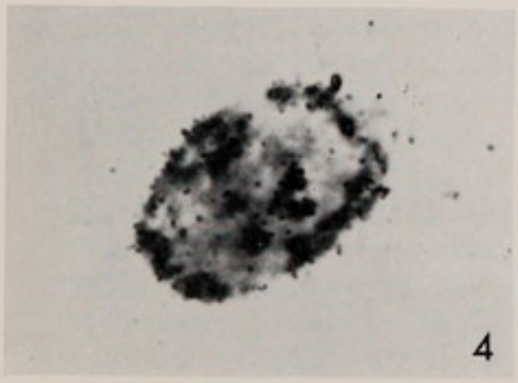
6, 7. Diploid and probably 16-ploid nuclei of the root of *Allium flavum*, showing RNA synthesis in euchromatin, and failure of ^{3}H-uridine incorporation in the chromocentres (5 min., 10 μCi/ml., exposure 7 days).

1

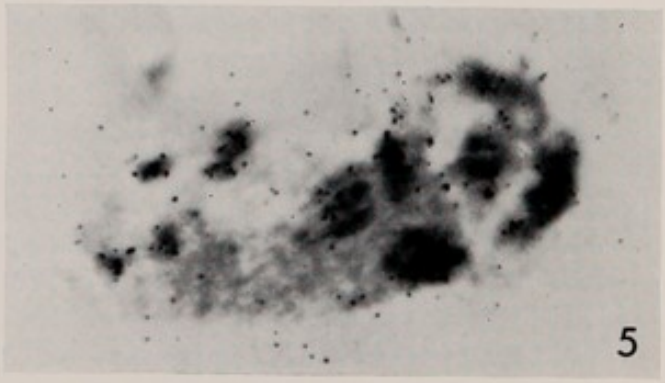


2

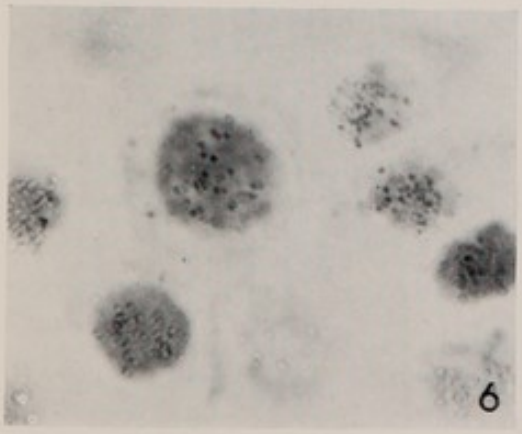
3



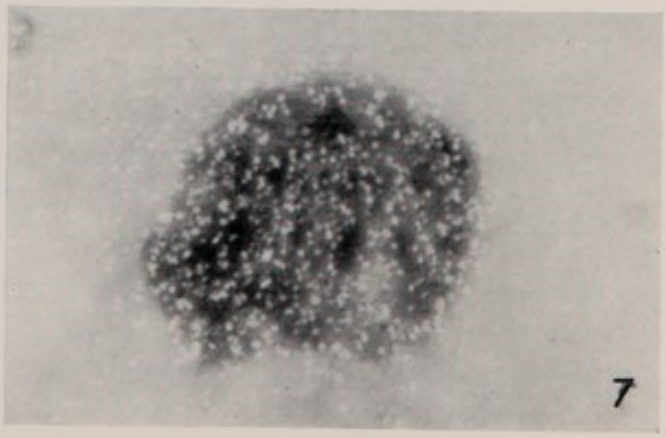
4



5



6



7

if a polytenic nucleus passes through a cycle with chromosome coiling, the polytene chromosomes break up into a high number of single sister chromosomes (Bier, 1959, compare also Nagl, 1970*b*). In any case, polyteny is only one particular type of interphasic nuclear structure which can be produced by the endomitotic short-cut of the cell cycle.

4. Nuclear volume and DNA content

In short, nuclear growth takes place discontinuously in two waves. The first occurs just before, during and immediately after endomitosis, the second directly follows DNA replication (Nagl, 1968). As a rule, the mean values of interphasic volume become nearly doubled between two levels of endopolyploidy. The DNA content becomes doubled during interphase, as in the mitotic cycle (Tschermak-Woess, 1959; Nagl, 1968). Therefore the nuclear DNA content as well as the nuclear volume can be used to determine the degree of endopolyploidy. However, using volume measurements, all three diameters of a nucleus should be measured to receive good values, and only nuclei from one and the same tissue, or cell row, can be compared (Tschermak-Woess and Hasitschka, 1953).

5. DNA replication

Although it had been shown by Tschermak-Woess (1959) that nuclei in the dispersion stage have already doubled their DNA, Altmann (1966) and Müller (1966) supposed that the dispersion of chromatin may be the expression of late DNA synthesis in the chromocentres. Since also several other authors have assumed that heterochromatin decondenses during the replication of its DNA, autoradiographic investigations were made in *Allium*. They revealed that there is no DNA synthesis during endomitosis (Nagl, 1970*c*, *e*). DNA replication is confined to the S period, which can be divided into three sections. During early S period, only the euchromatin becomes labelled with ^{3}H-thymidine; this euchromatin consists of small and weakly stained elements which are characteristic of G_1 nuclei. During the middle S period, both euchromatin and chromocentres incorporate the radioactive precursor, the chromatin structure can be classified only with difficulty. During the late S period, only the chromocentres show incorporation and the heterochromatin remains in a condensed state (plate, figs. 4 and 5). At this time, the euchromatin exhibits the denser appearance characteristic of G_2 nuclei (Nagl, 1970*c*).

DNA replication in condensed chromocentres was also found by Comings (1967), Pera and Wolf (1967), Citoler and Gropp (1969), and others. Moreover, the bands of polytene chromosomes replicate their DNA in the condensed state, too (Hägele, 1970).

6. RNA synthesis

During endomitotic interphase, most of the ^{3}H-uridine is incorporated into euchromatin. The chromocentres appear to be less or not labelled (plate, figs. 6 and 7). This corresponds with the findings of Hsu (1962) and Sieger *et al.* (1970) in animal cells. However, RNA synthesis is not interrupted by endomitosis as it is by mitosis, because chromosomes do not

condense. The pattern of incorporation of ^{3}H-uridine was not studied in detail, but the silver grains seem to be more regularly distributed during the dispersion stage than during interphase. Probably the decondensed heterochromatin becomes active in RNA synthesis, too.

The continuous activity of the chromosomes may be one of the most attractive advantages of endomitotic growth. It allows a high synthesis of ribosomal RNA due to the uninterrupt synthesis of nucleolar RNA, and it makes possible the early and rapid specialisation of the cell. This interpretation could be confirmed by the findings in the suspensor of *Phaseolus*, where the polytene chromosomes synthesise an extremely large nucleolus (Nagl, 1970*a*) and show puffed regions during the whole development of the suspensor and embryo (Nagl, 1969).

7. Suppression of mitosis to endomitosis

Experiments in *Allium* roots using actinomycin D and histone f1, both of which are known to inhibit RNA synthesis (Hurwitz *et al.*, 1962; Huang and Bonner, 1962), essentially resulted in suppression of mitosis to endomitosis (table 1; compare Nagl, 1970*d*). In every experiment mitosis was more

Table 1

Suppression of mitosis to endomitosis in the root tip meristem of Allium carinatum. *The roots were treated with 0·1 mg/ml. actinomycin D, recovery took place in tap water. Values in per cent. of nuclei examined (300-500 at every given time)*

		Hours							
	DNA	Actinomycin D					Recovery		
Stage	content	0	10	20	30	40	10	50	60
Mitosis	4C	31	18	5	3	0	0	0	0
Dispersion	4C	4	4	3	4	3	5	7	8
$2n/G_1$	2C	20	18	15	12	12	11	4	2
2n/S	2C-4C	30	29	25	16	5	6	13	15
$2n/G_2$	4C	15	31	52	65	80	75	65	60
$4n/G_1$	4C	0	0	0	0	0	3	10	10
4n/S	4C-8C	0	0	0	0	0	0	1	2
$4n/G_2$	8C	0	0	0	0	0	0	0	3

affected and inhibited than endomitosis (fig. 2). In general, endomitosis seems to be less sensitive to disturbances than mitosis (Tschermak-Woess, 1971). It can be concluded that the preparation for mitosis (including breakdown and new formation of the nuclear boundary, origin of the spindle apparatus, chromosome coiling, etc.) requires the synthesis of—perhaps specific—RNA not needed for endomitosis. In any case, it seems that the endomitotic short-cut of the chromosome cycle can be triggered by blocking mitosis-preparation RNA synthesis. These results are comparable to those found by Jain and Singh (1967) on suppression by actinomycin D of meiosis to mitosis in a locust, or to those found by Nuti Ronchi *et al.* (1965) and by Nasjleti and Spencer (1968) on suppression of mitosis to endoreduplication in pea roots and in lymphocytes, respectively, caused by 8-azaguanine and chloramphenicol, respectively.

8. Regulation

Mitosis occurs in the meristem of the root and of other organs, endomitosis in the differentiating regions. It is known that the nucleotide sequence of

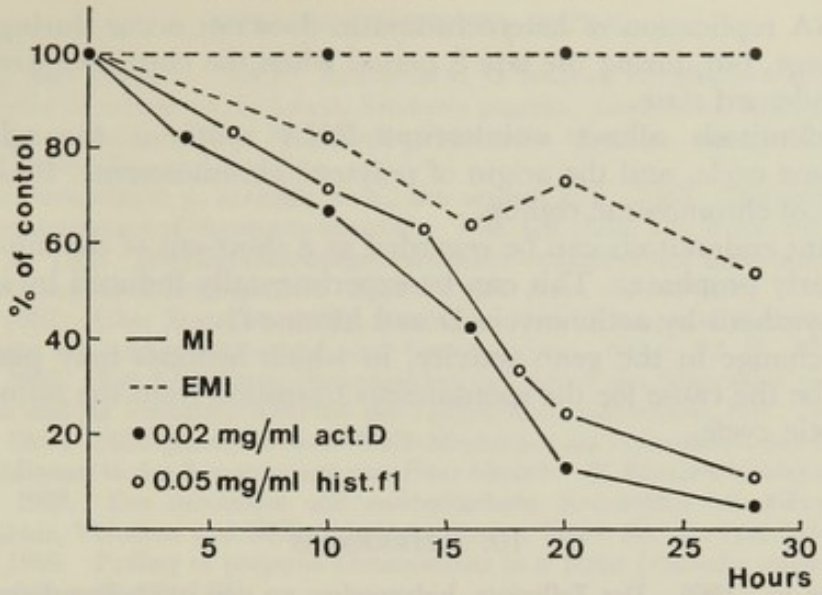


FIG. 2.—The differential inhibition of mitosis (in the root meristem) and endomitosis (in the differentiating region of the root) in *Allium carinatum* by treatment with actinomycin D or histone f1. MI mitotic index, EMI endomitotic index (each determination based on the examination of 1000 cells).

RNA in the meristem differs from that in other sections of the root (Heyes, 1963; Börner and Ramshorn, 1968). Evidently different genes are active in different regions and, therefore, during the different chromosome cycles occurring in them. On the other hand, development and differentiation may be accompanied by changing histone/DNA ratios (Gifford and Tepper, 1962; Sauter, 1969). Although the biological function of histones is still not well understood, the variable lysine-rich histones are sometimes assumed to be candidates for a repressor role (literature in Georgiev, 1969; Lima-de-Faria, 1969). Since the lysine-rich histones cause the condensation of chromatin (Littau *et al.*, 1965), and since condensed chromatin is relatively inactive in RNA synthesis, the histones might play an indirect role in the regulation of gene activity.

Too few factors are known to establish even a preliminary hypothesis on the control of the endomitotic short-cut of the chromosome cycle. We do not possess data on the amount of RNA, histones and other proteins in nuclei during the endomitotic cycle, and we do not know the role of phytohormones. However, a tentative assumption can be put forward as a step towards an understanding of endomitosis: the transformation of a mitotically active nucleus of a meristematic cell to an endomitotically growing nucleus of a differentiating cell might be caused by a change in the set of genes active in RNA synthesis. Histones are possibly involved in some way in the regulation of this step.

9. SUMMARY

1. The endomitotic chromosome cycle in angiosperms is characterised by a rhythmic increase of nuclear DNA content and nuclear volume.

2. The structure of nuclei differs in G_1 and G_2, at least in several species. Endomitosis itself can be recognised by the dispersion stage during which the chromocentres decondense, a change comparable with that during very early mitotic prophase.

3. DNA replication of heterochromatin does not occur during the dispersion stage, but during the late S period when the chromocentres remain in the condensed state.

4. Endomitosis allows uninterrupt RNA synthesis throughout the chromosome cycle, and the origin of polytene chromosomes. Both depend on failure of chromosome coiling.

5. Plant endomitosis can be regarded as a short-cut of the mitotic cycle in very early prophase. This can be experimentally induced by inhibition of RNA synthesis by actinomycin D and histone f1.

6. A change in the gene activity, in which histones may play a role, seems to be the cause for the spontaneous transition from the mitotic to the endomitotic cycle.

10. References

ALTMANN, H.-W. 1966. Der Zellersatz, insbesondere an den parenchymatösen Organen. *Verhdl. Deutsch. Ges. Pathol., 50*, 15-51.

BEERMANN, W. 1962. Riesenchromosomen. *Protoplasmatologia, VI. D.* Springer, Wien.

BIER, K. 1959. Quantitative Untersuchungen über die Variabilität der Nährzellkernstruktur und ihre Beeinflussung durch die Temperatur. *Chromosoma, Berl., 10*, 619-653.

BÖRNER, R., AND RAMSHORN, K. 1968. Untersuchungen über die Veränderung der Basenzusammensetzung der RNS an unterschiedlich differenziertem Wurzelgewebe von *Vicia faba*. *Biol. Zentralbl., 87*, 411-418.

CITOLER, P., AND GROPP, A. 1969. DNS-Replikation von autosomalem Heterochromatin. *Exptl. Cell Res., 54*, 337-346.

COMINGS, D. E. 1967. The duration of replication of the inactive X chromosome in humans based on the persistence of the heterochromatic sex chromatin body during DNA synthesis. *Cytogenetics, 6*, 20-37.

D'AMATO, F. 1952. New evidence on endopolyploidy in differentiated plant tissues. *Caryologia, 4*, 121-144.

D'AMATO, F. 1964. Endopolyploidy as a factor in plant tissue development. *Caryologia, 17*, 41-52.

ERBRICH, P. 1965. Über Endopolyploidie und Kernstrukturen in Endospermhaustorien. *Österr. Bot. Z., 112*, 197-262.

GEORGIEV, G. P. 1969. Histones and the control of gene action. *Ann. Rev. Genetics, 3*, 155-180.

GEITLER, L. 1948. Ergebnisse und Probleme der Endomitoseforschung. *Österr. Bot. Z., 95*, 277-299.

GEITLER, L. 1953. Endomitose und endomitotische Polyploidisierung. *Protoplasmatologia, VI. C.* Springer, Wien.

GIFFORD, E. M. JR., AND TEPPER, H. B. 1962. Histochemical and autoradiographic studies of floral induction in *Chenopodium album*. *Amer. J. Bot., 49*, 706-714.

HÄGELE, K. 1970. DNS-Replikationsmuster der Speicheldrüsen-Chromosomen von Chironomiden. *Chromosoma, Berl., 31*, 91-138.

HEITZ, E. 1929. Heterochromatin, Chromozentren, Chromomeren. *Ber. Deutsch. Bot. Ges., 47*, 274-284.

HEYES, J. K. 1963. The role of nucleic acids in cell growth and differentiation. *Symp. Soc. Exptl. Biol., 17*, 40-57.

HSU, T. C. 1962. Differential rate in RNA synthesis between euchromatin and heterochromatin. *Exptl. Cell Res., 27*, 332-334.

HSU, T. C., AND MOORHEAD, P. S. 1956. Chromosome anomalies in human neoplasms with special reference to the mechanisms of polyploidization and aneuploidization in the HeLa strain. *Ann. N.Y. Acad. Sci., 63*, 1083-1094.

HUANG, R. C., AND BONNER, J. 1962. Histone, a suppressor of chromosomal RNA synthesis. *Proc. natl. Acad. Sci., Wash., 48*, 1216-1222.

HURWITZ, J., FURTH, J. J., MALAMY, M., AND ALEXANDER, M. 1962. The role of DNA in RNA synthesis. III. The inhibition of the enzymatic synthesis of RNA and DNA by actinomycin D and proflavin. *Proc. natl. Acad. Sci., Wash., 48*, 1222-1229.

JAIN, H. K., AND SINGH, U. 1967. Actinomycin D induced chromosome breakage and suppression of meiosis in the locust, *Schistocera gregaria*. *Chromosoma, Berl., 21*, 463-471.

LIMA-DE-FARIA, A. (ed.). 1969. Handbook of Molecular Cytology. North-Holland Publ. Comp., Amsterdam and London.

LITTAU, V. C., BURDICK, C. J., ALLFREY, V. G., AND MIRSKY, A. E. 1965. The role of histones in the maintenance of chromatin structure. *Proc. natl. Acad. Sci., Wash., 54*, 1204-1212.

MÜLLER, H. A. 1966. Die Chromozentren in den Leberzellkernen der Maus unter normalen und pathologischen Bedingungen. *Ergebn. Pathol. Anat., 47*, 143-185.

NAGL, W. 1962. Über Endopolyploidie, Restitutionskernbildung und Kernstrukturen im Suspensor von Angiospermen und einer Gymnosperme. *Österr. Bot. Z., 109*, 431-494.

NAGL, W. 1965. Die SAT-Riesenchromosomen der Kerne des Suspensors von *Phaseolus coccineus* und ihr Verhalten während der Endomitose. *Chromosoma, Berl., 16*, 511-520.

NAGL, W. 1967. Mikrophotometrische DNS-Messungen an Interphase- und Ruhekernen sowie Mitosen in der Samenanlage von *Pinus silvestris*. *Z. Pflanzenphysiologie, 56*, 40-56.

NAGL, W. 1968. Der mitotische und endomitotische Kernzyklus bei *Allium carinatum*. I. Struktur, Volumen und DNS-Gehalt der Kerne. *Österr. Bot. Z., 115*, 322-353.

NAGL, W. 1969. Puffing of polytene chromosomes in a plant (*Phaseolus vulgaris*). *Naturwissenschaften, 56*, 221-222.

NAGL, W. 1970*a*. Temperature-dependent functional structures in the polytene chromosomes of *Phaseolus*, with special reference to the nucleolus organizers. *J. Cell Sci., 6*, 87-107.

NAGL, W. 1970*b*. Inhibition of polytene chromosome formation in *Phaseolus* by polyploid mitoses. *Cytologia, 35*, 252-258.

NAGL, W. 1970*c*. The mitotic and endomitotic nuclear cycle in *Allium carinatum*. II. Relations between DNA replication and chromatin structure. *Caryologia, 23*, 71-78.

NAGL, W. 1970*d*. Differential inhibition by actinomycin D and histone f1 of mitosis and endomitosis in *Allium carinatum*. *Z. Pflanzenphysiologie 63*, 316-326.

NAGL, W. 1970*e*. Correlation of chromatin structure and interphase stage in nuclei of *Allium flavum*. *Cytobiologie, 1*, 395-398.

NASJLETI, C. E., AND SPENCER, H. H. 1968. The effect of chloramphenicol on mitosis of phytohemagglutinin stimulated human leucocytes. *Exptl. Cell Res., 53*, 11-17.

NUTI RONCHI, V., AVANZI, S., AND D'AMATO, F. 1965. Chromosome endoreduplication (endopolyploidy) in pea root meristems induced by 8-azaguanine. *Caryologia, 18*, 599-617.

PERA, F., AND WOLF, U. 1967. DNS-Replikation und Morphologie der X-Chromosomen während der Synthese-Periode bei *Microtus agrestis*. *Chromosoma, Berl., 22*, 378-389.

SAUTER, J. J. 1969. Cytochemische Untersuchungen der Histone in Zellen mit unterschiedlicher RNS- und Proteinsynthese. *Z. Pflanzenphysiologie, 60*, 434-449.

SIEGER, M., PERA, F., AND SCHWARZACHER, H. G. 1970. Genetic inactivity of heterochromatin and heteropycnosis in *Microtus agrestis*. *Chromosoma, Berl., 29*, 349-364.

STREET, H. E. 1966. The physiology of root growth. *Ann. Rev. Plant Physiol., 17*, 315-344.

TORREY, J. G. 1965. Physiological bases of organization and development in the root. *Encycl. Plant Physiol., 15/1*, 1256-1327.

TSCHERMAK-WOESS, E. 1956. Karyologische Pflanzenanatomie. *Protoplasma, 46*, 798-834.

TSCHERMAK-WOESS, E. 1959. Die DNS-Reproduktion in ihrer Beziehung zum endomitotischen Strukturwechsel. *Chromosoma, Berl., 10*, 497-503.

TSCHERMAK-WOESS, E. 1963. Strukturtypen der Ruhekerne von Pflanzen und Tieren. *Protoplasmatologia, V. 1.* Springer, Wien and New York.

TSCHERMAK-WOESS, E. 1971. Endomitose. *Handb. Allgem. Pathol.* (in press). Springer, Berlin, Heidelberg, New York.

TSCHERMAK-WOESS, E., AND HASITSCHKA. 1953. Veränderungen der Kernstruktur während der Endomitose, rhythmisches Kernwachstum und verschiedenes Heterochromatin bei Angiospermen. *Chromosoma, Berl., 5*, 574-614.

WARDLAW, C. W. 1968. Morphogenesis in Plants. Methuen & Co., London.

THE RELATIONSHIP BETWEEN NUCLEAR DNA AMOUNT AND THE DURATION OF THE MITOTIC CYCLE

G. M. EVANS, H. REES, C. L. SNELL and S. SUN

Department of Agricultural Botany, University College of Wales, Aberystwyth

1. Introduction

Van't Hof and Sparrow (1963) and Van't Hof (1965) have shown that the duration of the complete mitotic cycle in comparable tissues of different species is dependent to a remarkable degree upon the nuclear DNA content. In general, the higher the DNA content the longer the duration of mitosis. The aims of the present work were, first, to compare in more detail the consequences of DNA change in diploids within different plant groups, viz. monocotyledons and dicotyledons and, second, to inquire into the effects on mitosis of DNA changes of different origin, in particular, those resulting from different kinds of numerical chromosome change.

2. Methods

Mitotic cycles. The method used to determine the duration of the mitotic cycle and its constituent phases was essentially that proposed by Quastler and Sherman (1959) and Wimber (1960). Actively growing roots were fed tritiated thymidine for 30 minutes and samples taken for autoradiography at two-hourly intervals. Counts of labelled prophases gave the typical double humped curve (see fig. 1). The best fitting line for each plot was calculated

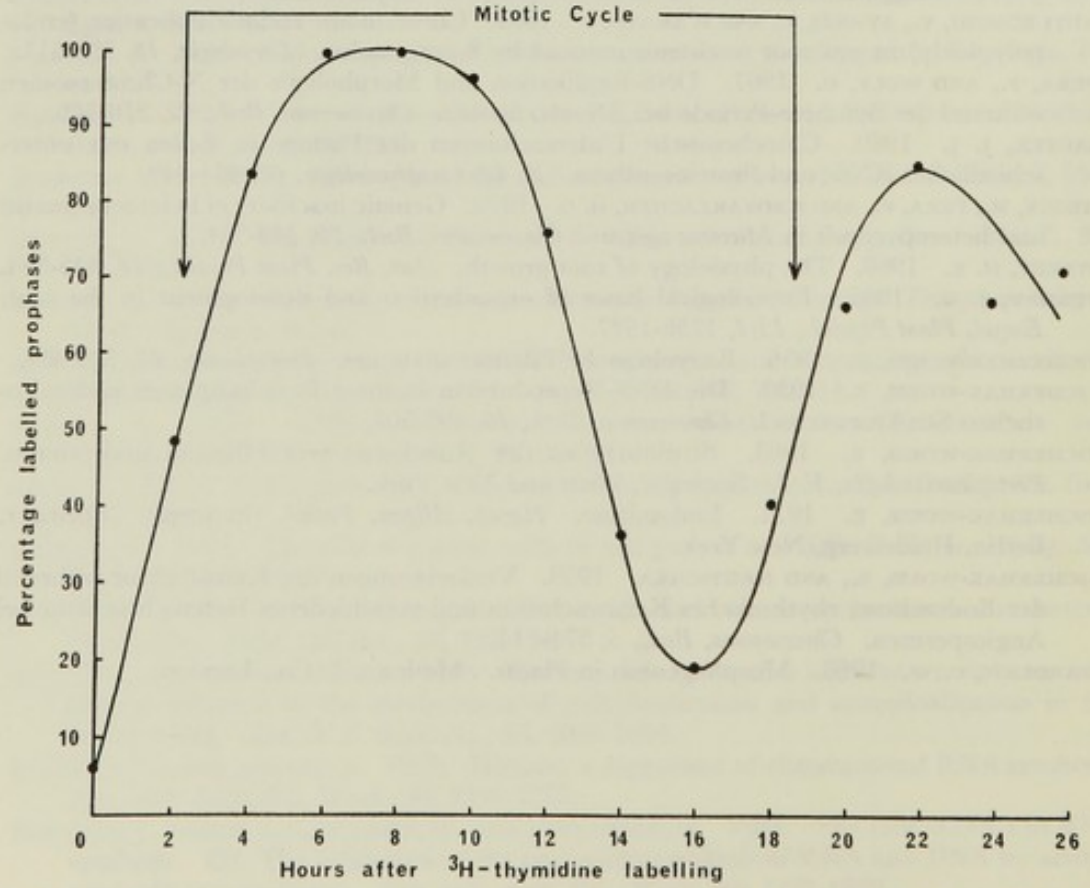


Fig. 1.—Percentage labelled prophases v's time after treatment with ^3H thymidine in root tips of *Nigella damascena*.

by means of a computer using the formula of orthogenal polynomials of least squares. The duration of the complete mitotic cycle is reflected directly by the interval between the 50 per cent. intercepts of the two humps in each curve. The time taken at synthesis was estimated by measuring the time interval between the halfway intercepts of the ascending and descending parts of the first peak of each graph.

DNA amounts. Estimates of nuclear DNA was by Feulgen photometry (see McLeish and Sunderland, 1961). All measurements were 2C nuclei which unlike DNA averages are not subject to environmental and physiological fluctuations. Conversion from arbitrary to absolute units was achieved by using Van't Hof's (1965) 2C value for *Allium cepa* as a standard, viz. $35{\cdot}5 \times 10^{-12}$ gm.

3. Results

A. *Diploids*

(i) *Rate of change in mitotic time.* Our estimates of the duration of the complete mitotic cycle and of the synthesis (S) phase, together with the 2C DNA values, for a range of diploid angiosperms are given in table 1 and

Table 1

The amount of DNA per 2C nucleus, mitotic cycle duration and length of synthesis in a series of diploid dicotyledons and monocotyledons

	DNA (10^{-12} gm.)	Mitotic cycle (Hours)	Synthesis (Hours)
DICOTYLEDONS			
Lycopersicum esculentum	3·9	10·6*	4·3*
Crepis capillaris	4·2	10·8*	3·25*
Linum usitatissimum	1·4	11·2	4·1
Lathyrus angulatus	8·9	12·25	3·9
Lathyrus articulatus	12·4	14·25	4·3
Lathyrus tingitanus	18·1	16·75	5·3
Lathyrus hirsutus	20·1	18·0	5·0
Nigella damascena	21·1	16·5	10·5
Vicia faba	23·9	19·3†	7·3†
MONOCOTYLEDONS			
Lolium perenne	9·9	8·6	4·2
Zea mays	11·0	10·5	4·25
Secale cereale	18·9	11·7	6·0
Allium fistulosum	26·3	15·3	7·1
Allium cepa	33·5	17·8	9·6
A. cepa × *A. fistulosum*	29·9	16·6	8·6
Tradescantia paludosa	33·8	20·0‡	10·8‡
Hyacinth	49·7	24·0	13·6

* From Van't Hof (1965).
† From Evans and Scott (1964).
‡ From Wimber (1960).

fig. 2. We have also included some results obtained by other workers. The data show quite clearly the general pattern of increasing duration of the mitotic cycle with increasing nuclear DNA. The rate of increase of 0·374 hours per picogram DNA for monocotyledons is almost identical to that for

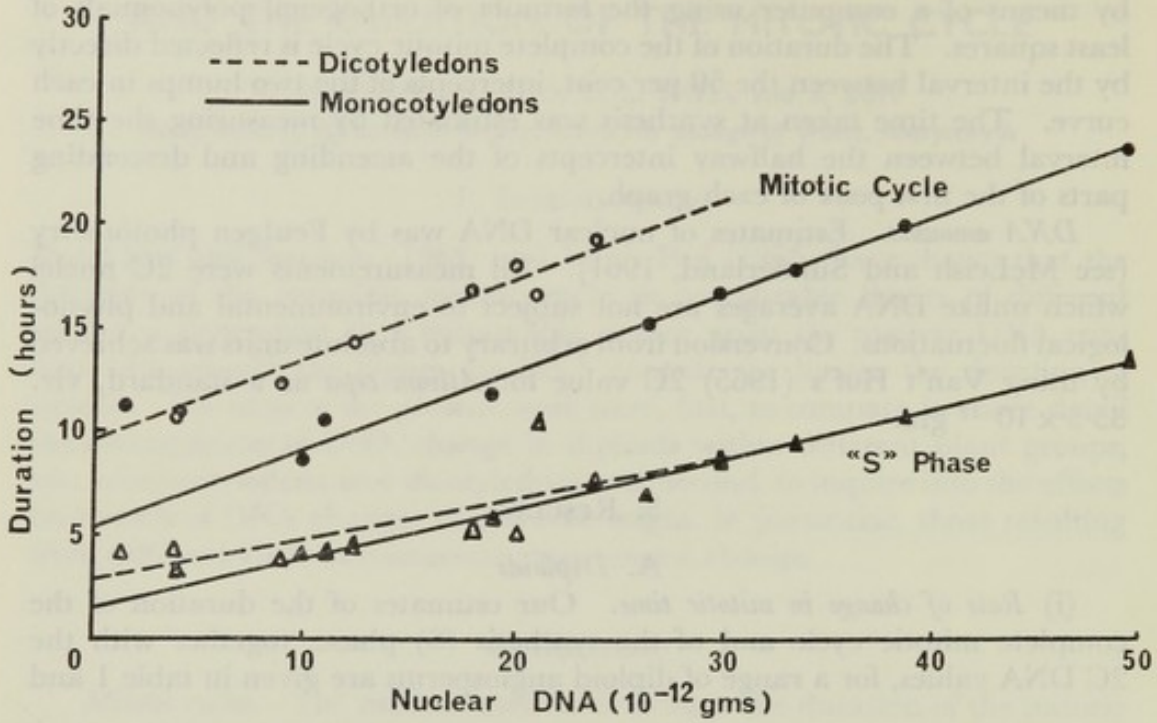


FIG. 2.—The relationship of the duration of the mitotic cycle and of synthesis to the 2C nuclear DNA amounts in diploid monocotyledons and dicotyledons. (Data from table 1.)

dicotyledons (0·385). These values are not significantly different from each other, nor do they differ significantly from the value of 0·300 hours/picogram given by Van't Hof (1965).

(ii) *Monocotyledons and dicotyledons.* Although the rates of increase of mitotic time per unit nuclear DNA are not different between monocotyledons and dicotyledons there is a large difference between the average duration of the mitotic cycles. Over the range of DNA values covered by these species the cycle times of dicotyledons are approximately four hours longer than those of monocotyledons of comparable DNA amounts. An analysis of covariance confirms that the difference is highly significant ($P = <0{\cdot}001$).

It is pertinent to inquire which of the phases of mitosis contribute to the difference in duration of the total cycle. It is evident, in so far as the duration of S is concerned, that monocotyledons and dicotyledons cannot be separated (see fig. 2). Analysis of covariance confirms that there is no significant difference between either the means or the slopes of the regression lines. Similarly, data on the duration of G2 and division show no uniform difference. It will be observed (table 2) that the difference in total cycle time between dicotyledons and monocotyledons is largely accounted for by the difference in G1, *i.e.* that part of the interphase preceding synthesis. It is appreciated that since the estimation of G1 is by subtraction of G2, division and synthesis from the complete mitotic cycle time, it will be the least accurate because it includes all the errors of these values. Nevertheless, the data in table 2 show that in no monocotyledonous species is the G1 phase longer than 1 hour which is in marked contrast to the dicotyledons which have G1 phases of appreciably longer duration. In this context we have evidence to indicate that this variation in G1 could be due, in part at least, to a difference in chromosome organization during division. The metaphase

TABLE 2

The amount of DNA per 2C nucleus, the duration of G1 and the DNA per unit volume ($\mu\mu g/\mu^3$) of metaphase chromosomes in diploid dicotyledons and monocotyledons

	DNA (10^{-12} gm.)	G1 (Hours)	DNA per unit vol. of chromosome ($\mu\mu g/\mu^3$)
DICOTYLEDONS			
Lathyrus angulatus	8·9	4·4	0·400
Lathyrus articulatus	12·4	3·9	0·508
Lathyrus tingitanus	18·1	6·3	0·574
Lathyrus hirsutus	20·1	7·8	0·454
Nigella damascena	21·1	1·5	
Vicia faba	23·9	4·9*	0·430
			Mean 0·473
MONOCOTYLEDONS			
Lolium perenne	9·9	0·5	0·352
Zea mays	11·0	0·5	0·364
Secale cereale	18·9	1·0	0·178
Allium fistulosum	26·3	0·5	0·194
Allium cepa	33·5	0·5	0·222
Hyacinth	49·7	0·8	0·234
			Mean 0·257

* From Evans and Scott (1964).

chromosomes of dicotyledons are on average almost twice as dense as those of monocotyledons (table 2). From this we conclude that the metaphase chromosomes of dicotyledons are more tightly coiled than those of monocotyledons and, further, that they require a longer time to uncoil prior to the initiation of synthesis, in other words a longer G1. This difference in organisation between the two angiosperm groups could be due to one of two causes,

1. A structural or molecular disparity in chromosome material.
2. A genotypic control exercised on chromosome organisation.

B. *Interspecific hybridisation*

The possibility of a genotypic control over the duration of mitosis was investigated by comparing the duration of the complete cycle in two species differing in nuclear DNA content, viz. *Allium cepa* and *Allium fistulosum* and in their F_1 hybrid *A. cepa* × *A. fistulosum*. As expected the mitotic cycle in *A. cepa* was longer than in *A. fistulosum* which has 30 per cent. less DNA and smaller chromosomes (table 1). However, the mitotic time and synthesis time in the hybrid is almost exactly intermediate between that of the parental values. It is related directly to the DNA content of the hybrid, *i.e.* the mean of the two parents. There is certainly no suggestion of a genotypic control by way of genes showing " dominance " or " heterosis ". At the same time, however, the results are ambiguous in so far as they do not rule out the possibility of control by genes of purely additive effect.

C. *Polyploidy*

For this comparison we used autopolyploids of maize, rye and garden hyacinth. The duration of the mitotic cycle and the S phase of both the

polyploids and their corresponding diploids are given in table 3. Also included are the values of Van't Hof (1965) for *Allium tuberosum* which is

TABLE 3

The amount of DNA per 2C nucleus, mitotic cycle duration and length of synthesis in a series of diploid and autopolyploid monocotyledons

		DNA value (10^{-12} gm.)	Mitotic cycle (Hours)	Synthesis (Hours)
Zea mays	2x	11·0	10·5	4·3
	4x	22·0	13·0	7·4
Secale cereale	2x	18·9	11·7	6·0
	4x	37·8	14·5	7·4
Allium tuberosum	4x	55·7	20·6*	11·8*
Hyacinth	2x	49·7	24·0	13·6
	3x	73·7	27·0	15·4
	4x −2	94·1	30·2	17·4

* From Van't Hof (1965).

a natural tetraploid. It is evident that autopolyploids have a longer mitotic cycle and S phase than their corresponding diploids and in the case of hyacinth the hypertetraploid has a longer cycle than the triploid. It is also clear from fig. 3 where both the duration of the complete cycle and S are

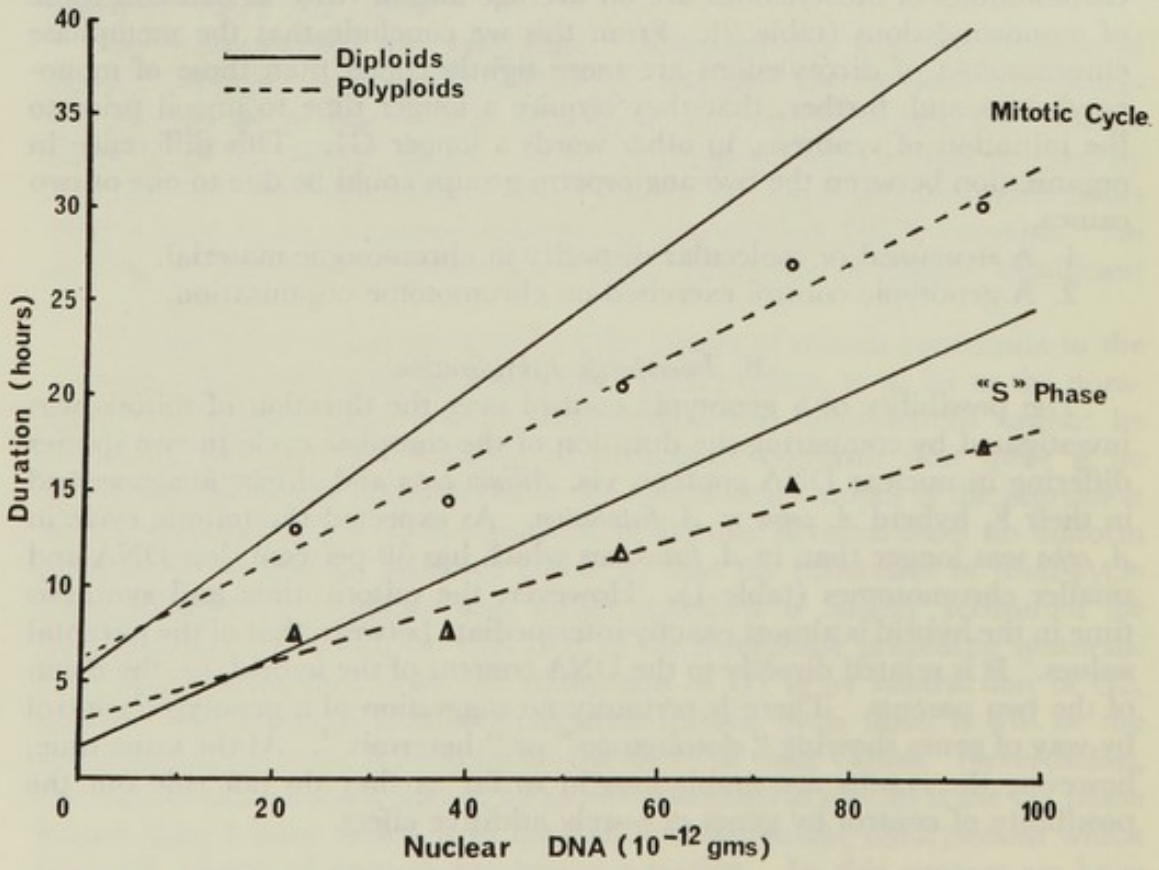


FIG. 3—Duration of the complete mitotic cycle and of synthesis in relation to the nuclear DNA amount in diploid and tetraploid monocotyledons. (Slope of the "diploid" regression line from fig. 2. Tetraploid data from table 3.)

plotted against nuclear DNA, that the *rate* of increase with increasing DNA is less in polyploids than diploids. This is in sharp contrast to that observed in some allopolyploids such as hexaploid wheat where the mitotic cycle duration is actually shorter than its diploid progenitors (Bennett, pers. comm.).

D. *Accessory chromosomes*

It has already been shown by Ayonoadu and Rees (1968) that the presence of B chromosomes causes an increase in the duration of the mitotic cycle in *Secale cereale*. The present data (table 4 and fig. 3) cover a wider

TABLE 4

The amount of DNA per 2C nucleus, mitotic cycle duration and length of synthesis in Lolium perenne, Zea mays *and* Secale cereale *containing B chromosomes*

		DNA ($\times 10^{-12}$ gm.)	Mitotic cycle (Hours)	Synthesis (Hours)
Lolium perenne	(0B)	9·9	8·6	4·2
	(3B)	12·0	10·0	5·5
Zea mays	(0B)	11·0	10·5	4·25
	(8B)	15·0	13·5	5·6
Secale cereale	(0B)	18·9	11·7	6·0
	(1B)	20·2	13·1	6·9
	(2B)	21·5	15·0	7·6
	(3B)	22·8	14·0	7·1
	(4B)	24·1	17·1	9·1

range of B chromosome classes in *Secale* and also cover two other species, viz. *Lolium perenne* and *Zea mays*. It will be observed that the rate of increase of both cycle time and S in relation to DNA is disproportionately high as compared with diploids with the addition of B's. Two possible explanations can be put forward to explain this divergence.

1. Late replication of B chromosome and
2. A genotypic control by B's.

Although both *Secale* and *Zea* have B chromosomes which are, to a certain degree at least, heterochromatic and late labelling, those of *Lolium* are not. Late labelling, therefore, is unlikely to explain completely the extension of S.

4. DISCUSSION

This survey together with those of Van't Hof (1963 and 1965) confirms that the duration of the mitotic cycle is a function of nuclear DNA amount. The duration of the cell cycle which, quite properly, can be said to be under genetic control, is to a remarkable extent, however, quite independent of any change in the quality of the DNA, although not entirely so as shown by the difference between monocotyledons and dicotyledons. There is, in short, an inevitability about the increasing duration of mitosis as DNA is added to the nucleus, irrespective, more or less, of the kind DNA added, the kind of genes it embodies.

At the molecular level it is now abundantly clear that chromosomes must have more than one site of initiation of DNA replication. Investigations, using DNA autoradiography and density label techniques, give

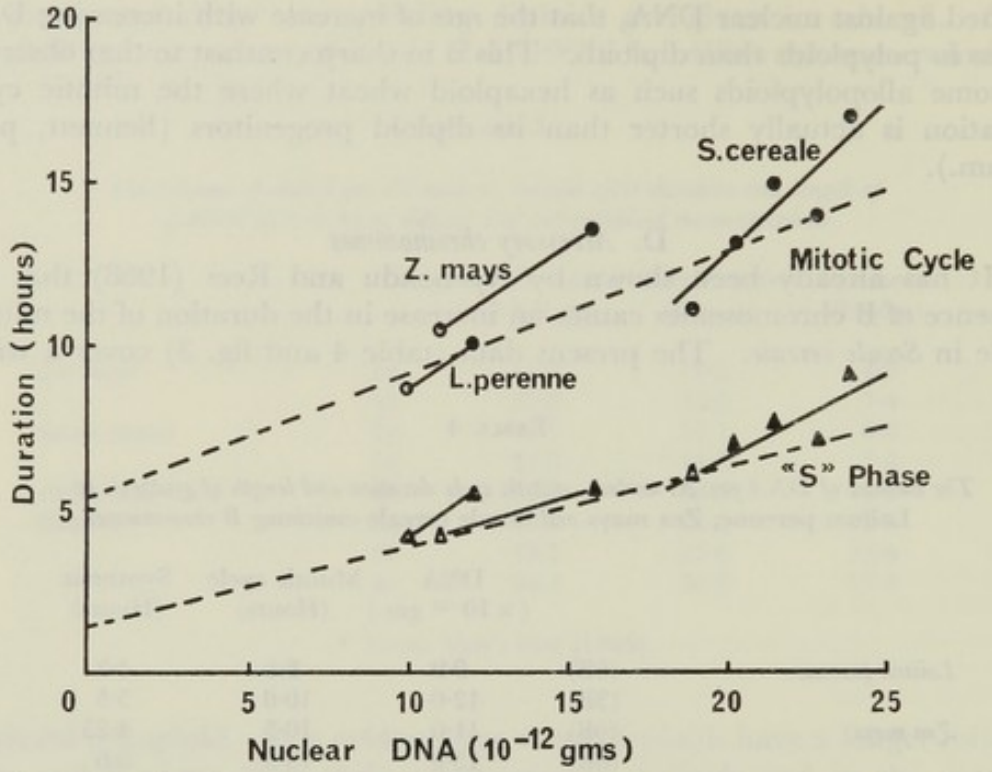


FIG. 4.—Effect of B chromosomes on the duration of the mitotic cycle and synthesis in *Zea mays*, *Lolium perenne* and *Secale cereale*. (*Z. mays* 0B and 8B; *L. perenne* 0B and 3B ; *S. cereale* 0B, 1B, 2B, 3B and 4B.) - - - - - Regression lines for diploid monocotyledons as in fig. 2.

estimates of the number of replicons in human (He La) cells as 5,000-10,000 (Painter *et al.*, 1966) and replication rates of from 0·5 μ to 5 μ per minute in single DNA double helices from a range of mammalian cells (Cairns, 1966 ; Painter *et al.*, 1966 ; Huberman and Riggs, 1968 ; Taylor, 1968 ; Painter and Schaefer, 1969 and Taylor, 1969). In this connection two questions remain unanswered. First, does an increase in chromosomal DNA involve a change in length or number of replicons per genome ? Second, in hybrids such as *Allium cepa* × *Allium fistulosum*, where the duration of mitosis is intermediate between the parental times, the larger chromosomes of *A. cepa* must complete their replication quicker and the shorter chromosomes of *A. fistulosum* slower than in the parental species. Does this involve in the hybrid a change in the number of replication sites (replicons) or does it involve a change in the rate of synthesis along the replicons as compared with the parental species ?

5. Summary

1. Variation in the duration of the mitotic cycle between diploid angiosperms is closely correlated with the amount of nuclear DNA. In general the higher the DNA content the longer the cycle.

2. There is however, on average, a difference of four hours in the length of the mitotic cycle between diploid monocotyledons and dicotyledons with comparable DNA amounts. The difference lies mainly in the G1 (presynthetic) phase.

3. The duration of mitosis in an *Allium* species hybrid is intermediate between that in the parents.

4. The *rate* of increase in the mitotic cycle with increasing DNA is lower in autopolyploids than in diploids.

5. In contrast, DNA increase due to the addition of B chromosomes causes a disproportionate increase in the duration of the mitotic cycles.

6. References

AYONOADU, U. A. U., AND REES, H. 1968. The regulation of mitosis by B-chromosome in rye. *Exptl. Cell Res.*, *52*, 284-290.

CAIRNS, J. 1966. Autoradiography of He La cell DNA. *J. Mol. Biol.*, *15*, 372-373.

EVANS, H. J., AND SCOTT, D. 1964. Influence of DNA synthesis on the production of chromatid aberrations by X-rays and Maleic Hydrazide in *Vicia faba*. *Genetics*, *49*, 17-38.

HUBERMAN, J. A., AND RIGGS, A. D. 1968. On the mechanism of DNA replication in mammalian chromosomes. *J. Mol. Biol.*, *32*, 327-341.

MCLEISH, J., AND SUNDERLAND, N. 1961. Measurements of deoxyribosenucleic acid [DNA] in higher plants by Feulgen photometry and chemical methods. *Exptl. Cell Res.*, *24*, 527-540.

PAINTER, R. B., JERMANY, D. A., AND RASMUSSEN, R. E. 1966. A method to determine the number of DNA replicating units in cultured mammalian cells. *J. Mol. Biol.*, *17*, 47-56.

PAINTER, R. B., AND SCHAEFER, A. W. 1969. Rate of synthesis along replicons of different kinds of mammalian cells. *J. Mol. Biol.*, *45*, 467-479.

QUASTLER, H., AND SHERMAN, F. G. 1959. Cell population kinetics in the intestinal epithelium of mouse. *Exptl. Cell. Res.*, *17*, 420-438.

TAYLOR, J. H. 1968. Rate of chain growth and units of replication in DNA of mammalian chromosomes. *J. Mol. Biol.*, *31*, 579-594.

TAYLOR, J. H. 1969. Replication and organization of chromosomes. Proc. XII Intern. Congr. Genetics, Vol. *3*, 177-189.

VAN'T HOF, J., AND SPARROW, A. H. 1963. A relationship between DNA content, nuclear volume, and minimum mitotic cycle time. *Proc. Natl. Acad. Sci. U.S.A. 49*, 897-902.

VAN'T HOF, J. 1965. Relationships between mitotic cycle duration, *S* period duration and the average rate of DNA synthesis in root meristems of several plants. *Exptl. Cell Res.*, *39*, 48-58.

WIMBER, D. E. 1960. Duration of the nuclear cycle in *Tradescantia paludosa* root tips as measured with ^{3}H thymidine. *Am. J. Bot.*, *47*, 828-834.

DNA CONTENT OF RELATED SPECIES

Dr D. P. FOX

Department of Genetics, University of Aberdeen, Aberdeen, Scotland

1. Introduction

One of the major outstanding problems in cell biology is the macromolecular organisation of the eukaryote chromosome. Not surprisingly, a great deal of attention has been given to the arrangement of DNA within this structure though it is a just testimony to our ignorance that we cannot yet say if the chromosome is basically a unineme or multineme.

Recent work on the renaturation kinetics of DNA from a wide variety of eukaryotes (Britten and Kohne, 1968) has shown that, with the possible exception of yeast, some base sequences are reiterated many times within one haploid genome while other sequences are apparently unique. This latter fact could be used to support the unineme model; certainly it excludes the possibility of many identical strands.

A much older but equally unexplained series of observations is that of the large quantitative differences in genomic DNA which often occur between closely related species. Some workers, *e.g.* Schrader and Hughes-Schrader (1956), Rothfels *et al.* (1966), Rothfels and Heimberger (1968), have interpreted their data in terms of a chromosome model with a variable number of strands but many results, *e.g.* Ullerich (1967, 1970), Rees and Hazarika (1969), Rees and Jones (1967) are not consistent with the addition or subtraction of whole strands.

This approach to the structure of the chromosome is really the analysis of nature's own experiments in chromosome evolution. Its importance lies in the evolutionary biologist's belief that differences between closely related species are likely to be of adaptive significance. It is for this reason that I have recently investigated the quantitative differences in genomic DNA between species of the beetle genus *Dermestes*.

2. The system

Dermestes is particularly suitable for study since its member species are very similar to both the taxonomist (Hinton, 1945) and the cytologist (John and Shaw, 1966). The six species used by John and Shaw (1966) all have $2n = 2x = 16+XY$ in the male with similar chromosome morphology between species. This karyotypic uniformity is more apparent than real for densitometric estimation of IC DNA values (Fox, 1969*a*) showed a 2·7 fold range. Surprisingly, the two extreme species with regard to DNA value (*D. haemorrhoidalis* and *D. peruvianus*) are those which are taxonomically most closely related.

Determination of chromosome volumes (see fig. 3, Fox, 1969*a*) indicated that when DNA was lost or gained during evolution, the changes affected most of the chromosomes in a similar manner. This trend is apparent in other work (*e.g.* Ullerich, 1967; Rees and Hazarika, 1969) and may be necessary to maintain a similar genetic balance.

John and Shaw (1966) noted that the meiotic and mitotic prophase chromosomes of *Dermestes* all had marked blocks of heterochromatin spanning their centromeres. Further, they were of the opinion that these blocks were larger in some species than in others. It seemed to me that variation in the heterochromatin might account for much of the variation in the genomic DNA content previously observed. In the following section, experiments are described which show that much, but not all of the DNA differences are due to variations in the amount of heterochromatic DNA.

3. The experiments

Six species of *Dermestes* (*haemorrhoidalis*, *frischii*, *maculatus*, *lardarius*, *ater* and *peruvianus*) were used in this investigation. They are the same species used by John and Shaw (1966) and were derived from the same source, the Pest Infestation Laboratory, Slough.

In interphase nuclei of somatic tissues of *Dermestes* a number of large chromocentres can be seen (fig. 4, Fox 1969*b*). These chromocentres probably arise by the fusion of the centric heterochromatic blocks. The distinct boundary of each chromocentre allows the partitioning of DNA into euchromatic and heterochromatic components by making density maps of nuclei stained by the Feulgen reaction. The method is a modification of that of Ross and Jans (1968) and will be described in detail elsewhere. The maps are made with the aid of the Joyce, Loebl recording densitometer (Joyce, Loebl Instruments, Gateshead-on-Tyne). Heterochromatic areas are outlined on tracing paper placed over a photographic enlargement of each nucleus and this tracing is projected on to the density map. Consequently an integrated extinction value can be obtained for each chromocentre and for the total euchromatin.

If the chromocentres of the interphase nuclei do correspond to the centric heterochromatin seen at prophase, then they should contain the same proportion of DNA in equivalent nuclei of different somatic tissues. Alternatively, they could correspond to chromatin which is not active in transcription in the sense that Harris (1967) has used the term condensed chromatin. In this case the proportion might vary considerably between different tissues.

In an attempt to differentiate between these two possibilities four tissues of *Dermestes maculatus* males, namely testis wall, fat body, Malpighian tubule and vas deferens, have been used. In each of these tissues the smallest nuclei present have the 2C DNA content (Fox, 1969*b*, 1970 and unpublished). Five nuclei presumed to be 2C were selected visually from each tissue type and analysed for the amount of DNA present in the euchromatin and heterochromatin (table 1). An analysis of variance of the total integrated

Table 1

DNA contents of four somatic tissues of D. maculatus *partitioned into euchromatic and heterochromatic components. Five 2C nuclei of each tissue were used. The DNA contents are in arbitrary units*

	Tissue			
	MT	VD	TW	F
Mean total DNA	2, 262·2	2148·6	2435·7	2185·4
Mean total DNA in euchromatin	1, 144·7	1037·2	1262·9	899·6
Mean total DNA in heterochromatin	1, 117·5	1, 111·4	1172·8	1285·8
Mean per cent. DNA in heterochromatin	49·4	51·7	48·2	58·8

Symbols: MT—Malpighian tubule; VD—vas deferens; TW—testis wall; F—fat body.

extinctions for the nuclei indicates no significant difference between tissues (V.R. (3, 16) = 2·231, P = 0·2–0·1). This is consistent with them all having the 2C DNA content. Segregation of extinction into euchromatic and heterochromatic components (fig. 1) does show some scatter of results,

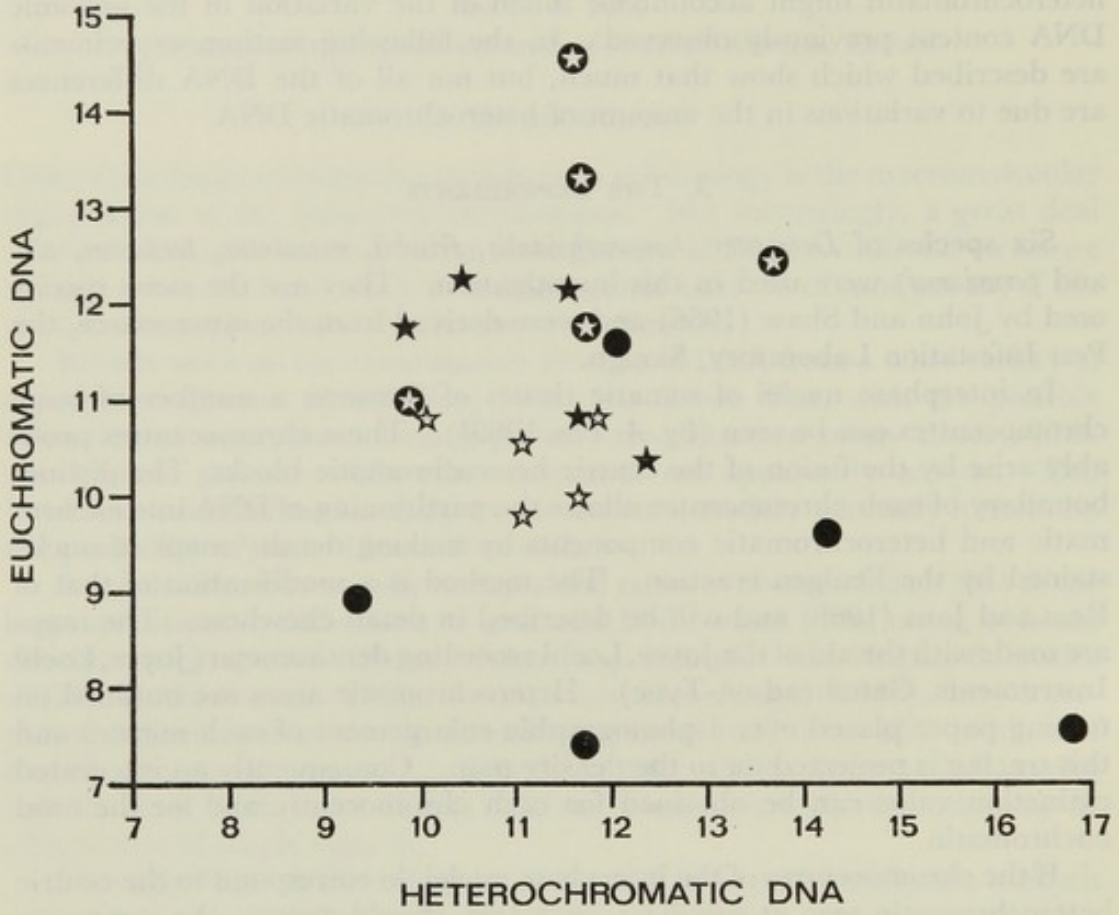


Fig. 1.—Plot of euchromatic DNA versus heterochromatic DNA for 2C nuclei of four tissues of *D. maculatus*. The DNA values are in arbitrary units $\times 10^{-3}$.

Symbols: ●—fat body, ✪—testis wall, ☆—vas deferens, ★—Malpighian tubule.

particularly for nuclei of the fat body. An analysis of variance of per cent. heterochromatin (transformed to angles) gave a significant difference between tissues (V.R. (3, 16) = 4·863, P = 0·05–0·01). However, the significance disappeared on removal of the fat body data from the analysis (V.R. (2, 12) = 1·718, P > 0·2). The anomalous behaviour of the fat body is unexplained. Good agreement is found between the other three tissues in the proportion of DNA present in the heterochromatin and it does tentatively suggest that this proportion is not a tissue characteristic. Consequently, the analysis was extended to presumptive 2C Malpighian tubule nuclei of males of all six species.

The data for Malpighian tubule nuclei are summarised in table 2, the species being ranked in order of increasing DNA content. This order is the same as that found previously for the germ line and soma of these species(Fox, 1969*a*, *b*, 1970). There are significant differences in total DNA content between all adjacent species pairs. With regard to the proportion of DNA found in heterochromatin (fig. 2) an analysis of variance shows that

TABLE 2

DNA contents of Malpighian tubule 2C nuclei of six species of Dermestes *partitioned into euchromatic and heterochromatic components. Five nuclei of each species were used*

	Species					
	H	F	M	L	A	P
Mean total DNA	1334·3	1721·5	2262·2	2699·6	3084·7	3626·4
Mean total DNA in euchromatin	872·6	939·6	1144·7	1229·3	1646·1	1324·5
Mean total DNA in heterochromatin	461·7	781·6	1117·5	1470·3	1438·6	2301·9
Mean per cent. DNA in heterochromatin	34·6	45·4	49·4	54·5	46·6	63·5

Symbols: H—*haemorrhoidalis*; F—*frischii*; M—*maculatus*; L—*lardarius*; A—*ater*, and P—*peruvianus*.

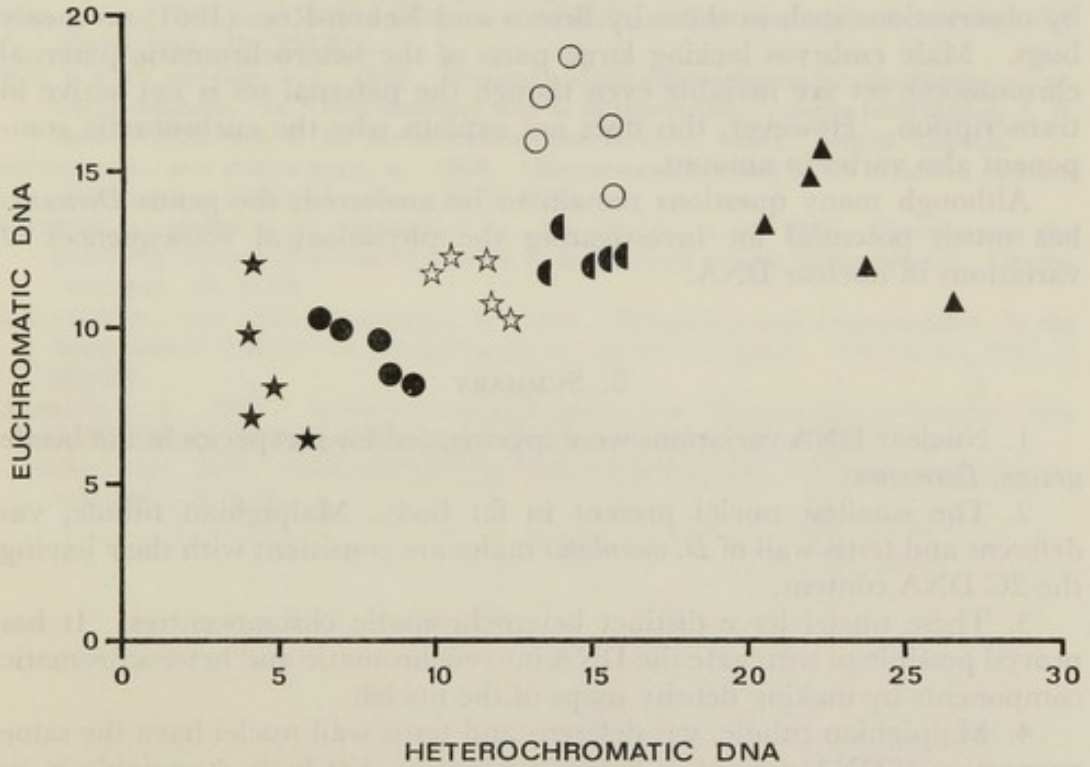


FIG. 2.—Plot of euchromatic DNA versus heterochromatic DNA for 2C nuclei of six species of *Dermestes*. The DNA values are in arbitrary units $\times 10^{-3}$.

Symbols: ▲—*D. peruvianus*, ○—*D. ater*, ◖—*D. lardarius*, ☆—*D. maculatus*, ●—*D. frischii*, ★—*D. haemorrhoidalis*.

D. frischii, *D. maculatus* and *D. ater* do not differ significantly. *D. haemorrhoidalis* has a significantly lower proportion of its DNA in heterochromatin than the former group while *D. lardarius* and *D. peruvianus* have a significantly higher proportion than that group. *D. peruvianus* has a significantly greater proportion of its DNA in heterochromatin than *D. lardarius*.

4. THE IMPLICATIONS

In my original paper (Fox, 1969*a*) on *Dermestes* DNA values, I concluded that the evidence did not fit a model of the chromosome in which variation

of DNA content could occur by adding or removing strands. This conclusion is reinforced by the data presented here. If strand number were the only variable responsible for DNA value variation then *the proportion of DNA found in heterochromatin should be constant and independent of total genomic DNA content.* The results clearly differ from this proposition and do so in a fairly regular way. When the genome increases its DNA content both euchromatic and heterochromatic DNA increase but, as a general rule, *the heterochromatic component increases at a faster rate.*

The fact that the euchromatic component only varies by 2-fold between the species while the heterochromatic component varies by 5-fold perhaps goes some way towards explaining the taxonomic similarity of the species. However, it raises even more forcibly the question of what the heterochromatin is doing. Perhaps it is involved in some kind of control mechanism in the cell such that a change in the euchromatin : heterochromatin ratio alters the balance of this control. This type of interpretation is supported by observations such as those by Brown and Nelson-Rees (1961) on mealy bugs. Male embryos lacking large parts of the heterochromatic paternal chromosome set are inviable even though the paternal set is not active in transcription. However, this does not explain why the euchromatic component also varies in amount.

Although many questions remain to be answered, the genus *Dermestes* has much potential for investigating the physiological consequences of variations in nuclear DNA.

5. Summary

1. Nuclear DNA variations were investigated for six species in the beetle genus, *Dermestes*.

2. The smallest nuclei present in fat body, Malpighian tubule, vas deferens and testis wall of *D. maculatus* males are consistent with their having the 2C DNA content.

3. These nuclei have distinct heterochromatic chromocentres. It has proved possible to segregate the DNA into euchromatic and heterochromatic components by making density maps of the nuclei.

4. Malpighian tubule, vas deferens and testis wall nuclei have the same proportion of DNA present in heterochromatin. Fat body is variable in its behaviour but generally has a higher proportion than the other tissues.

5. It is tentatively concluded that the chromocentres are derived from the centric blocks of heterochromatin, though the data on fat body nuclei do not necessarily support this view.

6. The proportion of DNA found in heterochromatin of 2C Malpighian tubule nuclei of males of the six species varied between 35 per cent. and 63 per cent. and increased with the total genomic DNA content, with the single exception of *D. ater*.

7. These observations support previous evidence that gross changes in DNA content in this genus are achieved by some mechanism other than an alteration of the number of lateral strands in the chromosome.

Acknowledgments.—I should like to thank Mr D. Hannay for his help in making the density maps. The Joyce, Loebl densitometer used in this investigation was purchased with a grant from the Science Research Council.

6. References

BRITTEN, R. J., AND KOHNE, D. E. 1968. Repeated sequences in DNA. *Science, 161*, 529-540.

BROWN, S. W., AND NELSON-REES, W. A. 1961. Radiation analysis of a lecanoid genetic system. *Genetics, 46*, 983-1007.

FOX, D. P. 1969*a*. The relationship between DNA value and chromosome volume in the Coleopteran genus *Dermestes*. *Chromosoma, Berl., 27*, 130-144.

FOX, D. P. 1969*b*. DNA values in somatic tissues of *Dermestes* (Dermestidae : Coleoptera). I. Abdominal fat body and testis wall of the adult. *Chromosoma, Berl., 28*, 445-456.

FOX, D. P. 1970. DNA values in somatic tissues of *Dermestes* (Dermestidae : Coleoptera). II. The Malpighian tubules of the adult male. *Chromosoma, Berl.* (in press).

HARRIS, H. 1967. The reactivation of the red cell nucleus. *J. Cell Sci., 2*, 23-32.

HINTON, H. E. 1945. A monograph of the beetles associated with stored products: No. 1: British Museum (Natural History) London.

JOHN, B., AND SHAW, D. D. 1966. Karyotype variation in Dermestid beetles. *Chromosoma, Berl., 20*, 371-385.

REES, H., AND HAZARIKA, M. H. 1969. Chromosome evolution in *Lathyrus*. In " Chromosomes Today ", 158-165, Suppl. to *Heredity, 24*.

REES, H., AND JONES, R. N. 1967. Structural basis of quantitative variation in nuclear DNA. *Nature, Lond., 216*, 825-826.

ROSS, K. F. A., AND JANS, D. E. 1968. The study of cell differentiation by quantitative microscopic methods. In " Cell structure and its interpretation ", essays presented to J. R. Baker, F.R.S. (eds. S. M. McGee-Russell and K. G. A. Ross). Arnold, London.

ROTHFELS, K., AND HEIMBERGER, M. 1968. Chromosome size and DNA values in sundews (*Droseraceae*). *Chromosoma, Berl., 25*, 96-103.

ROTHFELS, K., SEXSMITH, E., HEIMBERGER, M., AND KRAUSE, M. O. 1966. Chromosome size and DNA content of species of *Anemone L.* and related genera (*Ranunculaceae*). *Chromosoma, Berl., 20*, 54-74.

SCHRADER, F., AND HUGHES-SCHRADER, S. 1956. Polyploidy and fragmentation in the chromosomal evolution of various species of *Thyanta* (*Hemiptera*). *Chromosoma, Berl., 7*, 469-496.

ULLERICH, F.-H. 1967. Weitere Untersuchungen über Chromosomenverhältnisse und DNS—Gehalt bei *Anuren* (*Amphibia*). *Chromosoma. Berl., 21*, 345-368.

ULLERICH, F.-H. 1970. DNA—Gehalt und Chromosomenstruktun bei Amphibien. *Chromosoma, Berl., 30* 1-37.

THE MOLECULAR STRUCTURE OF NUCLEOHISTONE

J. F. PARDON and B. M. RICHARDS

Searle Research Laboratories, Lane End Road, High Wycombe, Buckinghamshire

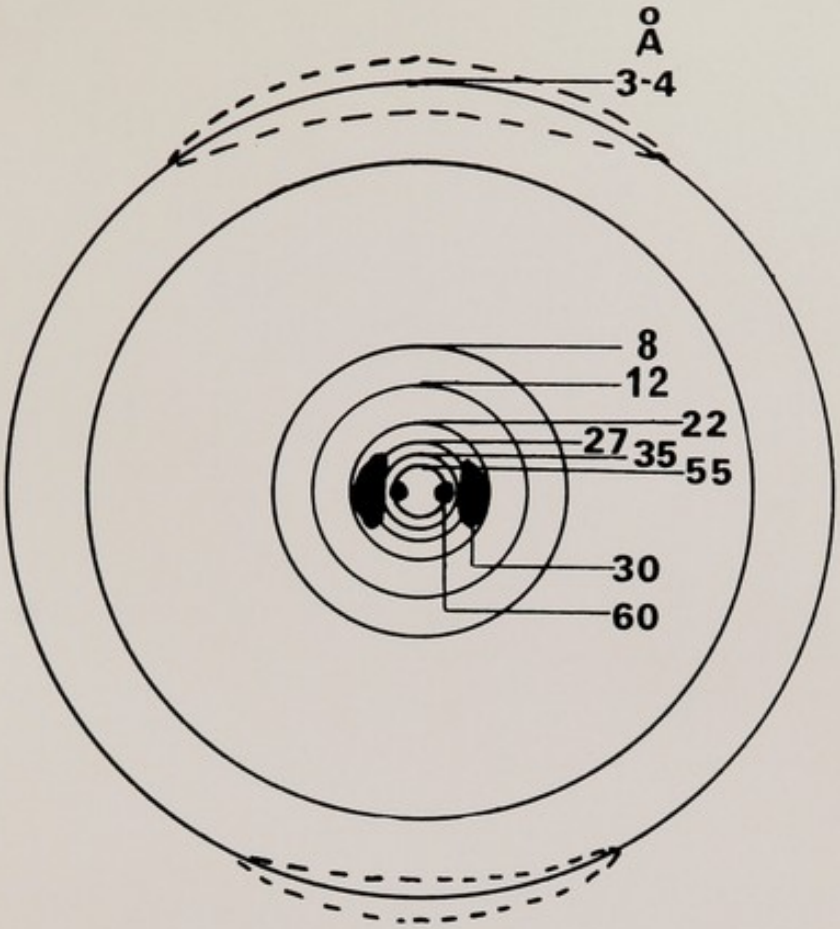
1. Introduction

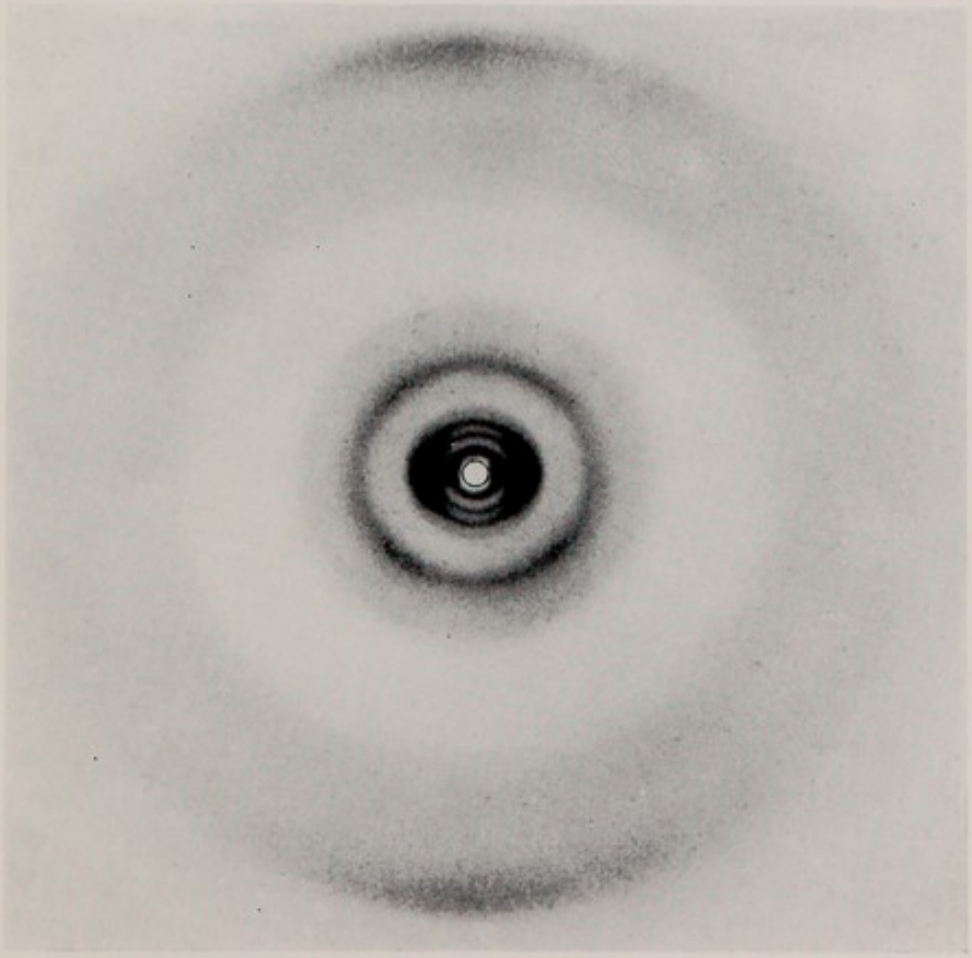
Efforts to determine the molecular structure of chromosomes have been mainly confined to the use of genetic and microscopic techniques. Genetic techniques have been very successful in mapping chromosomes, especially of lower organisms. In both higher and lower organisms, however, much of what is known about the mechanics of chromosome replication sheds little light on the sub-microscopic or molecular structure of the chromosomes. Light microscopic techniques give an indication of the overall morphology of the chromosome and electron microscopic studies reveal a " unit thread " of nucleoprotein as a basic unit of the chromosome. However, these techniques have not provided a model for the macromolecular structure of the nucleoprotein from which the chromosome is formed, or the relationship, if any, between the " unit thread " and the gross microscopic morphology of the metaphase chromosomes.

We have used X-ray diffraction to study the molecular structure of nucleohistone since we believe that a detailed knowledge of nucleohistone structure may be the best approach to an understanding of chromosome structure and, ultimately, chromosome function. Diffraction patterns obtained from intact nuclei isolated both from chick erythrocytes and calf thymus are essentially similar to those obtained from isolated nucleohistone prepared by the Zubay-Doty (1959) method. This proves that nucleohistone remains intact during isolation, having a structure which is relevant to any discussion of the chromosome. The diffraction results establish the existence of a regular tertiary structure in nucleohistone and the data obtained can be explained by a model in which the DNA double helix is constrained into a regular supercoil configuration. We have been interested in finding out which of the histone types possess the ability to supercoil the DNA and have investigated the structure of nucleohistones reconstructed from DNA and fractionated histones. The invariance in amino acid sequence between one of the histone types, fraction F2a1, isolated from both calf thymus and pea embryo tissue (De Lange *et al.*, 1969; Ogawa *et al.*, 1969), suggests that the structure of the entire protein is important to its role. We have built molecular models based on the amino acid sequence of this histone to investigate possible conformations of the free molecule and when complexed with DNA (Richards and Pardon, 1970). In particular we have considered those arrangements of this histone relative to the DNA which might induce supercoiling.

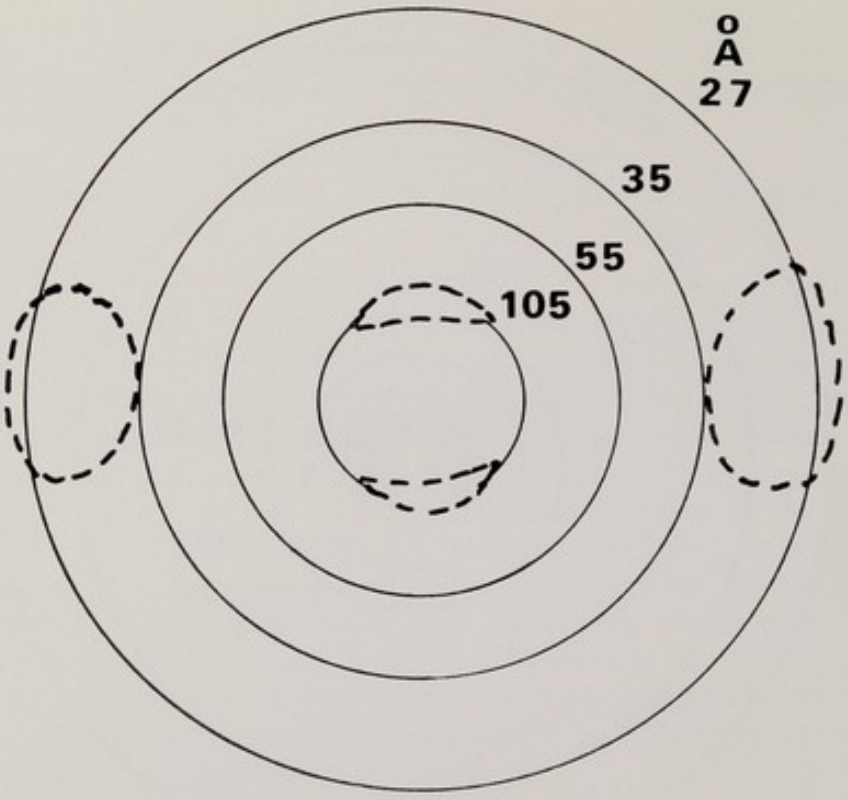
2. X-ray diffraction studies of nucleohistone

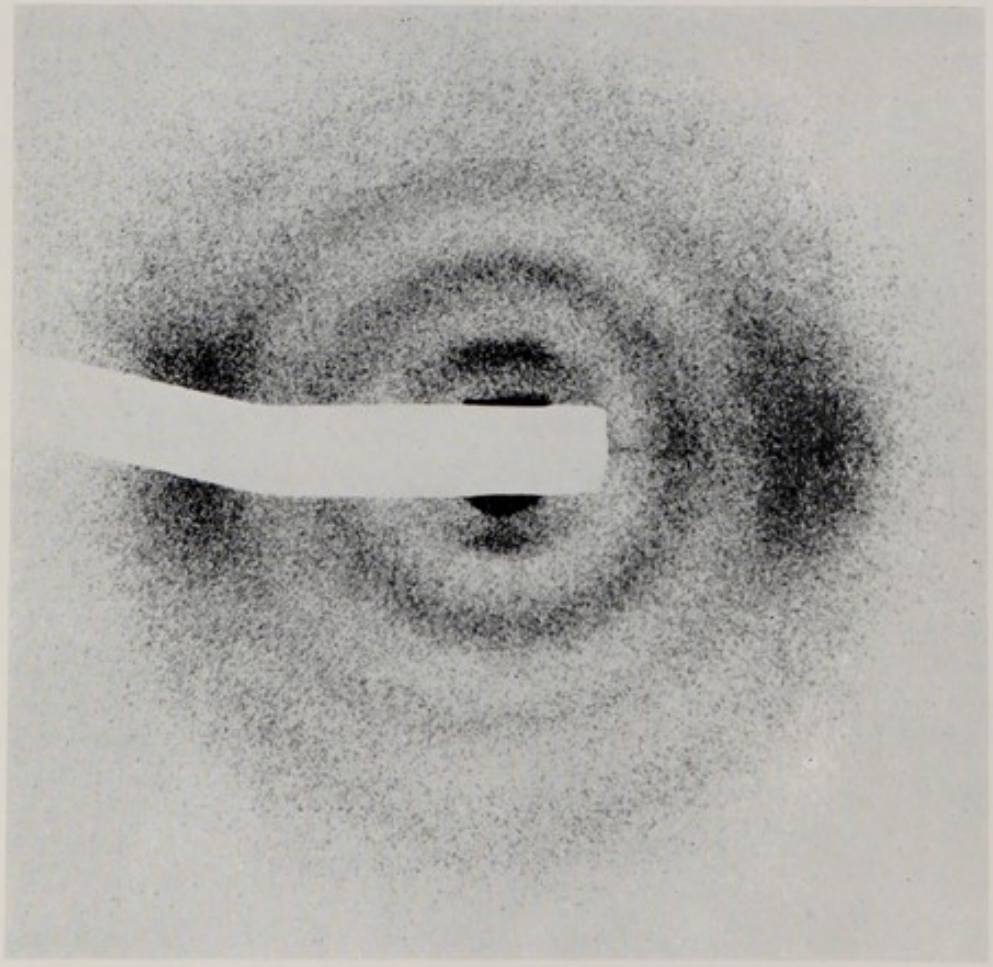
Most of the diffraction work has been with nucleohistone extracted from calf thymus using the techniques originally described by Zubay and Doty (1959) for the material which sediments at 75,000 g. from dilute water



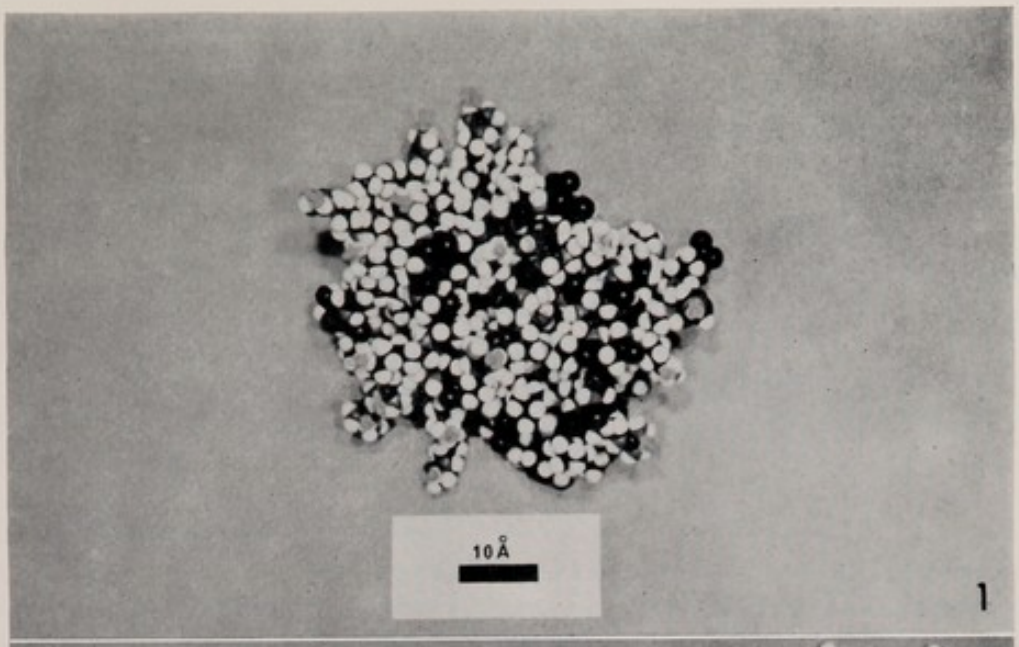


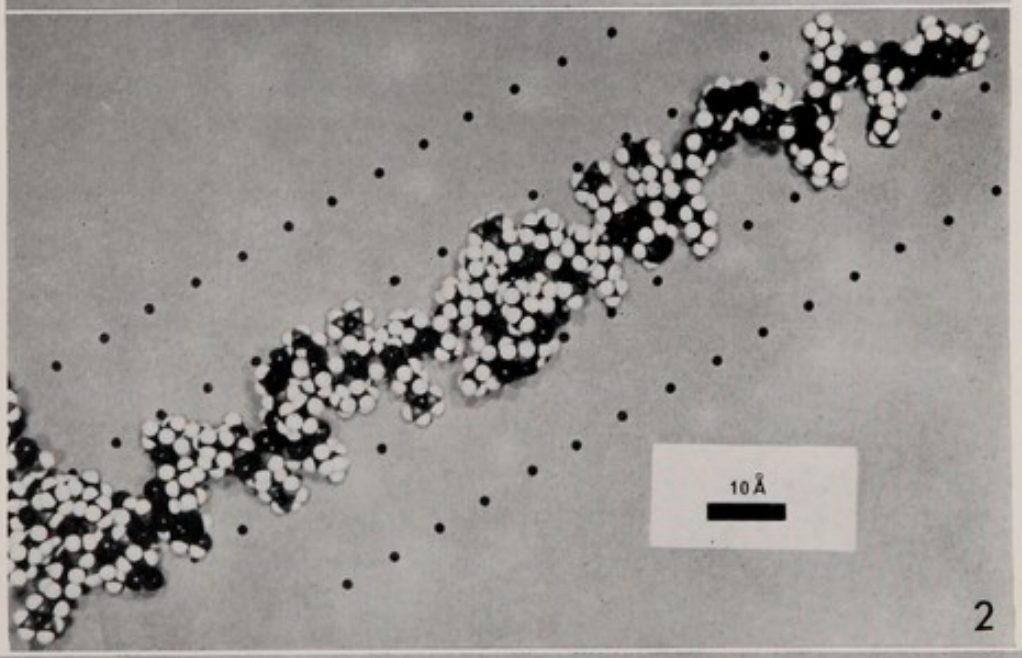
High angle diffraction pattern from a fibre specimen of nucleohistone at 98 per cent. relative humidity, the accompanying diagram shows the spacings of the diffraction maxima.

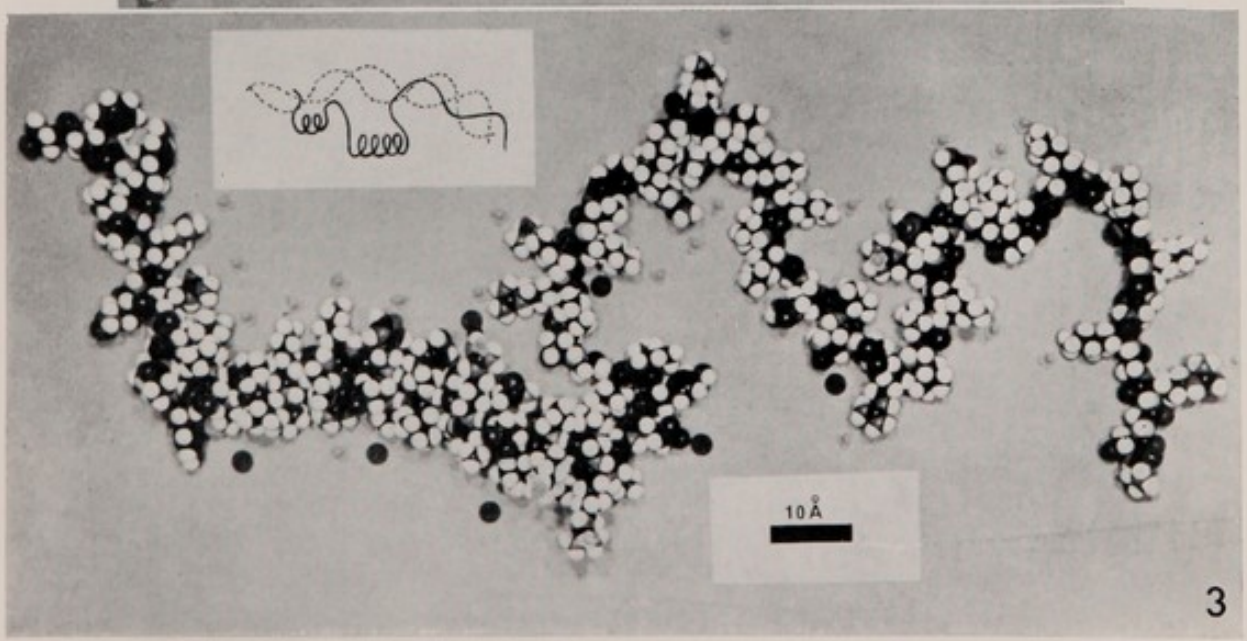




Low angle diffraction pattern of a nucleohistone gel at a concentration exceeding 100 per cent. relative humidity, obtained using a Franks double-mirror focussing camera. The gel was formed from a fibre specimen in a capillary tube by adding water, thus maintaining some alignment of the individual molecules. The diffraction ring at 105 Å is meridionally oriented.







Molecular models for histone type F2a1 using CPK space filling components.

FIG. 1.—A possible globular configuration with most of the hydrophobic residues inside and charged groups on the surface of the molecule.

FIG. 2.—The N-terminal portion of the molecule positioned on a cylindrical projection of the DNA phosphate groups. With the histone occupying the larger groove no more than 60 per cent. of the phosphates can be neutralised.

F one F2a1 when attached to DNA. The protein
l regions loop away from the double helix.

dispersed gel. The pellet contains a high molecular weight material with a DNA-histone ratio of 1 : 1·1. Fibre specimens are readily drawn from this material using techniques similar to those originally developed for DNA and oriented gels produced in capillary tubes by immersing the fibres in either distilled water or buffer solution. Diffraction patterns have been recorded over a range of relative humidities (r.h.) and concentrations, using high-angle cameras with pin hole collimation and low-angle cameras with Franks double-mirror focussing.

Typical diffraction patterns obtained from a fibre (plate I) and oriented gel (plate II) of nucleohistone include features which can be described under three headings.

(a) *Diffraction from the DNA double helix*

The high angle diffraction pattern shown in plate I was obtained from a fibre specimen maintained at 98 per cent. r.h. It includes diffuse rings which are characteristic of diffraction from double helical DNA. The most prominent is a ring or diffuse meridional arc at 3·4 Å arising from the stacking of the base pairs and rings at 8 and 12 Å corresponding to the third and second layer lines of helix diffraction. In some better oriented specimens the 3·4 Å arc is well oriented and concentrations of intensity appear in the positions of the cruciform set of reflections characteristic of the double helix of DNA. There is also a strong equatorial reflection which varies in position with relative humidity between 22 and 35 Å arising from the side-by-side packing of the molecules in the fibre specimen. The orientation in the diffraction from DNA in nucleohistone never approaches that obtained from fibres of isolated deproteinised DNA.

(b) *Diffraction from a lipid component*

An equatorial reflection at 60 Å is sometimes observed which arises from a lipid contaminant, probably nuclear membrane. The main difference between diffraction from isolated nucleohistone and intact nuclei is in the intensity of this reflection which is very strong in diffraction from nuclei.

(c) *Low angle diffraction pattern*

The series of reflections which we describe as the low angle rings are present in plates I and II. The complete series comprises reflections at 105, 55, 35, 27 and 22 Å. In plate I the ring at 105 Å is absent while in plate II it is clearly visible oriented in a meridional direction. In this diffraction pattern the 22 Å ring is not visible. In other examples meridional orientation is observed in the rings at 55 and 35 Å. The intensity distribution in the low angle diffraction pattern depends on the concentration of the specimen.

The poor orientation observed in the diffraction from the DNA suggests that the molecules are not capable of alignment parallel to the axis of the fibre. The series of low angle diffraction maxima, sometimes meridionally oriented, suggests that a regular tertiary structure is present. These considerations led Wilkins to suggest that a regular supercoiled structure exists in wet specimens of nucleohistone. Calculations in which the Fourier transforms of helices of various dimensions were studied (Pardon, 1966)

show that a helix with pitch 120 Å and radius 50 Å gives good agreement between observed diffraction and that calculated (see text fig. 1). Further,

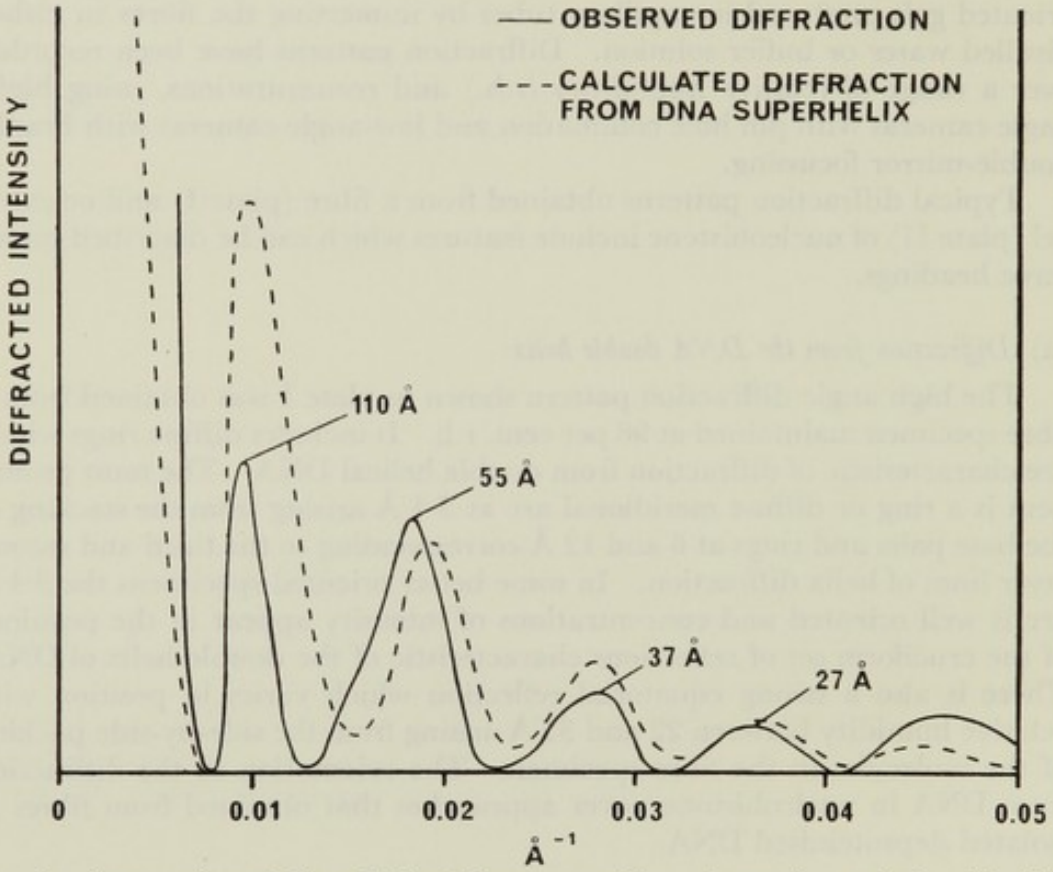


Fig. 1.—A comparison of the calculated Fourier transform of a continuous helix with pitch 120 Å and radius 50 Å with the observed diffraction from a nucleohistone gel. In this illustration the first order maxima in the observed diffraction is less than the calculated intensity. This reduction in intensity is due to packing together of the supercoiled molecules.

the supercoil model accounts for the observed tendency towards semi-meridional orientation. The changes in relative intensity within the low angle rings when the specimen concentration is changed can be explained by a consideration of the packing together of the helical molecules. Fig. 2 illustrates the dimensions of the super helix, the continuous lines representing the loci of the phosphate groups on a two dimensional projection.

3. The role of the different histone types in generating and maintaining the supercoil

In order to find out which histones are responsible for generating the supercoil we have studied nucleohistones which do not contain the full complement of histone types (Richards and Pardon, 1970). Nucleoproteins were reconstituted using DNA and fractionated histones. The structure of nucleohistones partially depleted of histones by semi-selective dissociation with various concentrations of salt were also examined. The results of reconstitution experiments are summarised in table 1. Nucleohistones reconstructed from whole histones and from whole histone minus type F1 always give diffraction patterns indistinguishable from native nucleohistone.

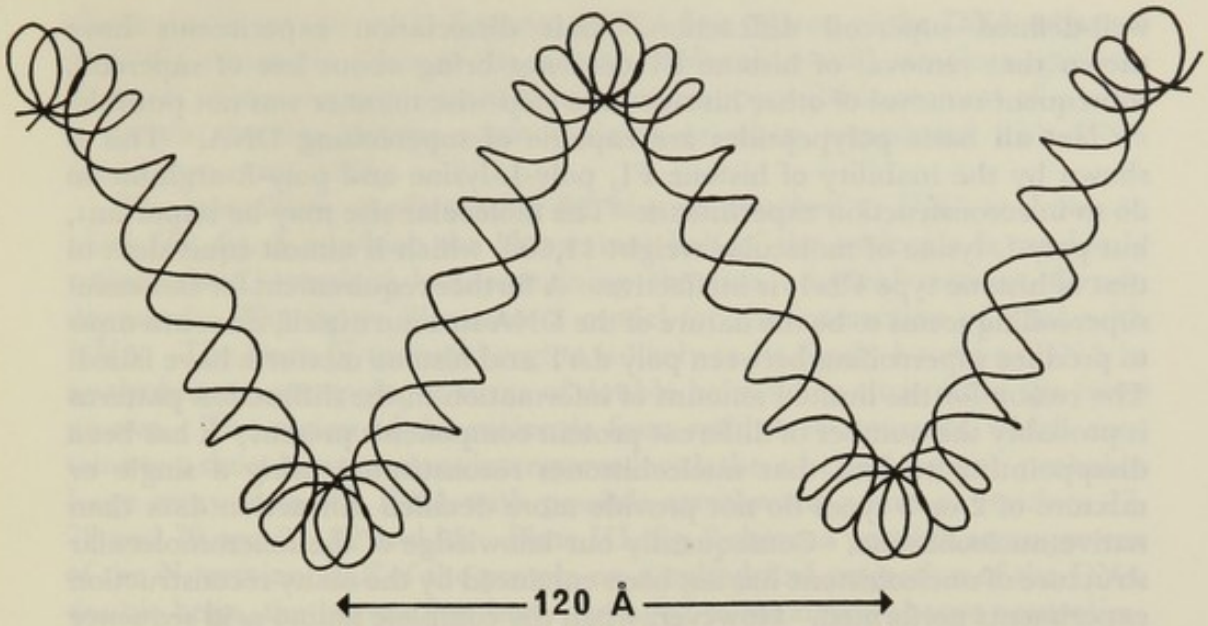


FIG. 2.—Diagram of the supercoil model for nucleohistone. The lines represent a planar projection of the phosphate groups of DNA in the supercoil configuration. The helix pitch is 120 Å and the centre to centre diameter of the DNA strand is 100 Å.

The " lysine rich " histone F1 prepared by the acid titration method of Murray (1966) never reconstitutes with DNA to give a diffraction pattern with low angle supercoil rings. None of the single histone types fractionated by the methods of Johns or Bonner have given supercoiled diffraction patterns but some success has been obtained using mixtures of 2 or 3 histone types. Firstly a mixture of F3, F2a1 and F2a2 prepared in our laboratory and secondly a mixture of types F3 and F2a2, with only a trace of histone F2a1, prepared for us by Dr Johns. Both gave diffraction patterns with

TABLE 1

A summary of results obtained from reconstruction experiments. Nucleohistones were reconsituted from DNA and various histone fraction mixtures, the presence or absence of tertiary structure in the resulting complexes is indicated

Histone	Method of histone preparation	Source of DNA	Tertiary structure present in complex
1. Whole histone	Salt extraction from DNH	Calf thymus	Yes
2. Whole histone less F1	Acid titration from DNH-*p*H 1·2 and 0·6... fractions	Calf thymus	Yes
3. Mainly F1	Acid titration from DNH-*p*H 1·8 fraction	Calf thymus	No
4. F3+F2a1+F2a2	Chemical fractionation	Calf thymus	Yes
5. F3+F2a2 (trace F2a1)	Chemical fractionation	Calf thymus	Yes
6. Poly-L-lysine	Obtained from Miles Labs. Inc.	Calf thymus	No
7. Poly-L-arginine	Obtained from Miles Labs. Inc.	Calf thymus	No
8. Whole histone minus F1 (as 2)	As 2	Poly dAT (Miles Labs. Inc.)	No

well-defined supercoil diffraction. Salt dissociation experiments have shown that removal of histone F1 does not bring about loss of supercoil, subsequent removal of other histones in a step-wise manner was not possible.

Not all basic polypeptides are capable of supercoiling DNA. This is shown by the inability of histone F1, poly-L-lysine and poly-L-arginine to do so in reconstruction experiments. The molecular size may be important, but poly-L-lysine of molecular weight 11,000, which is almost equivalent to that of histone type F2a1, is ineffective. A further requirement for successful supercoiling seems to be the nature of the DNA structure itself, since attempts to produce supercoiling between poly dAT and histone mixtures have failed. The reason for the limited amount of information in the diffraction patterns is probably the number of different protein components present; it has been disappointing to find that nucleohistones reconstituted using a single or mixture of 2 or 3 types do not provide more detailed diffraction data than native nucleohistone. Consequently our knowledge of the macromolecular structure of nucleohistone has not been enhanced by the many reconstruction experiments performed. However, when the complete amino acid sequence of histone F2a1 became available (De Lange *et al.*, 1969; Ogawa *et al.*, 1969) closely followed by that of histone F2b (Iwai *et al.*, 1970), we were able to examine possibilities for the atomic interaction between DNA and these histone fractions.

Model building structures

We have built the complete amino acid sequence for histone F2a1 using CPK space filling atomic models, investigated various conformations for the protein and eliminated those conformations which do not permit extensive neutralisation of the charged phosphate groups of the DNA molecule. We have thus considered possible arrangements for the histone to the DNA which might impose specific constraints leading to supercoil formation.

A brief survey of the amino acid sequence shows that the N-terminal half of histone F2a1 is very basic with 17 basic side chains in the first 45 residues. In contrast the C-terminal portion of the molecule from residue 45-102 is not particularly basic although there are two short basic regions from residue 77 to 79 and residues 91 to 95. De Lange *et al.* noted that any spontaneous secondary structure in the molecule is likely to be confined to the C-terminal half owing to the presence of hydrophobic and hydrogen bonding hydroxyl groups. This was confirmed by an analysis which we (1970) and others (Boublik *et al.*, 1970) have made showing that the sequence has a potential for α-helix formation between residues 57-74 and 80-90 whilst the N-terminal half of the molecule, by virtue of its high charge density, is unlikely to adopt any α-helix conformation and must consequently exist in a random coil form.

Plate III, fig. 1 shows the model of histone F2a1 in a globular conformation with the majority of the hydrophobic groups in the central region and most of the charged groups pointing outwards. The overall diameter of this model is only 40 Å and it can attach to only a single turn of DNA helix. Even if the molecule is allowed to make contact with two adjacent DNA double helices it is not capable of neutralising many of the phosphate groups when equal weights of DNA and protein are present. A completely extended

chain would occupy up to 6 turns of DNA but regions of the DNA adjacent with the non-basic parts of the histone molecule would remain negatively charged. Experimental results show that an appreciable amount of α-helix is present in this histone when isolated or when complexed with DNA (Bradbury *et al.*, 1965).

The most likely conformation for histone attached to DNA is shown in plate III, fig. 3 in which the N-terminal half of the molecule is randomly coiled, the C-terminal half containing the two α-helical sections already discussed. The figure illustrates a model for the interaction of F2a1 with DNA. The basic N-terminal region is likely to be firmly bound to DNA in nucleohistone occupying 2 turns of double helix when situated in the large groove. The region of the molecule from residue 46 onwards, which contains two short basic sections interspersed with the α-helical regions, probably loops away from the DNA with possible attachment points at residues 77, 78 and 79 and 91, 92 and 95. Plate III, fig. 2 shows a possible arrangement of the N-terminal half of the protein on a cylindrical projection of the DNA double helix, the histone has been arranged to maximise charge neutralisation. It is readily apparent that only 60 per cent. of the phosphate groups can be neutralised in the vicinity of the N-terminal half of the molecule, if the remainder of the molecule is arranged such that the other attachment points closely follow the N-terminal region there is no reduction in the 60 per cent. of phosphate groups neutralised. The mass ratio of DNA : Histone for the protein attached to the DNA in this way is 1 : 0·65, a figure significantly less than the experimentally determined ratio of 1 : 1·1.

It is interesting to speculate about possible mechanisms which might induce supercoil formation. If the spacings between positive sidechains of histone are slightly different from those of the phosphate groups, the two sets of charged groups will not be able to match without some distortion of one or other of the components. The result of such an interaction could lead to the linear double helix adopting a super-helix conformation. A similar argument can equally well be applied if either hydrophobic or hydrogen bonds are involved between some side chains and atoms within the grooves of the double helix. The model building studies indicate that there is no regular distribution of charged groups on either F2a or F2b and there is no evidence for any mismatch that might induce regular coiling although the possibility cannot be ruled out. Johns (1966) suggested that the apolar amino-acid sequences may form loops extending away from the double helix and form hydrophobic links with similar loops situated further along the molecule, he suggested that such interactions might lead to condensation or supercoiling of the DNA. A third mechanism capable of introducing tertiary coiling requires both restraints along the DNA and also a change in the pitch of the DNA duplex. In closed circular viral DNA a change in helix pitch brought about by a change in buffer does introduce supercoiling (Bauer and Vinograd, 1968; Vinograd *et al.*, 1968). If histones form links at regular points along the DNA, either between adjacent molecules or different parts of a single molecule, a situation analogous to closed circular DNA exists. Slight distortions in the DNA brought about by histone-DNA interaction would lead to the introduction of supercoiling. We have examined the ability of histone F2a1 to form cross links via its C-terminal end as shown in fig. 3*a* and *b*. Clearly this histone fraction only has the ability to interact with at most a single gyre of the supercoil.

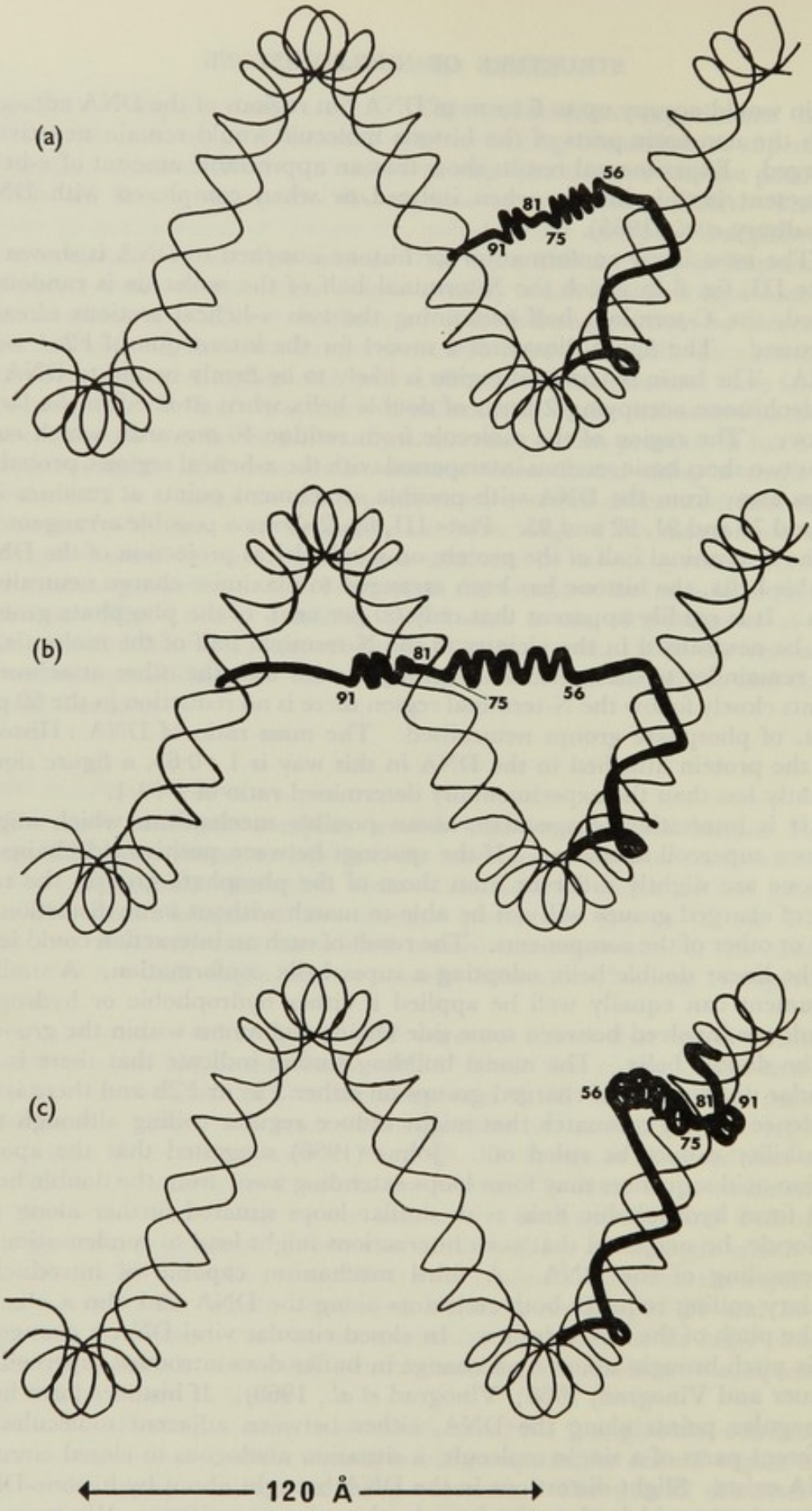


FIG. 3.—Alternative arrangements for the attachment of histone type F2a1 to supercoiled. DNA in which the C-terminal half of the protein forms an intra-molecular crosslink. The histone is represented by the thick line and the α-helical regions are included. Histone F2a1 is unlikely to form links across a complete gyre of the supercoil either diametrically (a) or parallel to the supercoil axis (b). The most likely arrangement, in which the α-helix forms small loops away from the DNA, is shown in (c).

4. The relationship between the structure of nucleohistone and chromosome structure

Any comparison that is made between the macromolecular structure of nucleohistone and that of chromosomes must involve a comparison of results obtained by X-ray diffraction and those obtained by electron microscopy.

Electron microscopic investigation of both thin sections and spread preparations reveal in chromosome material the existence of a unit thread (Davies, 1968). There is some controversy about the actual diameter of the thread, measurements made with sections give diameters within the range 100-150 Å (Wolfe and Martin, 1968; DuPraw, 1965; Wolfe, 1965; Ris, 1968). The 250 Å thread may consist of a single 100 Å thread folded back upon itself (Ris, 1968). It should be borne in mind that all samples studied in the electron microscope are dry and that all biological macromolecules require the co-existence of water for the maintenance of their natural configuration. Indeed X-ray diffraction studies have shown that the nucleohistone supercoil diffraction is no longer present in patterns obtained from specimens maintained at relative humidities less than 75 per cent., even if the nucleohistone has been fixed using the techniques in general use by electron microscopists. It is almost certainly for this reason alone that regular supercoiled nucleohistone molecules have not been observed in the electron microscope. However, it is tempting to look upon the 100 Å thread as a collapsed form of what was originally a regular helical structure with an outer diameter of between 130 and 140 Å. It is hoped that parallel studies by electron microscopy and X-ray diffraction may in future clarify the position of nucleohistone as the basic unit of chromosome structure.

Clearly a determination of the macromolecular structure of the unit thread only provides the initial step towards a knowledge of chromosome structure. Major efforts will be needed to define the organisation of the unit thread within the chromosome especially during chromosome reduplication, transcription and cell division.

5. Summary

1. The structure of interphase chromosomes can be considered in terms of nucleohistone structure because these materials give similar results in X-ray diffraction studies.

2. A regular supercoil structure is very likely to occur in nucleohistone.

3. Molecular models of histone F2a1 have been built in order to examine interactions between histone and DNA.

Acknowledgments.—We wish to thank Dr A. J. Hale for encouragement, discussion and provision of facilities, and Mr Zoltan Gabor for the excellent photographic work, Mr Jack Hayward for making our X-ray cameras and Mrs Elizabeth Hirst and Mr Tim Knight for technical help.

6. References

BAUER, W., AND VINOGRAD, J. 1968. The interaction of closed circular DNA with intercalative dyes. I. The superhelix density of SV40 DNA in the presence and absence of dye. *J. Mol. Biol.*, *33*, 141.

BOUBLIK, M., BRADBURY, E. M., AND CRANE-ROBINSON, C. 1970. An investigation of the conformational changes of Histones F1 and F2a1 by proton magnetic resonance spectroscopy. *Eur. J. Biochem., 12,* 258.

BRADBURY, E. M., CRANE-ROBINSON, C., PHILLIPS, D. M. P., JOHNS, E. W., AND MURRAY, K. 1965. Conformational investigations of histones. *Nature, 205,* 1315.

DAVIES, H. G. 1968. Topographical observations on the envelope-limited sheets of chromatin associated with the cell nucleus. *4th Europ. Regional Conf. on Electron Microscopy,* 175.

DAVIES, H. G., AND SMALL, J. V. 1968. Structural units in chromatin and their orientation on membranes. *Nature, 217,* 1122.

DE LANGE, D. J., FAMBROUGH, D. M., SMITH, E. L., AND BONNER, J. 1969. Calf and pea Histone IV. II. The complete amino acid sequence of calf thymus histone IV. Presence of —N-acetyllysine. *J. Biol. Chem., 244,* 319.

DUPRAW, E. J. 1965. The organisation of nuclei and chromosomes in honey bee embryonic cells. *Proc. Natl. Acad. Sci., 53,* 161.

IWAI, K., ISHIKAWA, K., AND HAYASHI, H. 1970. Amino-acid sequence of slightly lysine-rich histone. *Nature, 226,* 1056.

JOHNS, E. W. 1966. The fractionation and characterization of histones and their interactions with deoxyribonucleic acid. *Biochem., J., 101,* 23p.

KAYE, J. S., AND MCMASTER-KAYE, R. 1966. The fine structure and chemical composition of nuclei during spermiogenesis in the house cricket. *J. Cell Biol., 31,* 159.

MURRAY, K. 1966. The acid extraction of histones from calf thymus DNP. *J. Mol. Biol., 15,* 409.

OGAWA, Y., QUAGLIAROTTI, G., JORDAN, J., TAYLOR, C. W., STANBUCK, W. C., AND BUSCH, H. 1969. Structural analysis of the glycine-rich, arginine-rich histone. III. Sequence of the amino terminal half of the molecule containing the modified lysine residues and the total sequence. *J. Biol. Chem., 244,* 4387.

PARDON, J. F. 1966. Coiled-coil models for nucleohistone. X-ray diffraction studies and Fourier transform calculations. *Ph.D. Thesis, University of London.*

PARDON, J. F., AND RICHARDS, B. M. 1971. The structure of nucleoprotein systems. *Biological Macromolecules,* vol. 4 (in press) (ed. Timasheff and Fasman, Arnold, London).

RICHARDS, B. M., AND PARDON, J. F. 1970. The molecular structure of nucleohistone (DNH). *Exptl. Cell Research, 62,* 184.

RIS, H. 1968. The molecular organisation of chromosomes. *The Handbook of Molecular Cytology* (ed. Lima-de-Faria), 222.

VINOGRAD, J., LEBOWITZ, J., AND WATSON, R. 1968. Early and late helix-coil transitions in closed DNA, the number of superhelical turns in polyoma DNA. *J. Mol. Biol., 33,* 173.

WOLFE, S. G. 1965. The fine structure of isolated chromosomes. *J. Ultrastruct. Res., 12,* 104.

WOLFE, S. L., AND MARTIN, P. G. 1968. The ultrastructure and strandedness of chromosomes from two species of *Vicia*. *Exptl. Cell Res., 50,* 140.

ZUBAY, G., AND DOTY, P. 1959. The isolation and properties of deoxyribonucleoprotein containing single nucleic acid molecules. *J. Mol. Biol., 1,* 1.

CHROMOSOME STRUCTURE STUDIED BY NUCLEIC ACID HYBRIDISATION IN CYTOLOGICAL PREPARATIONS *

MARY LOU PARDUE and JOSEPH G. GALL

M.R.C. Epigenetics Research Group, Department of Genetics, University of Edinburgh, and Department of Biology, Yale University

1. Introduction

Biochemical studies (Marmur and Lane, 1960; Doty, Marmur, Eigner and Schildkraut, 1960; Hall and Spiegelman, 1961) have established that single stranded nucleic acids, both RNA and DNA, can associate with their complementary sequences to form hybrid double helical molecules. Techniques have now been developed which permit the formation of such hybrid molecules between an isolated nucleic acid and its complementary sequences within the DNA of an intact cytological preparation (Gall and Pardue, 1969; Pardue and Gall, 1969; John, Birnstiel and Jones, 1969; Buongiorno-Nardelli and Amaldi, 1970). Autoradiographic detection of tritium-labelled hybrids allows the spatial localisation of specific DNA sequences.

In developing a technique for cytological hybridisation we have used oocytes of the toad *Xenopus laevis* as a test system. Pachytene oocytes in *Xenopus* have a large mass of extra DNA forming a cap over the chromosomes. This DNA mass contains many extra copies of the genes coding for ribosomal RNA and is produced by differential replication of these sequences after the chromosomes have completed their premeiotic doubling (Brown and Dawid, 1968; Evans and Birnstiel, 1968; Gall, 1968, 1969; Macgregor, 1968). The follicle cells of the ovary do not undergo amplification of the ribosomal cistrons. Thus, squash preparations of the *Xenopus* ovary have the cytologically distinct cap regions in the oocyte which should hybridise extensively with *Xenopus* ribosomal RNA. In addition these preparations have pachytene chromosomes and follicle cells which serve as control regions. We would expect to detect the unamplified ribosomal genes in the follicle cells only after very long autoradiographic exposures.

2. Methods

We have published a detailed account of our technique (Gall and Pardue, 1970). Briefly the procedure is as follows. Conventional cytological squash preparations of *Xenopus* ovary tissue were treated with dilute acid to remove basic proteins and then with RNase to digest endogenous RNA. The DNA of the preparations was next denatured with alkali and the slides were incubated with tritiated *Xenopus* ribosomal RNA. After this hybridisation step the preparations were again treated wth RNase to remove any non-specifically bound RNA and then coated with autoradiographic emulsion. After the appropriate time autoradiographs were developed and the chromosomes were stained with Giemsa stain through the emulsion.

* Research supported by USPHS grants GM 12427 and GM 397 from the National Institute of General Medical Sciences.

3. Results

Plate I, fig. 1 shows an autoradiograph of a pachytene oocyte after hybridisation. Tritiated ribosomal RNA has bound to the extra DNA of the cap but not to the chromosomes. Plate I, fig. 2 shows follicle cells from a *Xenopus* ovary squash preparation which has been given a very long autoradiographic exposure. This slide shows localised labelling over each nucleus. It is frequently possible to see a nucleolus under the label, suggesting that we are detecting the ribosomal cistrons of the unamplified genome.

The hybridisation in these slides is confined to locations which might be expected from the biochemical studies on the origin of ribosomal RNA. Thus the binding shows the specificity expected of true nucleic acid hybridisation. We have other evidence that we are seeing true hybrid molecules. (1) If DNA is removed with DNase prior to hybridisation no RNA is bound to the slide. (2) The RNA in the hybrids is resistant to RNase digestion. (3) There is almost no hybridisation unless the DNA of the preparation is first treated with a denaturing agent. (4) The most convincing proof that these preparations show true hybridisation comes from the fact that the reaction discriminates between different types of RNA. Non-radioactive *Xenopus* ribosomal RNA competes with tritiated *Xenopus* ribosomal RNA for binding sites, but non-radioactive ribosomal RNA from *Escherichia coli* does not.

We have also used the *Xenopus* oocyte as a test system in developing a technique for the hybridisation of isolated DNA fractions to the DNA of cytological preparations. In this case the hybridising material was tritiated *Xenopus* DNA from which the ribosomal cistrons had been removed. As shown by Wallace and Birnstiel (1966) the ribosomal genes in *Xenopus* are more dense in CsCl gradients than the bulk of the DNA. By discarding the fractions of a CsCl gradient which were more dense than the main band of DNA we obtained a DNA which contained essentially all of the *Xenopus* genome except for the ribosomal cistrons. Xenopus ovary preparations hybridised with this DNA gave the converse of the picture obtained when the hybridisation was done with ribosomal RNA. Label was found over pachytene chromosomes (plate I, fig. 4) and interphase follicle cells (plate I, fig. 3) but not over the cap of ribosomal cistrons in the pachytene oocyte (plate I, fig. 4).

For our first attempts at examining the organisation of genes within chromosomes we chose the polytene chromosomes of Dipteran salivary glands. The genes which we studied were again those coding for ribosomal RNA. We have not been able to obtain Dipteran RNA with sufficient radioactivity for our experiments. Instead we have used *Xenopus* ribosomal RNA in these hybridisations. Sinclair and Brown (1969) showed that *Xenopus* ribosomal RNA hybridised with the DNA of all of the eukaryotes which they tested. We have used such heterologous hybridisations in a number of types of experiments in our laboratory. All the evidence indicated that the *Xenopus* RNA is actually binding to the ribosomal genes in the Dipteran DNA (Gerbi, 1970; Pardue, Gerbi, Eckhardt and Gall, 1970). In some experiments we have used tritiated RNA which we prepared by *in vitro* transcription with *E. coli* RNA polymerase. This procedure yields a complementary RNA of much higher specific radioactivity than can be obtained *in vivo*. The template for the *in vitro* transcription was *Xenopus*

Plate I

FIGURES 1-4. Autoradiographs of nuclei from the ovary of the toad, *Xenopus* after cytological hybridisation with radioactive nucleic acids. All slides are stained with Giemsa.

FIG. 1.—Late pachytene oocyte nucleus hybridised with radioactive *Xenopus* ribosomal RNA. Grains are seen over the mass of extra ribosomal cistrons but not over the chromosomes. The DNA of the nucleus was denatured on the slide with 0·07 N NaOH. The slide was then incubated with rRNA at 2 μg/ml. in 6 × SSC at 66° C. for 16 hours. After the hybridisation step, non-specifically bound RNA was removed with RNase. The specific activity of the RNA was 500,000 c.p.m./μg. Exposure 52 days. × 1200.

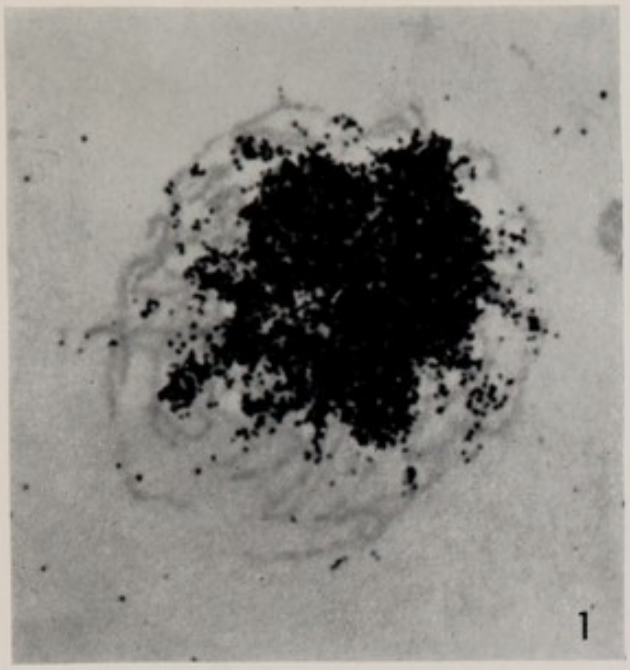
FIG. 2.—Interphase follicle nuclei hybridised with the same ribosomal RNA used in fig. 1. After an autoradiographic exposure of 147 days the localised binding shows the presence of the unamplified ribosomal cistrons of the normal diploid complement. Exposure 147 days. × 1100.

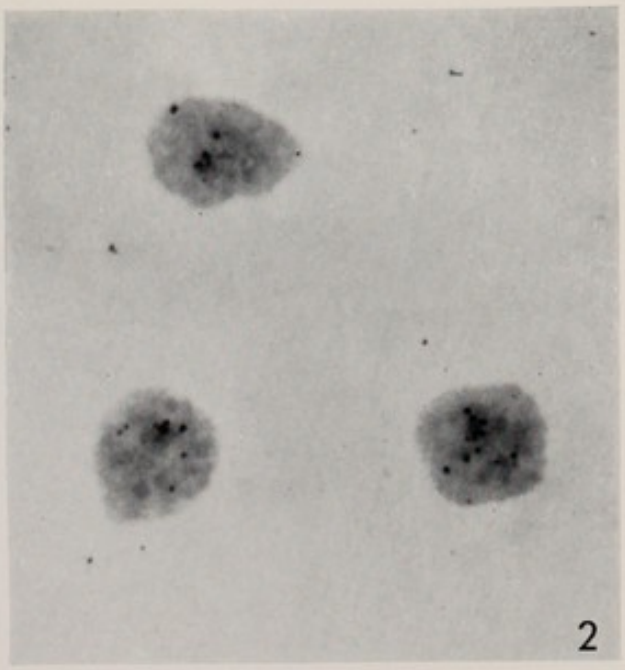
FIG. 3.—Follicle nuclei after hybridisation with radioactive *Xenopus* DNA from which the ribosomal cistrons had been removed. Label is spread over the nuclei. The DNA of the cytological preparation was denatured with 0·07 N NaOH. The slide was then incubated with denatured radioactive DNA at 2 μg/ml. in 2 × SSC at 66° C. for 15 hours. After the annealing step the slide was washed 5 times in 2 × SSC at 60° C. to remove non-specifically bound DNA. The specific activity of the DNA was 130,000 c.p.m./μg. Exposure 12 days. × 1200.

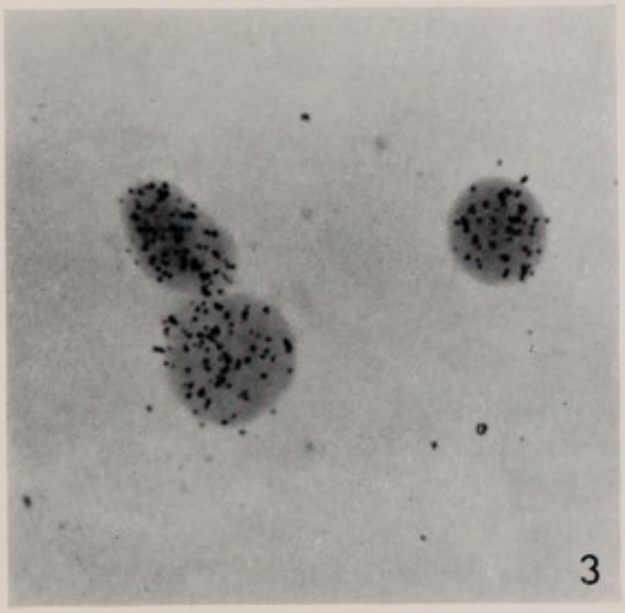
FIG. 4.—Pachytene oocyte nucleus hybridised with the same radioactive *Xenopus* DNA used in fig. 3. The test DNA has bound to the chromosomes but not the cap of amplified rDNA. The conditions of denaturation and hybridisation are those described for fig. 3. Exposure 16 days. × 1200.

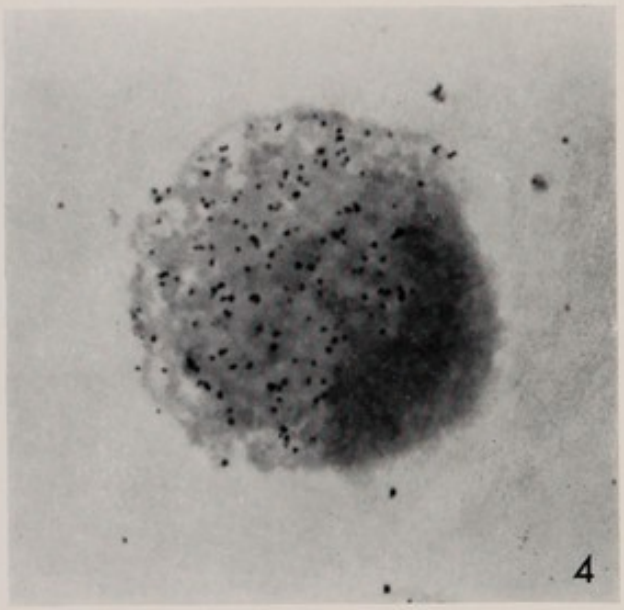
FIG. 5.—Autoradiograph of a salivary gland preparation from a larva of *Drosophila hydei* showing hybridisation of radioactive ribosomal RNA to the DNA within the nucleolus. The DNA of the preparation was denatured with 0·07 N NaOH for 20 minutes and then hybridised with radioactive *Xenopus* ribosomal RNA for 14 hours. The RNA had a specific activity of 5×10^5 c.p.m./μg. The slide was stained with Giemsa. Exposure 94 days. × 1300.

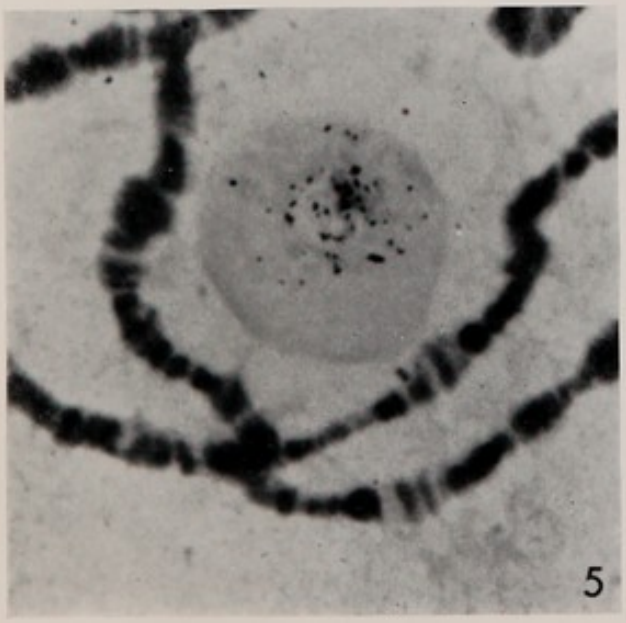
FIG. 6.—Autoradiograph of an X chromosome from a salivary gland nucleus of *Rhynchosciara hollaenderi*. Complementary RNA transcribed *in vitro* from the *Xenopus* satellite DNA (which contains the ribosomal cistrons) is hybridised to the heterochromatic end of the chromosome. The DNA of this slide was denatured with 0·07 N NaOH for 2 minutes and then hybridised with complementary RNA for 10 hours. The RNA had a calculated specific activity of 7×10^7 d.p.m./μg. Slide stained with Giemsa. Exposure 10 days. × 670.


1


2

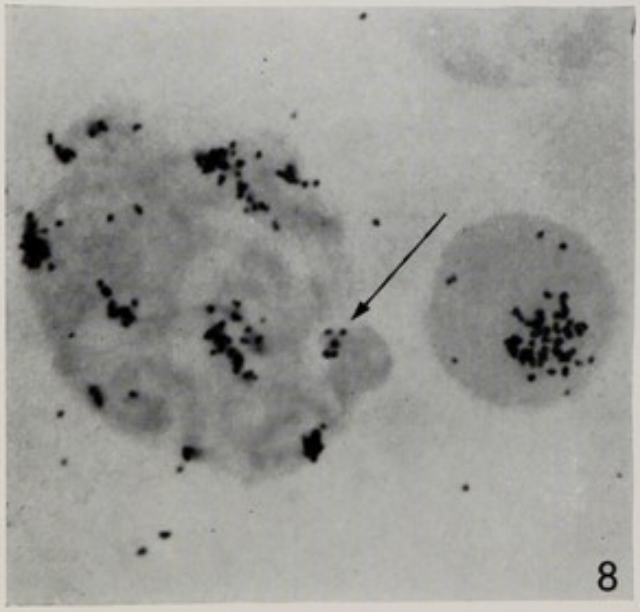

3

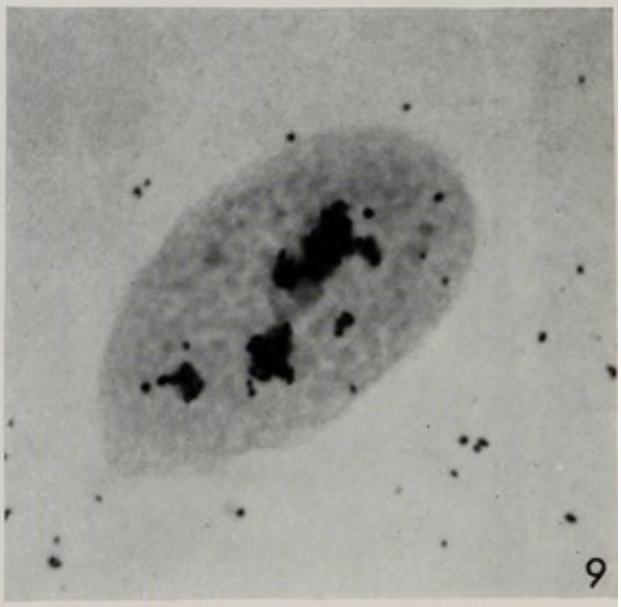

4


5

6

7


8


9

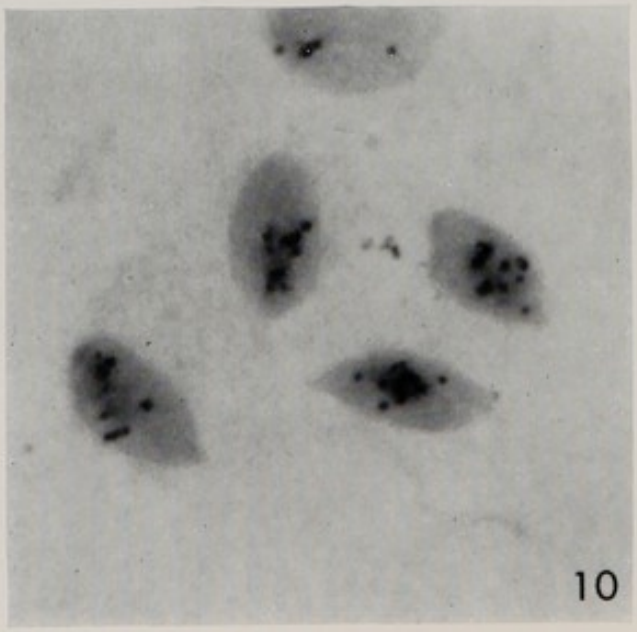

10

Plate II

FIG. 7.—Autoradiograph of chromosomes from mouse tissue culture line SV3T3 after cytological hybridisation with radioactive RNA copied *in vitro* from mouse satellite DNA. The RNA has bound to its complementary sequences in the DNA of the chromosomes, showing that these sequences are localised in the centromeric heterochromatin. The DNA of the cytological preparation was denatured *in situ* and the slide was incubated with the radioactive RNA for 10 hours at 66° C. in 2 × SSC. The RNA had a calculated specific activity of 7×10^7 d.p.m./μg. Slide stained with Giemsa. Exposure 5 days. × 2600.

FIG. 8.—Autoradiograph of a mouse pachytene spermatocyte and a spermatid hybridised with radioactive RNA copied *in vitro* from mouse satellite DNA. In the pachytene nucleus the densely stained heterochromatic regions of the autosomes are heavily labelled, but the sex vesicle, containing the heterochromatic X and Y chromosomes, shows only a few grains and these are localised over the tip (*arrow*). The centromeric heterochromatin of the spermatid is tightly localised. The conditions of denaturation and hybridisation are the same as for fig. 7. Exposure 10 days. Giemsa stain. × 1500.

FIG. 9.—Autoradiograph of a Sertoli cell from a mouse testis preparation which has been hybridised with radioactive RNA complementary to mouse satellite DNA, as described for fig. 7. Heavy label is seen over the two basophilic bodies of the " compound nucleolus " and over one other densely staining region, indicating the associations of the centromeric regions in these cells. Exposure 10 days. Giemsa stain. × 1900.

FIG. 10.—Autoradiograph of elongating spermatids from a mouse testis preparation hybridised with radioactive RNA copied from mouse satellite DNA, as described for fig. 7. The centromeric localisation seen in the early spermatid in fig. 8 is still apparent in these cells. Exposure 2 days. Giemsa stain. × 1800.

ovarian satellite DNA which contains the ribosomal cistrons (Pardue, Gerbi, Eckhardt and Gall, 1970; Reeder and Brown, 1970). The product of the *in vitro* transcription hybridised to the same regions of the Dipteran chromosomes as did *Xenopus* ribosomal RNA.

In *Drosophila*, the biochemical experiments of Ritossa and Spiegelman (1965) have shown that the ribosomal cistrons are located at or very near to the nucleolus organiser on the X and Y chromosomes. We have done cytological hybridisations on salivary chromosomes from *Drosophila hydei*. These experiments showed that the ribosomal genes are actually in the body of the nucleolus (plate I, fig. 5). There was no binding of ribosomal RNA to any chromosomal band. The hybridisation tended to follow the distribution of DNA in the nucleolus. It is sometimes possible to see strands of hybridised DNA extending from the nucleoli but, since the nucleoli are usually separated from the chromosomes during squashing, we have not been able to follow these strands back to the chromosomes. The results suggest that the nucleolus might be an exaggerated puff with the nucleolar material surrounding the chromosomal strands which contain the ribosomal genes. These strands would be part of the linear continuity of the chromosome in the intact cell but easily separated from the chromosome in squashing.

We have done similar experiments on the salivary gland nuclei of two other Diptera, *Rhynchosciara* and *Sciara*. Here the organisation of the nuclear material is somewhat different from that of *Drosophila*. In *Rhynchosciara* salivary glands the tip of the X chromosome bears a prominent nucleolus in young larvae but this nucleolus tends to disperse in later larval life. These salivary gland nuclei also contain a second type of nucleolar material in the form of " micronucleoli ". Micronucleoli are small bodies scattered among the chromosomes. They resemble the nucleolus in fine structure and have been shown to contain DNA as well as RNA (Eckhardt, personal communication; Gabrusewycz-Garcia, personal communication).

The experiments on *Rhynchosciara* were done in collaboration with Dr Ronald Eckhardt. We found that *Xenopus* ribosomal RNA hybridised to the nucleolar organiser on the X chromosome (plate I, fig. 6), to the micronucleoli, and to the end of the C chromosome where no nucleolar organiser has as yet been described.

Xenopus ribosomal RNA hybridised to the heterochromatic region which is found on one end of the *Rhynchosciara* X chromosome. When portions of the nucleolus were still attached to the X chromosome, hybridised DNA was seen to be splayed out into the nucleolar material forming a sort of puff around the heterochromatic knob. It may be that the micronucleoli arise by fragmentation of parts of this nucleolar organiser. Ribosomal RNA also hybridised with a heterochromatic knob on one end of the C chromosome of *Rhynchosciara*. Although we have seen no evidence of a nucleolus associated with this region in the stages which we have examined, the C chromosome might bear a nucleolus in other stages or in other tissues.

Hybridisation experiments on salivary gland nuclei from *Sciara*, done in collaboration with Dr Susan Gerbi, showed that ribosomal DNA was present in the nucleolar organiser and the micronucleoli.

We had an additional reason for doing cytological hybridisation studies on the salivary chromosomes of sciarid flies. The polytene chromosomes of both *Rhynchosciara* and *Sciara* contain several DNA puffs. These regions

IMPERIAL CANCER
LINCOLN'S INN
FIELDS
LIBRARY

undergo more rounds of DNA replication than other regions of the polytene chromosomes (Breuer and Pavan, 1955; Rudkin and Corlette, 1957; Swift, 1962; Crouse and Keyl, 1968). None of the DNA puffs in either *Rhynchosciara* or *Sciara* bound ribosomal RNA in our experiments. This result shows that genes other than those coding for ribosomal RNA can undergo gene amplification.

We are also using cytological hybridisation to study the chromosomal localisation of some of the fractions of multiply repeated DNA sequences which have been found in the genomes of eukaryotes (Britten and Kohne, 1968). Although all higher organisms appear to contain families of repetitive DNA, nothing is known of the function of this type of DNA. Perhaps the most thoroughly studied fraction of repetitive DNA is the mouse satellite DNA (Kit, 1961; Flamm, McCallum and Walker, 1967). For this reason we chose it for our first experiments. We have studied the mouse satellite DNA both by DNA-DNA hybridisation and by RNA-DNA hybridisation using RNA copied *in vitro* from mouse satellite DNA (Pardue and Gall, 1969, 1970). In both types of experiment the results were the same. Mouse satellite DNA sequences were localised in the blocks of heterochromatin adjacent to the centromeres (plate II, fig. 7). In our preparations only one chromosome was consistently unlabelled. Because this chromosome was one of the smallest in the testis preparations and was more heteropycnotic than the rest we have tentatively identified it as the Y chromosome. As a control we have done parallel experiments with the main peak of the mouse DNA. Main peak DNA hybridisation was widely distributed over the mouse chromosomes indicating that the euchromatic regions had been denatured and were capable of binding complementary nucleic acid sequences.

Jones (1970) has also reported that mouse satellite binds to the centromeric region of metaphase chromosomes from mouse tissue culture cells. The results from *in situ* hybridisation confirm and extend the findings from two biochemical studies on the localisation of mouse satellite DNA. Maio and Schildkraut (1969) fractionated mouse chromosomes on the basis of size. They found that each size class contained approximately the same proportion of satellite DNA as did the entire genome, suggesting that satellite DNA makes up a constant proportion of the DNA of the individual chromosomes. Yasmineh and Yunis (1969) showed that isolated heterochromatin from mouse cells contained satellite DNA while isolated euchromatin did not.

In our experiments with both tissue culture and testis preparations mouse satellite DNA bound to the centromeric heterochromatin in all cells in which the chromosomes were condensed. In other nuclei the positions of the centromeres could be followed by the localisation of satellite hybridisation. The deeply staining chromocentres of interphase nuclei preferentially bound satellite DNA. In the Sertoli cells of the testis, hybridisation of satellite DNA indicated that the centromeres are closely associated into a few small bodies near the nucleolus (plate II, fig. 9). In spermatocytes the centromeres make up a single mass in the centre of the nucleus. This configuration persists during the differentiation of the spermatids (plate II, fig. 10).

Mouse satellite DNA hybridises specifically with mouse chromosomes. In collaboration with Dr Frank Ruddle (Ruddle, Chapman, Chen and Klebe, 1970) we have done experiments using satellite hybridisation to detect the mouse chromosomes in tissue culture lines derived from the fusion of

mouse and human cells. In these cells only the chromosomes of mouse origin bound mouse satellite DNA.

These cytological hybridisation experiments serve to emphasise again the difference between constitutive and facultative heterochromatin. In the mouse testis both the X and Y chromosomes show facultative heterochromatinisation yet satellite DNA bound only to a small portion of the body formed by these two chromosomes (Plate II, fig. 8). Presumably that portion represents the centromeric heterochromatin of the X chromosome. It is possible that all heterochromatin, both constitutive and facultative, might be rich in highly repetitive DNA sequences and that the characteristics of heterochromatin are a reflection of the redundant nature of the DNA. However, the nucleotide sequence of the centromeric heterochromatin in the mouse seems to be unique to that particular heterochromatin.

4. Summary

1. A technique is described for the formation of molecular hybrids between either DNA or RNA in solution and the DNA of cytological preparations. Autoradiographic detection of radioactive hybrids permits the localisation of specific DNA sequences at the chromosome level.

2. In *Xenopus* oocytes, ribosomal RNA binds strongly to the mass of extra DNA which forms a cap over the chromosomes at pachytene and which contains many extra copies of genes coding for ribosomal RNA.

3. In the salivary glands of *Drosophila* the ribosomal cistrons are located within the nucleolus. In sciarid flies they are present both in the nucleolar organising regions of the chromosomes and in micronucleoli scattered throughout the nucleus.

4. The extra DNA in the DNA puffs of sciarid flies does not hybridise with ribosomal RNA, indicating that genes other than those coding for ribosomal RNA can undergo amplification.

5. Mouse satellite DNA sequences are localised in the centromeric heterochromatin of each of the chromosomes with the possible exception of the Y chromosome. Centromere associations in several types of interphase cells of the mouse can be followed by studying the binding of satellite DNA in these cells.

5. References

BREUER, M. E., AND PAVAN, C. 1955. Behavior of polytene chromosomes of *Rhynchosciara angelae* at different stages of larval development. *Chromosoma, 7*, 371-386.

BRITTEN, R. J., AND KOHNE, D. E. 1968. Repeated sequences in DNA. *Science, 161*, 529-540.

BROWN, D. D., AND DAWID, I. B. 1968. Specific gene amplification in oocytes. *Science, 160*, 272-280.

BUONGIORNO-NARDELLI, M., AND AMALDI, F. 1970. Autoradiographic detection of molecular hybrids between RNA and DNA in tissue sections. *Nature, 225*, 946-948.

CROUSE, H. V., AND KEYL, H.-G. 1968. Extra replications in the " DNA puffs " of *Sciara coprophila*. *Chromosoma, 25*, 357-364.

DOTY, P., MARMUR, J., EIGNER, J., AND SCHILDKRAUT, C. L. 1960. Strand separation and specific recombination in deoxyribonucleic acids: physical chemical studies. *Proc. Nat. Acad. Sci., Wash., 46*, 461-476.

EVANS, D., AND BIRNSTIEL, M. L. 1968. Localization of amplified ribosomal DNA in the oocyte of *Xenopus laevis*. *Biochim. Biophys. Acta., 166*, 274-276.

FLAMM, W. G., MCCALLUM, M., AND WALKER, P. M. B. 1967. The isolation of complementary strands from a mouse DNA fraction. *Proc. Nat. Acad. Sci., Wash., 57*, 1729-1734.

GALL, J. G. 1968. Differential synthesis of the genes for ribosomal RNA during amphibian oogenesis. *Proc. Nat. Acad. Sci., Wash., 60,* 553-560.

GALL, J. G. 1969. The genes for ribosomal RNA during oogenesis. *Genetics, 61* (Suppl.), 121-132.

GALL, J. G., AND PARDUE, M. L. 1969. Formation and detection of RNA-DNA hybrid molecules in cytological preparations. *Proc. Nat. Acad. Sci., Wash., 63,* 378-383.

GALL, J. G., AND PARDUE, M. L. 1970. Nucleic acid hybridization in cytological preparations. *Methods in Enzymology* (eds. Grossman and Moldave). Academic Press, New York (in press).

GERBI, S. A. 1971. Localization and characterization of the ribosomal RNA cistrons in *Sciara coprophila. J. Mol. Biol.* (in press).

HALL, B. D., AND SPIEGELMAN, S. 1961. Sequence complementarity of T2-DNA and T2-specific RNA. *Proc. Nat. Acad. Sci., Wash., 47,* 137-146.

JOHN, H. A., BIRNSTIEL, M. L., AND JONES, K. W. 1969. RNA-DNA hybrids at a cytological level. *Nature, 223,* 582-587.

JONES, K. W. 1970. Chromosomal and nuclear location of mouse satellite DNA in individual cells. *Nature, 225,* 912-915.

KIT, S. 1961. Equilibrium sedimentation in density gradients of DNA preparations from animal tissues. *J. Mol. Biol., 3,* 711-716.

MACGREGOR, H. C. 1968. Nucleolar DNA in oocytes of *Xenopus laevis. J. Cell Sci., 3,* 437-444.

MAIO, J. J., AND SCHILDKRAUT, C. L. 1969. Isolated mammalian metaphase chromosomes. II. Fractionated chromosomes of mouse and Chinese hamster cells. *J. Mol. Biol., 40,* 203-216.

MARMUR, J., AND LANE, D. 1960. Strand separation and specific recombination in deoxyribonucleic acids: biological studies. *Proc. Nat. Acad. Sci., Wash., 46,* 453-461.

PARDUE, M. L., AND GALL, J. G. 1969. Molecular hybridization of radioactive DNA to the DNA of cytological preparations. *Proc. Nat. Acad. Sci., Wash., 64,* 600-604.

PARDUE, M. L., AND GALL, J. G. 1970. The chromosomal localization of mouse satellite DNA. *Science, 168,* 1356-1358.

PARDUE, M. L., GERBI, S. A., ECKHARDT, R. A., AND GALL, J. G. 1970. Cytological localization of DNA complementary to ribosomal RNA in polytene chromosomes of *Diptera. Chromosoma, 29,* 268-290.

REEDER, R. H., AND BROWN, D. D. 1970. Transcription of the ribosomal RNA genes of an amphibian by the RNA polymerase of a bacterium. *J. Mol. Biol., 51,* 361-377.

RITOSSA, F. M., AND SPIEGELMAN, S. 1965. Localization of DNA complementary to ribosomal RNA in the nucleolus organizer region of *Drosophila melanogaster. Proc. Nat. Acad. Sci., Wash., 53,* 737-745.

RUDDLE, F. W., CHAPMAN, V. M., CHEN, T. R., AND KLEBE, R. J. 1970. Linkage between human lactate dehydrogenase A and B and peptidase B. *Nature, 227,* 251-257.

RUDKIN, G. T., AND CORLETTE, S. L. 1957. Disproportionate synthesis of DNA in a polytene chromosome region. *Proc. Nat. Acad. Sci., Wash., 43,* 964-968.

SINCLAIR, J. H., AND BROWN, D. D. 1969. Comparative studies of genes for ribosomal RNA in eukaryotes. *Yb. Carnegie Inst., Wash., 67,* 404-413.

SWIFT, H. 1962. Nucleic acids and cell morphology in Dipteran salivary glands. *The Molecular Control of Cellular Activity* (ed. Allen), McGraw-Hill Book Co. Inc., New York.

WALLACE, H., AND BIRNSTIEL, M. L. 1966. Ribosomal cistrons and the nucleolar organizer. *Biochim. Biophys. Acta., 114,* 296-310.

YASMINEH, W. G., AND YUNIS, J. J. 1969. Satellite DNA in mouse autosomal heterochromatin. *Biochem. Biophys. Res. Comm., 35,* 779-782.

REGULATION OF RNA SYNTHESIS IN THE EVALUATION OF BREAD WHEAT

H. K. JAIN and R. N. RAUT

Division of Genetics, Indian Agricultural Research Institute, New Delhi-12

1. Introduction

Considerable progress has been made in recent years towards an understanding of the nature and regulation of gene activity. Most studies have been made on microorganisms as higher organisms, in general, do not offer many opportunities for studies of this type. However, *Triticum* and other genera showing various levels of ploidy provide suitable material for studying the interaction of gene loci which are known to perform a common function. Thus, in the case of wheat, diploid, tetraploid and hexaploid species and a number of aneuploid lines show considerable variation in the number of nucleolar organising loci. All these loci can be shown to be active in the synthesis of RNA.

2. Experiments

Of the 42 chromosomes in hexaploid wheat, chromosomes 1A, 1B, 6B and 5D carry nucleolar organising loci (Morris and Sears, 1967). In the course of the present studies the root tip cells of different materials (table 1) were simultaneously fed with tritium labelled uridine (2·5 μC/ml., sp. act. 2·2 C/mM for 4 hours) and the autoradiographic technique discussed fully elsewhere (Raut and Jain, 1970) was used for studying the turnover of RNA. Three different sets of materials were used. The first set consisted of the three diploid progenitors of bread wheat, namely, *T. monococcum*, *Aegilops speltoides* and *Ae. squarrosa*, contributing A, B and D genomes respectively. *T. monococcum* with a single nucleolar organising locus, shows a greater degree of incorporation than *Ae. speltoides* which has two such loci. Also the incorporation in *T. monococcum* and *Ae. speltoides* is significantly greater than in *Ae. squarrosa*, carrying the D genome with its single organiser (table 1).

In the second set, observations on uridine incorporation were made on the tetraploid species *T. durum* and the hexaploid species *T. aestivum*. It was observed that the tetraploid species formed twice the number of grains in its cells compared to the diploid species, but there was not much increase in grain formation in the hexaploid species over the tetraploid level (table 1).

The third set of materials consisted of the ditelosomic and monotelosomic lines for the long arm of chromosomes, 1A, 1B, 6B and 5D. The missing short arm in each of these chromosomes in the above lines is known to carry a nucleolar organiser. In other words, each one of these ditelosomes is deficient for the nucleolar arm concerned in both the homologous chromosomes, while the monotelosomes have the arm carrying the nucleolar organiser missing in one of the two chromosomes of the pair. Cells of the ditelosomic line $1A^1$ show significantly less incorporation of label compared to cells of all other ditelosomic lines (plate, fig. 2). In contrast, cells of the other ditelosomic lines show a similar level of incorporation, and one

TABLE 1

Genomic and Chromosomal constitution of the different species and varieties and incorporation of RNA label in their root-tip cells

Species and varieties	Genomes	2*n*	Total no. of N.O.	Chromosomes with N.O.	*Mean no. of grain per cell	†Ratio of means
T. monococcum	A	14	2	1A, 1A	84·9	1·00
Ae. speltoides	B	14	4	1B, 1B, 6B, 6B	76·7	0·90
Ae. squarrosa	D	14	2	5D, 5D	53·4	0·63
T. durum	AB	28	6	1A, 1A, 1B, 1B, 6B, 6B	171·2	2·02
T. aestivum var. Chinese Spring	ABD	42	8	1A, 1A, 1B, 1B, 6B, 6B, 5D, 5D	181·8	2·14
T. aestivum lines:						
‡Ditelosomic $1A^1$	ABD	40+2 (1/2)	6	1B, 1B, 6B, 6B, 5D, 5D	122·6	1·44
Monotelosomic $1A^1$	ABD	41+1 (1/2)	7	1A, 1B, 1B, 6B, 6B, 5D, 5D	188·9	2·22
Ditelosomic $1B^1$	ABD	40+2 (1/2)	6	1A, 1A, 6B, 6B, 5D, 5D	168·9	1·98
Monotelosomic $1B^1$	ABD	41+1 (1/2)	7	1A, 1A, 1B, 6B, 6B, 5D, 5D	186·3	2·19
Ditelosomic $6B^1$	ABD	40+2 (1/2)	6	1A, 1A, 1B, 1B, 5D, 5D	175·2	2·06
Monotelosomic $6B^1$	ABD	41+1 (1/2)	7	1A, 1A, 1B, 1B, 6B, 5D, 5D	179·8	2·11
Ditelosomic $5D^1$	ABD	40+2 (1/2)	6	1A, 1A, 1B, 1B, 6B, 6B	176·5	2·07
Monotelosomic $5D^1$	ABD	41+1 (1/2)	7	1A, 1A, 1B, 1B, 6B, 6B, 5D	181·2	2·13

* Means based on grain counts over 300 cells, taking 100 randomly selected cells from each of three root tips.

† Ratio is calculated by taking grain count of *T. monococcum* as 1·00.

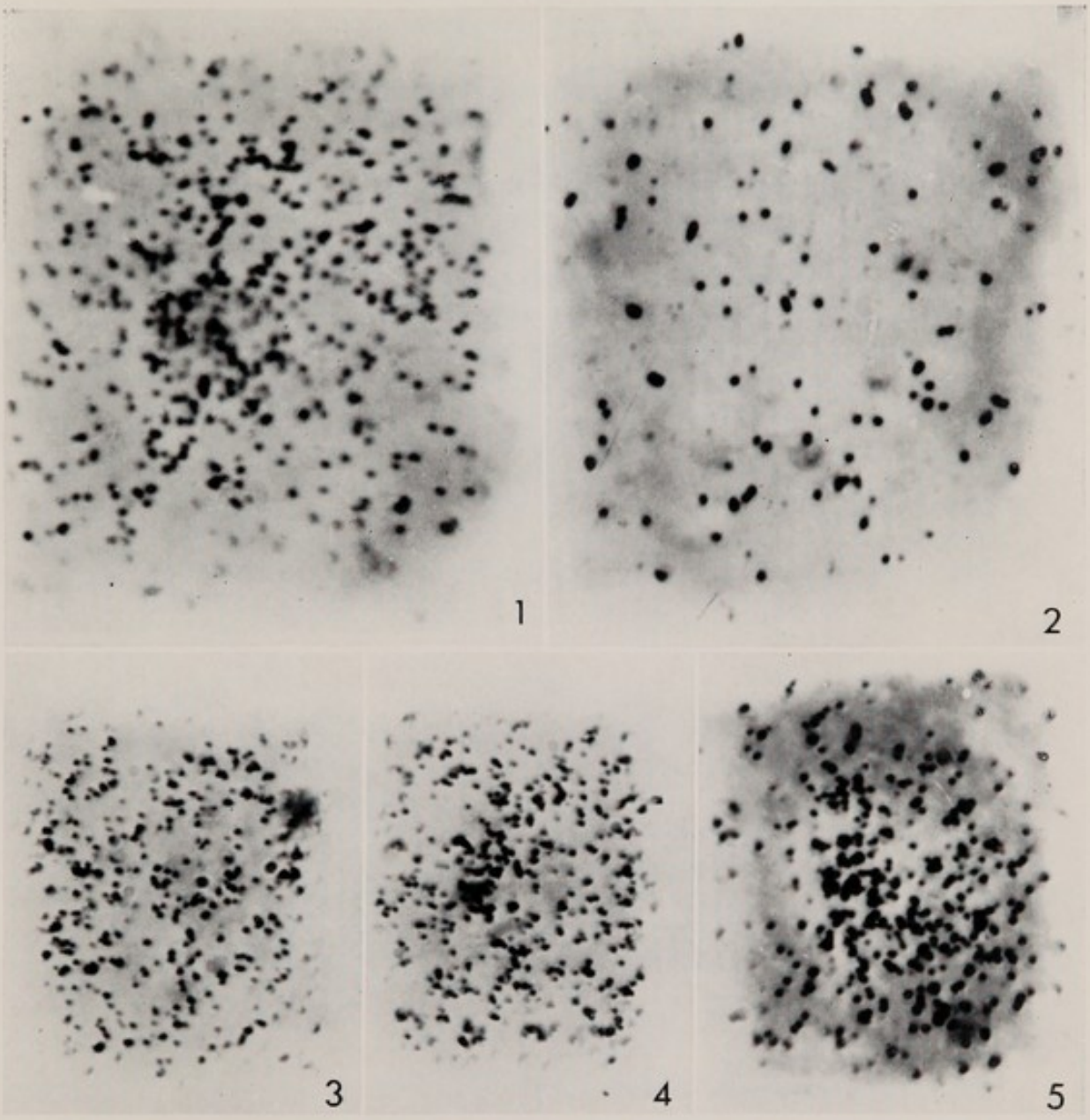
‡ Superscript 1 refers to the long chromosome arm. 1 (1/2) or 2 (1/2) means one or two telocentric chromosomes. The missing short arm in each case carries the nucleolar organiser.

comparable to that in cells of the normal hexaploid variety, Chinese Spring, from which they have been derived (plate, figs. 1, 3 and 4). The incorporation in all the monotelosomes is on a par with that in the variety Chinese Spring (table 1 and plate, fig. 5).

3. Discussion

The present results on RNA synthesis in the different wheat species, varieties and lines provide support for our earlier conclusion (Jain *et al.*, 1969) that the nucleolar organiser is a very active locus for RNA synthesis and that the bulk of the cellular RNA may be of nucleolar origin. The results further show that the organisers present in the different genomes differ in their capacity to synthesise RNA. Thus, it is found that cells of *T. monococcum* synthesise as much or more RNA as cells of *Ae. speltoides*, even though the latter species has two organising loci present in its chromosomes. In contrast, cells of *Ae. squarrosa* having the D genome show a significantly reduced degree of incorporation of the label.

A second observation of interest is that the contributions of the three genomes to RNA synthesis in the hexaploid species are not additive. This seems to indicate that when the genomes are brought together in a polyploid nucleus they interact with regard to their RNA turnover. The most striking evidence of this interaction is that the ditelosomes $6B^1$ and $5D^1$ show as much RNA synthesis as control plants having all four organisers. It is interesting, however, to find that this does not happen in the case of the ditelosomes $1A^1$, and $1B^1$. There is a sharp drop in the synthetic activity of the cells in the root tips of ditelosomic line $1A^1$. This observation supports the conclusion that the A genome is the most active and indispensable as



FIGS. 1-5.—Autoradiographs from RNA labelled root tip cells of the different lines of *T. aestivum*.

1. Var. Chinese Spring. ×2500.
2. Ditelosome for chromosome 1A^1. ×2500.
3. Ditelosome for chromosome 6B^1. ×1500.
4. Ditelosome for chromosome 5D^1. ×1500.
5. Monotelosome for chromosome 1A^1. ×1500.

far as RNA synthesis in the hexaploid cells is concerned. It is possible that it is the A genome which synthesises extra RNA in the ditelosomes for chromosomes 6B and 5D.

In addition to the intergenomic interaction, the occurrence of an intragenomic interaction is indicated by a comparison of the ditelosomic and monotelosomic lines. The monotelosomic lines show no reduction in RNA synthesis compared to the normal hexaploid varieties, indicating that a loss of one of the organisers is probably compensated by increased activity of its alleles in the homologous chromosome.

The present study is of interest in the context of the evolution of bread wheat. It clearly shows that the A genome is the most active in the synthesis of RNA (earlier work shows it to be ribosomal RNA) and this fact could contribute to its wider ecological adaptation. The behaviour of the tetraploid and hexaploid species so far as their RNA synthesis is concerned parallels their adaptability. The D genome is the least active in RNA synthesis, and as shown by several authors (Shebaski, 1959; Riley and Bell, 1959), it contributes very little to adaptability either. The analysis further suggests that the D genome can be substituted without much adverse effect on the adaptation of the wheat plant, if adaptability is determined, among other things, by ribosomal RNA.

4. Summary

1. Of the three diploid progenitors of wheat, the nucleolar organiser present in the A genome is far more active than the corresponding loci present in the other genomes.

2. RNA synthesis in the polyploid nuclei is regulated so that loss of one of the pairs of organisers is compensated by increased activity of the others.

3. However, in the evolution of bread wheat the A genome appears to be particularly important because the nucleolar organising loci present in it cannot be compensated by similar loci in the other genomes.

5. References

JAIN, H. K., RAUT, R. N., AND NERWAL, S. K. 1969. Nucleolar organiser as a hyper active locus for RNA synthesis. *Heredity*, *24*, 59-67.

MORRIS, R., AND SEARS, E. R. 1967. The cytogenetics of wheat and its relatives. In *Wheat and Wheat Improvement* (ed. K. S. Quisenberry and L. P. Reitz). *Am. Soc. Agron.*, 19-87.

RAUT, R. N., AND JAIN, H. K. 1970. RNA synthesising loci and evolution of bread wheat. *Indian Journal of Botany*, Golden Jubilee Number (in press).

RILEY, R., AND BELL, G. D. H. 1959. The evaluation of synthetic species. *Proc. 1st Int. Wheat Genet. Symp.*, 161-180.

SHEBASKI, L. H. 1959. Speculations on the impact of the D genome. *Proc. 1st. Int. Wheat Genet. Symp.*, 237-241.

ACIDIC PROTEIN AND RNA IN THE INITIATION OF PUFF FORMATION

Th. K. H. HOLT

Cell Biology Section, Department of Zoology University of Nijmegen, The Netherlands

1. Introduction

The application of agents effective in the activation of agent-specific gene loci in *Drosophila* has provided some information about factors which are involved in the process of puff formation (Berendes, 1968). Though the actual initiation mechanism, the relationship between the inducing agent and the genome site responding to it, is far from resolved, a variety of events occurring at the genome level during the process of puff initiation could be distinguished. It was established that during initiation and subsequent growth of a puff a local change in the structural organisation of the chromosome as well as changes in macromolecular composition takes place. The structural changes consist of a decondensation of chromatin of one or more bands. The alterations in macromolecular composition include a change in dye binding to protein (Rudkin, 1962; Swift, 1962; Berendes, 1968) at an early stage of puff formation, the synthesis of RNA (Pelling, 1964; Daneholt *et al.*, 1969), and the metabolisation and/or assembly of RNA with proteins (Stevens and Swift, 1966).

With respect to the changes in dye binding to proteins, it seems that non-histone proteins are responsible for this phenomenon. So far, no indications were obtained for a change in histone quantity in the puff region (Gorovsky and Woodard, 1967), nor for a possible change in configuration of histones due to acetylation, methylation, ethylation or phosphorylation (Clever and Ellgaard, 1970; Benjamin and Goodman, 1969; Holt, 1970).

Relatively few data are available with respect to the quantitative aspect of the changes in dye binding to proteins. The kinetics of this process could provide some clues to the functional significance of this event in puff formation. The present report attempts to evaluate the kinetics of the local changes in dye binding capacity to proteins during the process of puff formation by comparing cytophotometric data with those obtained from dry mass determinations with the interference microscope.

In addition, the possibility of a local accumulation of pre-existing RNA as a factor involved in puff initiation will be discussed. The suggestion that RNA could be involved in the regulation of gene activity in higher organisms was originally made by Frenster (1965). At present, there is some evidence that RNA-protein complexes might play a role in the dissociation of DNA-histone (Huang and Bonner, 1965). These findings were recently taken into consideration as a possible support for the hypothesis that a special type of RNA molecules should be involved in the regulation of gene activity in higher organisms (Britten and Davidson, 1969).

2. Materials and methods

All experiments were carried out with salivary gland chromosomes of late third instar larvae of *Drosophila hydei*. The *in vivo* induction of specific

puffs was achieved by transferring the larvae from 25° to 37° C. A description of the effect of this temperature treatment on the activity of the genome has been reported previously (Berendes, van Breugel and Holt, 1965).

The methods applied for quantification of dye binding to chromosomal protein and of the amount of solid material were the following. After dissection of the salivary glands, squashes were made following a 2 min. fixation in neutral 7·5 per cent. formaldehyde. The staining procedure applied for cytophotometry included the Feulgen reaction on DNA and naphthol yellow S binding to protein as described by Deitch (1955). Quantitative absorption measurements on the protein and DNA were performed with a Zeiss cytospectrophotometer (UMSP1) as reported previously (Holt, 1970). The quantitative determination of the dry mass of certain active chromosome regions was carried out with a Leitz interference microscope equipped with a photoelectric device. The interferometric as well as the cytophotometric measurements were made on two puff regions, 2-48C and 4-81B, during an inactive period and 5, 10, 30, 60 and 120 min. after the onset of a temperature treatment.

The interferometric measurements were performed on formaldehyde fixed and squashed material. The material was submitted to RNA digestion by incubation of the slides in a 1 mg./ml. RNase solution for 2 hours at 37° C. (Sigma, type I-A) before it was mounted in glycerol (Merck, analytical grade, 87 per cent. aq. sol. $n = 1{\cdot}453$) and used for investigation. In all measurements on the puff regions a particular non-puffed chromosome region was used as a reference. The full details of the method employed for dry mass determination in giant chromosomes are published elsewhere (Holt, 1971; see also: Berendes and Ross, 1963; Paul and Mateyko, 1970).

The possible role of RNA in puff initiation was studied by the microinjection of RNase into nuclei of isolated salivary glands prior to the onset of a temperature treatment. The RNase (Sigma, type III-A) was dissolved in a solution composed of 6·5 gm. NaCl, 0·14 gm. KCl, 0·2 gm. $NaHCO_3$, 0·01 gm. NaH_2PO_4, 0·203 gm. $MgCl_2$ in 1000 ml. H_2O (*p*H 7·3). By testing a series of RNase concentrations it was established that the nuclei retained the capacity of *de novo* RNA synthesis, after applying concentrations up to $2{\cdot}10^{-7}$ μg of RNase to a nucleus. Higher concentrations of RNase resulted in a complete inhibition of RNA synthesis. The amount of solution injected into individual nuclei was $5{\cdot}10^{-5}$ ($\pm 1{\cdot}10^{-5}$) μl.

In order to consider the leakage of injected macromolecules from the nuclei after withdrawal of the injection needle, a solution of the dye Evans blue was injected and its leakage from the nuclei estimated. Diffusion of the dye into the cytoplasm appeared to be negligible.

Five minutes after the RNase had been injected into the nuclei, a temperature shock was applied by raising the temperature of the incubation medium from 25° to 37° C. Squash preparations were made after 15 min. of incubation at 37° C. and the puffing pattern was analysed.

For the autoradiographic study on RNA synthesis uridine-5-T (spec. act. 28·4 C/mM) was used.

3. Results

(a) *Dye binding to protein in puffed regions*

Measurements of integrated absorption values were made on the puff

regions according to the scanning principle. A wavelength of 430 nm. was used for quantifying the naphthol yellow S binding to protein and of 550 nm. for the Feulgen reaction of the DNA. The ratio of the integrated absorption values at 430 nm. and 550 nm. reflects for each chromosome region a relative ratio of protein and DNA. Since this ratio has to be compared with similar ratios for the same chromosome region at different stages of activity, but the levels of polyteny of the chromosomes used may be different, an internal standard for the possible variations in DNA content of the same region has to be introduced.

Preliminary measurements of the protein/DNA ratio of inactive bands revealed that within *one* nucleus this ratio is in general constant for all regions (see: Paul and Mateyko, 1970). However, striking differences can be observed if different nuclei of the same preparation or nuclei of different preparations are compared. This feature inherent to the method makes it impossible to compare protein/DNA ratios from different nuclei directly. This difficulty can be overcome, however, if the ratio of a particular puff is considered relative to the ratio of a standard non-puffed chromosome band. The quotients of the protein/DNA ratio in a puff over that of a non-puffed band can be directly compared. In this quotient the protein/DNA ratio of the reference band is a constant value for all non-active chromosome regions in the same nucleus. Further, the integrated absorption values of the DNA of the reference band and that of the puff region should have a constant relation provided that (*a*) the puffing process does not include a specific DNA synthesis, and (*b*) that there is no difference in the replication period of the DNA in the reference band and that in the puff region. So far, there is no evidence for a specific DNA synthesis associated with puffing in *Drosophila*. It therefore seems fair to exclude this factor as one that could lead to incorrect evaluation of the measurements. However, the possibility that the duration of DNA synthesis in the puff region and the reference band are different, may give rise to variations in the integrated absorption values for the DNA if these are obtained from replicating nuclei. Since the glands used in this investigation were from late third instar larvae, 10-20 per cent. of the nuclei are in replication phase (Berendes, 1966). It, therefore, cannot be excluded that part of the variations in integrated absorption values for DNA as observed are derived from replicating nuclei (see fig. 1). Even so, it was found that the quotients calculated on the basis of protein/DNA ratios from the two inactive puff regions and their reference bands varied only slightly and were significantly different from the quotients obtained when the puffs were active. At successive stages after the onset of the temperature treatment up to 30 min. the quotient was increased significantly if compared with a previous time interval. At a stage of maximum expansion of the puff the quotient value was approximately twice that obtained from the same puff at an inactive stage (fig. 1). Because extensive changes in the amount of DNA during the puffing process are unlikely, this increase in the quotient must be due to a raise in the integrated absorption value for the protein in the puff region. A similar figure was obtained for both the puff regions investigated.

The increase in the integrated absorption values of the protein during the process of puff formation might result either from a change in the configuration of proteins already present, providing an increase in the number of binding sites for the dye, or from an actual increase in local protein con-

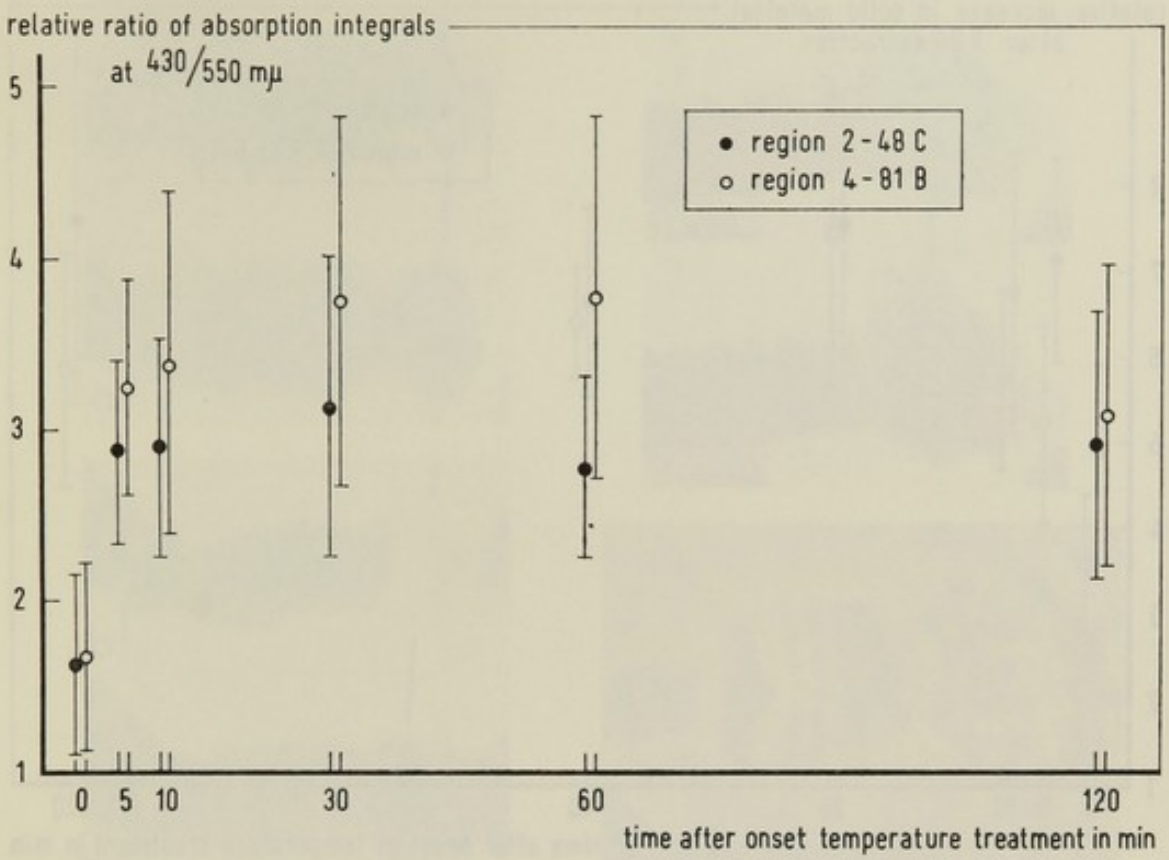


FIG. 1.—Quotients of the protein/DNA ratios in two puff regions over that of a reference band at subsequent stages of puff growth induced by a temperature treatment.

centration. In order to distinguish between these two alternatives, interference measurements were performed on the same puff regions at identical time intervals after the onset of the temperature treatment.

(b) *Dry mass determinations*

The dry mass determinations on the two puff regions were performed after ribonuclease treatment. Removal of the RNA from the puff regions was checked by staining with gallocyanin or toluidin blue. It was established that no staining for RNA could be detected after RNase treatment and so the dry mass of the puff and reference regions should be derived entirely from DNA and protein. On the basis of the considerations discussed in the foregoing section, it is unlikely that the relative quantity of the DNA in the puff region as compared with the reference band is subject to appreciable changes during the puffing process. Therefore, differences in the values obtained from the dry mass determinations on the same puff at successive stages after the onset of induction most likely reflect changes in local protein concentration. The interference measurements revealed that in both puffs, the quantity of solid material increases gradually during puff formation to a level which is approximately 200 per cent. higher than that at an inactive state (fig. 2). If the interferometric data are compared with those obtained cytophotometrically, it is clear that a striking similarity exists. On account of this similarity it may be concluded that the increase in protein staining intensity during puff formation reflects an actual increase in local protein concentration rather than a change in protein configuration.

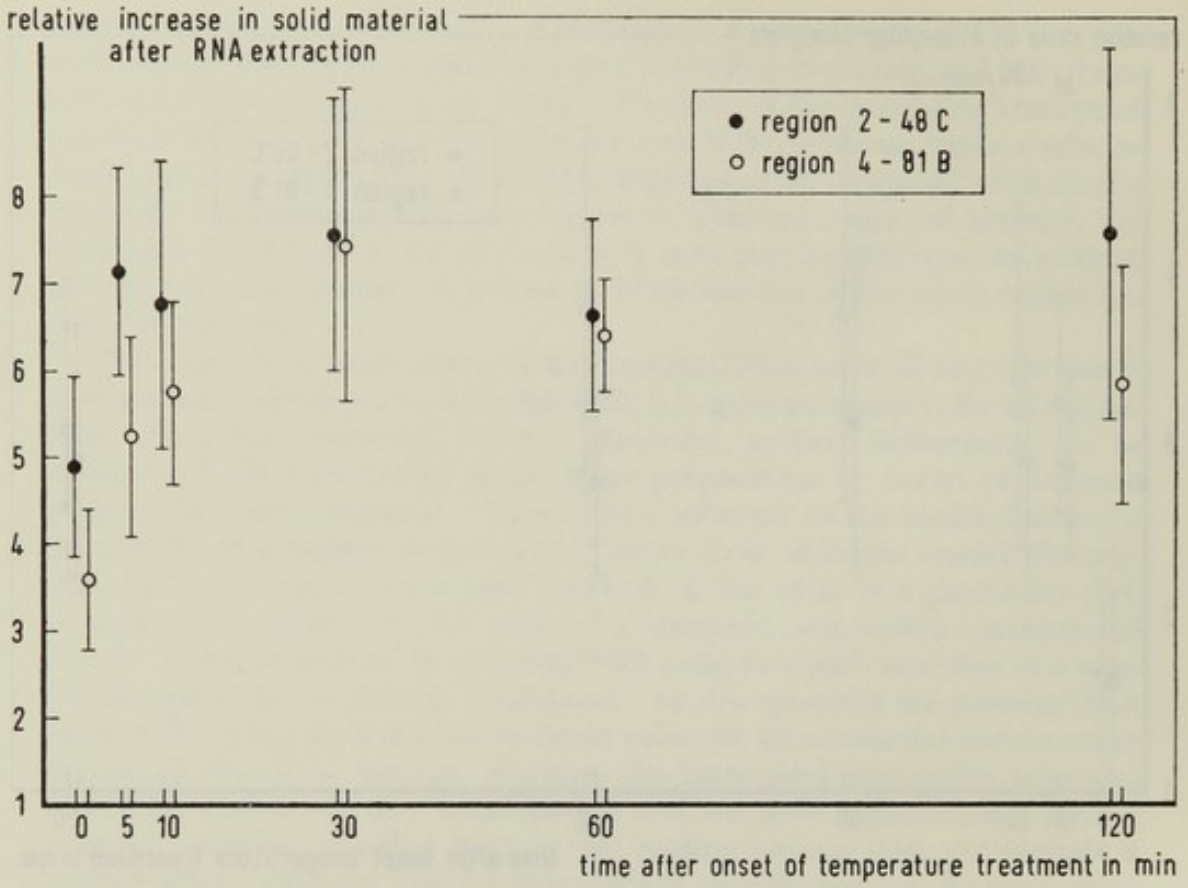


FIG. 2.—Relative values of the quantity of dry mass in two puff regions at subsequent stages of puff growth induced by a temperature treatment (obtained by interference microscopy). These data are derived from material which was submitted to RNA digestion.

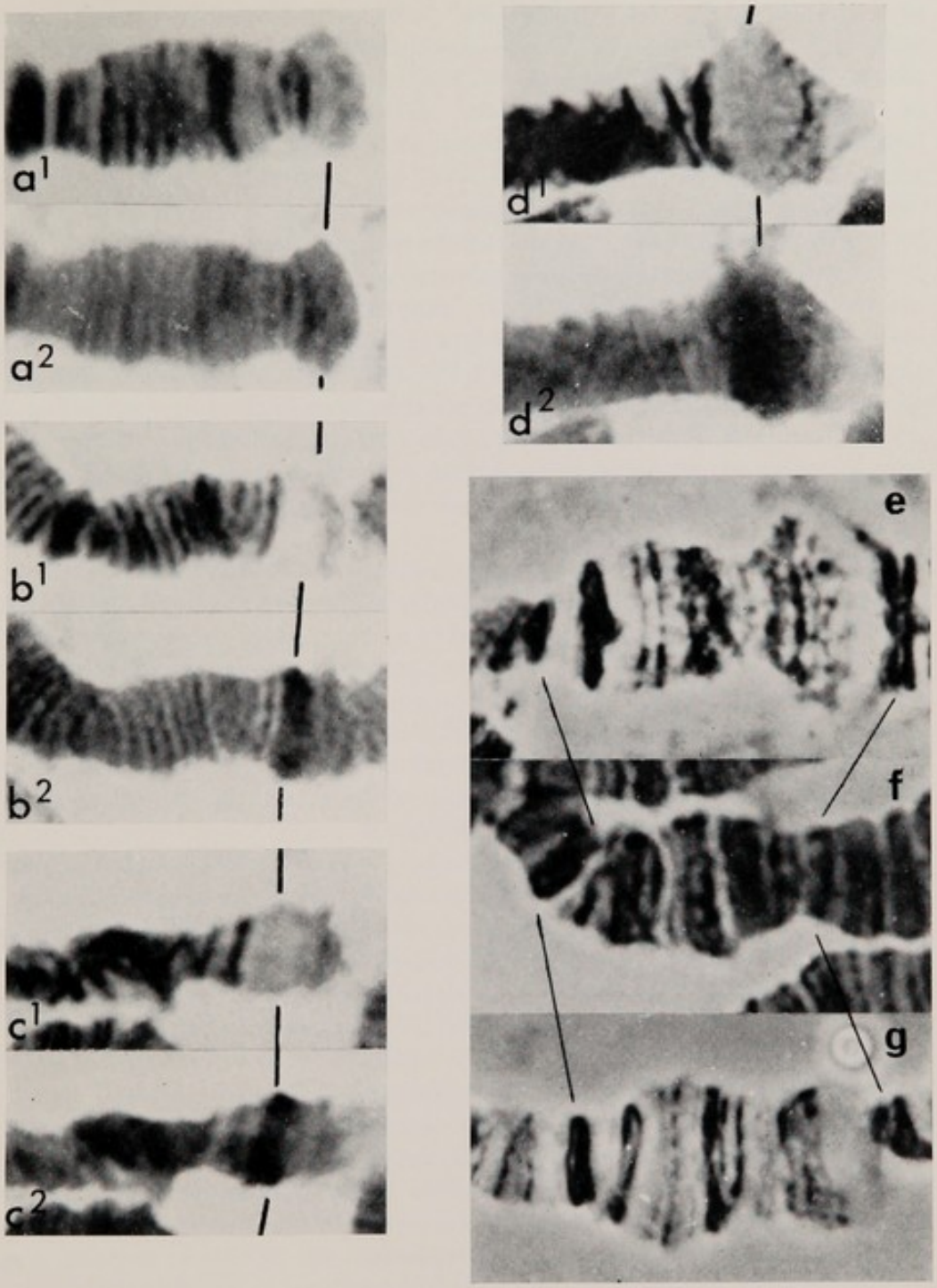
(c) *Inhibition of puff induction by ribonuclease*

Temperature treatments on isolated glands some nuclei of which had been injected with a solution containing $2 \cdot 10^{-7}$ μg. RNase revealed an obvious difference in the genome response of injected and non-injected nuclei. Whereas in all non-injected nuclei the temperature sensitive loci showed a normal response in displaying large-sized puffs, the ribonuclease injected nuclei did not (plate I, figs. *e-g*).

In order to test whether the failure of response of the injected nuclei was due to mechanical damage as a consequence of the injection procedure, control experiments were carried out in which the nuclei were injected with the solvent only. These nuclei, however, showed a perfect response to the temperature treatment. As further controls the effect of injection of oxidised RNase (plate I, fig. *g*) and another basic protein (protamine) were tested with regard to their influence on the response of the nuclei to puff induction. Neither of these substances, applied in the same quantity as active ribonuclease, interfered with the initiation of puff formation. It therefore seems that ribonuclease activity is required in order to obtain inhibition of experimentally induced puff formation.

4. Discussion

Early investigations on the cytochemical features of the puffing phenomenon revealed a drastic change in dye binding capacity to proteins in puffed regions (Swift, 1962). However, so far, neither the character nor the



FIGS. *a-d*.—Series of successive stages of development of puff 2-48C showing a gradual increase in binding of acidic fast green.

a, region 2-48C in an inactive state. (1) photographed with green filter. (2) photographed with red filter.

b, same region, 5 min. after onset of temperature treatment.

c, same region, 10 min. after onset.

d, same region, 60 min. after onset.

FIGS. *e-g*.—The effect of injection of active RNase and oxidised RNase on the responsiveness of the genome to a temperature treatment.

e, puff 2-32A in control nuclei after 15 min. of temperature treatment (37°) of the isolated gland.

f, the same region in a nucleus of the same gland as in (*a*) which was injected with $2 \cdot 10^{-7}$ μg RNase.

g, The same region in a nucleus of a different gland injected with $2 \cdot 10^{-7}$ μg oxidised RNase.

kinetics of this phenomenon has been resolved in detail. The present data reveal that the increase in dye binding capacity which occurs gradually during puff formation is due to an actual increase in local protein concentration. Indications for an increased protein concentration in a puffed region as compared with the same region in an inactive state were originally presented by Rudkin (1962). However, the level of increase in these cases was far less than that observed in the experimentally activated puffs in *Drosophila hydei*. The increase in protein concentration in a region undergoing puff formation seems to be due to an accumulation of non-histone proteins rather than to a *de novo* synthesis of protein. It is unlikely that *de novo* protein synthesis is required for the initiation and subsequent growth of a puff. Pretreatments of salivary glands with effective inhibitors of protein synthesis such as puromycin or cycloheximide do not inhibit either puff formation or the increase in dye binding capacity of the puffs (Clever and Romball, 1966). Further, administration of labelled amino acids prior to the onset of puff induction does not result in a specific incorporation of the labelled precursor in the puff regions.

So far, the function of the accumulating protein in the process of puffing is unknown. Even the question of whether the accumulating protein consists of only one species or more remains to be elucidated. As possible candidates for accumulation during the puffing process at least RNA polymerase and a class of proteins required for the protection of the newly synthesised RNA (proteins which associate with the RNA at the chromosomal level to form the characteristic RNP-particles) have to be considered. Both types of protein molecules are likely to be present in large quantities within the nucleus at any time. Their accumulation in the puff region, therefore, would be independent of *de novo* synthesis.

Apart from protein accumulation during puff formation, the accumulation of preformed RNA may occur at an early stage in the process of puff initiation. An indication for accumulation of RNA was obtained from experiments performed with *Chironomus* larvae. If larvae of *C. thummi* are grown in water containing actinomycin D, large puffs develop which are inactive in RNA synthesis. These puffs, however, display a definite staining reaction for RNA (Panitz, personal communication). The failure of puff formation in nuclei injected with ribonuclease might be interpreted in terms of a degradation of RNA which is required for the initiation of puff formation and normally accumulates at an early stage after the onset of induction. Though this interpretation is attractive with respect to the presumed regulatory function of special chromosomal RNA species (Dahmus and Bonner, 1970), more evidence is required before it can be definitely concluded that RNA is involved in the process of initiation of gene activity in *Drosophila*.

5. Summary

1. A cytophotometric study on the changes in protein dye binding capacity during puff formation revealed a gradual increase in dye binding to protein up to approximately 200 per cent. for each of two puffs induced by a temperature treatment.

2. The kinetics of this increase were identical with the kinetics of an increase in dry mass of the same regions after extraction of RNA, as was revealed by interferometric measurements.

3. The possibility that preformed RNA is involved in the process of puff initiation was indicated by the failure of nuclei injected with ribonuclease to respond to puff induction by a temperature treatment.

Acknowledgments.—The author is indebted to Professor Dr H. D. Berendes for his continuous support and interest. Thanks are further due to Mr W. v. d. Velden who performed the ribonuclease injections, to Professor Dr H. Galjaard for providing him with the facilities to perform the interferometric measurements and to Dr J. B. Bijlsma for the opportunity to use the UMSP1.

6. References

BENJAMIN, W. B., AND GOODMAN, R. M. 1969. Phosphorylation of Dipteran chromosomes and rat liver nuclei. *Science, 166*, 629-631.

BERENDES, H. D., AND ROSS, K. F. A. 1963. Measurements of the total solid material in the bands and interbands of the polytene chromosomes in living cells of *Chironomus*. *Chromosoma, 14*, 111-122.

BERENDES, H. D., VAN BREUGEL, F. M. A., AND HOLT, TH. K. H. 1965. Experimental puffs in salivary gland chromosomes of *Drosophila hydei*. *Chromosoma, 16*, 35-46.

BERENDES H. D. 1966. Differential replication of male and female X-chromosomes in *Drosophila*. *Chromosoma, 20*, 32-43.

BERENDES H. D. 1968. Factors involved in the expression of gene activity in polytene chromosomes. *Chromosoma, 24*, 418-437.

BRITTEN, R. J., AND DAVIDSON, E. H. 1969. Gene regulation for higher cells: a theory. *Science, 165*, 349-358.

CLEVER, U., AND ROMBALL, C. G. 1966. RNA and protein synthesis in the cellular response to a hormone, ecdysone. *Proc. nat. Acad. Sci., Wash., 56*, 1471-1476.

CLEVER, U., AND ELLGAARD, E. G. 1970. Puffing and histone acetylation in polytene chromosomes. *Science, 169*, 373-374.

DAHMUS, M. E., AND BONNER, J. 1970. Nucleoproteins in regulation of gene function. *Feder. Proc., 29*, 1255-1260.

DANEHOLT, B., EDSTRÖM, J. E., EGYHÁZI, E., LAMBERT, B., AND RINGBORG, U. 1969. RNA synthesis in a Balbiani ring in *Chironomus tentans* salivary gland cells. *Chromosoma, 28*, 418-429.

DEITCH, A. D. 1955. Microspectrophotometric study of the binding of the anionic dye, naphthol yellow S by tissue sections and by purified proteins. *Lab. Invest., 4*, 324-351.

FRENSTER, J. H. 1965. Nuclear polyanions as de-repressors of synthesis of ribonucleic acid. *Nature, 206*, 680-683.

GOROVSKY, M. A., AND WOODARD, J. 1967. Histone content of chromosomal loci active and inactive in RNA synthesis. *J. Cell. Biol., 33*, 723-728.

HOLT, TH. K. H. 1970. Local protein accumulation during gene activation. I. Quantitative measurements on dye binding capacity at subsequent stages of puff formation. *Chromosoma, 32*, 64-78.

HOLT, TH. K. H. 1971. Local protein accumulation during gene activation. II. Interferometric measurements of the amount of solid material in temperature-induced puffs of *Drosophila hydei*. *Chromosoma, 32*, 428-435.

HUANG, C. C., AND BONNER, J. 1965. Histone-bound RNA, a component of native nucleohistone. *Proc. nat. Acad. Sci., Wash.*, 960-967.

PAUL, J. S., AND MATEYKO, G. M. 1970. Quantitative interference microscopy of polytene chromosomes. I. Cytophysical studies on refractive index and dry mass concentration. *Exptl. Cell Res., 59*, 237-243.

PELLING, C. 1964. Ribonukleinesäure—Synthese der Riesenchromosomen. Autoradiographische Untersuchungen an *Chironomus tentans*. *Chromosoma, 15*, 71-122.

RUDKIN, G. T. 1962. Nucleic acid metabolism in giant chromosomes of *Drosophila melanogaster*. *Ann. Histochim. Suppl., 2*, 77-84.

STEVENS, B. J., AND SWIFT, H. 1966. RNA transport from nucleus to cytoplasm in *Chironomus* salivary glands. *J. Cell Biol., 31*, 55-77.

SWIFT, H. 1962. Nucleic acids and cell morphology in Dipteran salivary glands. In: *The Molecular Control of Cellular Activity* (ed. J. Allen), 73-125. McGraw Hill, New York.

INFLUENCE OF UV MICROBEAM ON SPINDLE FINE STRUCTURE AND ANAPHASE CHROMOSOME MOVEMENTS

ANDREW BAJER

Department of Biology, University of Oregon, Eugene, Ore. 97403, U.S.A.

1. Introduction

Interpretations of the effects of irradiation of the mitotic spindle by UV microbeams play an increasingly important role in explaining the mechanism of the chromosome movements. They are emphasised in stimulating reviews by Luykx (1970) and Nicklas (1971) and play a crucial role in the speculations of Forer (1966, 1969). In most experiments, kinetochores, spindle fibres or both have been irradiated. Effects of irradiation on chromosome movements have been followed by micrographic records (Inoué, 1964; Forer, 1965, 1966), camera lucida drawings (Izutsu, 1961) and only in a few cases (Zirkle, 1964; Bajer and Molè-Bajer, 1969; Bajer, 1969) by time-lapse techniques. Very few data on the fine structure of irradiated chromosomes (Bloom and Özarslan, 1965) and spindles (Bajer, 1969) are reported. Therefore, combined *in vitro* observations and detailed studies on fine structure after UV microbeam irradiation are urgently needed. Extensive studies on the correlation between fine structure and the behaviour of chromosomes (Bajer and Molè-Bajer, unpublished) and changes of birefringence after irradiation (Sato, Molè-Bajer and Bajer, unpublished) explain why some controversy exists in the interpretations of results.

The present experiments covered the whole cycle of mitosis. However, only a few concerning anaphase will be reported here. The data illustrate well the characteristic effects of the UV microbeam on chromosome movements.

2. Material and methods

Endosperm of *Haemanthus katherinae* Bak. was used. The methods for the location of selected parts of the cell in LM (light microscope) and EM (electron microscope) were as described before (Molè-Bajer and Bajer, 1968). Sections were made with an LKB ultratome and EM micrographs taken with a Siemens Elmiskop IA operated at 80 KV.

The UV microbeam equipment designed by Voss (1966) and produced by Zeiss (Jena) was used exclusively. In all experiments the reflecting condenser (N.A. 0·6) was used for irradiation. HBO 200 mercury arc was used as a source of UV. Observations were made with 40× dry phase objective. Wet filter (4 cm. thick, 240 g/litre $NiSO_4.6H_2O$ and 45 g/litre $CoSO_4.7H_2O$—Jagger 1967) was used between the lamp collector and focusing aperture. This filter transmits about 60 per cent. at λ 240-320 nm. In some experiments an interference filter λ 280 nm. (Baird Atomic, Cambridge, Mass.) was used. The latter increased considerably the time of irradiation needed to produce detectable results. The intensity of UV source (with wet filter) at the plane of irradiation was of the order $3{\cdot}6 \times 10^{-5} W/\mu m^2$.

In the equipment used the UV spot was rectangular. The size of the rectangle varied in different experiments (4-6 μm × 4-6 μm and 10-20 μm × 10-20 μm). In numerous experiments the slit (4 μm × 90 μm) was used. The image of the slit crossed the whole cell. The cells were fixed at different times after irradiation (5 sec.-30 min.) or observed for a few hours. It should be stressed, however, that it is not known when precisely the cell was killed if, for example, fixation started 5 sec. after irradiation. It is assumed that in the perfusion chamber used, 10-15 sec. are required to kill the cell. The thickness of the perfusion chamber (from the quartz cover slip to the cell) was about 0·5 mm. Chromosome behaviour was recorded with the 16 mm. time-lapse Wild equipment or a 35 mm. still camera. So far very few micrographic records have been made during irradiation and, therefore, there is usually a gap of about 30 sec.-1 min. in the time-lapse records. Over 400 cells were observed under various experimental conditions and fine structure was studied in about 50 cells.

3. General remarks

It appeared that heterochromatic UV microbeam (wet filter) irradiation for 3-5 sec. was sufficient to produce sticky bridges in anaphase, if chromosomes are irradiated in prometaphase-metaphase. A dose 2-4 times higher is required to produce an effect on chromosome movements after part of the spindle is irradiated. Paling of chromosomes (Zirkle, 1964) usually occurs after slightly higher doses than are required for the formation of bridges. Three important points should be emphasised:

1. Individual response of cells due to cell shape.
2. Precision of focusing and shallow depth of field of condenser.
3. Effects of UV diffraction and focusing on birefringence.

1. The response of cells to UV is often somewhat variable, as may be the dose required to produce a given effect. Variations in dose are negligible in producing bridges and pale spots, but vary considerably (even 2-3 fold) for effects on chromosome movements. This variation depends to a great extent on the thickness of the cell and chromosome arrangements. Results on chromosome movements are constantly repeatable in flattened cells where the spindle is not thicker than about 10 μm. In unflattened cells where numerous spindle fibres (composed from MTs—microtubules, Bajer, 1968) are arranged above each other, irradiation with a UV microslit gives easily repeatable results, while irradiation with the microbeam always affects a larger area (*i.e.* regions above and below the focal plane) than the selected kinetochore fibre and the results vary.

2. The intensity of the microbeam changes rapidly on either side of the focal point and, due to the shallow depth of field of the reflecting condenser through which the irradiation is done, an irradiated region of only about 2 μm thickness receives the dose at high intensity. It should also be stressed that the diameter of the beam in and out of focus changes. It is not surprising that most uniform results on chromosome movements are obtained if through focus technique (moving of condenser during irradiation) is used. This problem will be discussed in detail elsewhere (Sato, Molé-Bajer and Bajer, in preparation). If the irradiation is done through the condenser (Forer, 1965, 1966; Inoué, 1964 and experiments reported here) the thickness of

the chamber is of great importance. Well localised irradiation of a part of the cell may not be possible in a thicker chamber due to diffraction of the beam.

3. Reduction of birefringence which follows irradiation (Inoué, 1964; Forer, 1965, 1966; Sato, Molè-Bajer and Bajer, in preparation) is very rarely complete. Even if it appears to be complete, it is an open question whether some remaining birefringence is present but not detected. The borders between the unaffected region of the spindle and the zone of reduced birefringence is never sharp in LM. The latter fact may be partly due to difficulties in precise focusing and to the size of the beam below and above the focal point mentioned above.

4. Results

Only some experiments using moderate, non-lethal doses, which modify or stop chromosome movements in anaphase, will be reported here.

(i) *Observations* in vitro

A. *UV microslit*

Irradiation of half-spindle, in early and mid-anaphase, close to the kinetochores, *i.e.* between kinetochores and the poles, or in the kinetochore area, slows down or stops temporarily only the chromosome group with the irradiated half-spindle. In some cases after irradiation, the rate of movement of the sister chromosome group does not change at all. Slightly higher doses, which slow also the opposite chromosome group, will not be discussed here. It is of special importance that a chromosome group with an irradiated half-spindle may move a short distance (1-3 μm) backwards after irradiation, before continuation of poleward migration. Backward movement does not last long (2-3 min.) and is rather difficult to notice unless time-lapse technique followed by frame-by-frame analysis is used. Such effects are not observed if the irradiation is in late anaphase.

Irradiation of the interzonal region in early or mid-anaphase slows down or stops temporarily two sister chromosome groups. Slightly higher doses stop chromosome movements permanently and a restitution nucleus is formed.

B. *UV microbeam*

Irradiation of kinetochores, or a half-spindle, *i.e.* selected spindle fibres adjacent to the kinetochores, with a 3-5 μm spot usually does not affect chromosome movements in early anaphase. If, however, irradiation is in mid- or late anaphase, a chromosome with an irradiated kinetochore fibre may move towards the equator immediately after irradiation. Often however no clear effect is observed. If, however, because of a bridge a chromosome is under tension towards the opposite pole, it reacts in a different way. In these experiments 2-4 μm spot was used to irradiate chromosome arms, usually close to kinetochore. Often more than one chromosome arm was irradiated in late prometaphase or metaphase. Such irradiation resulted in the formation of sticky bridges in anaphase. If the bridge is close to the kinetochores it is stretched considerably in mid-anaphase. When the bridge is stretched, microbeam of 5-10 μm (usually 5 μm—the size depending on

the number of kinetochores involved) was used to irradiate the half-spindle region close to one of the kinetochores connected by a bridge. A high dose—*i.e.* one 3-4 times higher than needed to form a bridge, was used. The purpose was to irradiate kinetochore fibres of chromosomes connected by a bridge. The effect could be noticed as a rule immediately (10-15 sec.) after the end of irradiation, or even occasionally during irradiation. The kinetochores with irradiated kinetochore fibres moved as a rule rapidly backward towards the equator and were included in opposite sister nucleus (non-disjunction). Plate I illustrates such behaviour. If a lower dose was applied, kinetochores with irradiated fibres only slowed down temporarily. In all these cases the movements of sister kinetochores with non-irradiated kinetochore fibres were unaffected.

(ii) *Studies on fine structure*

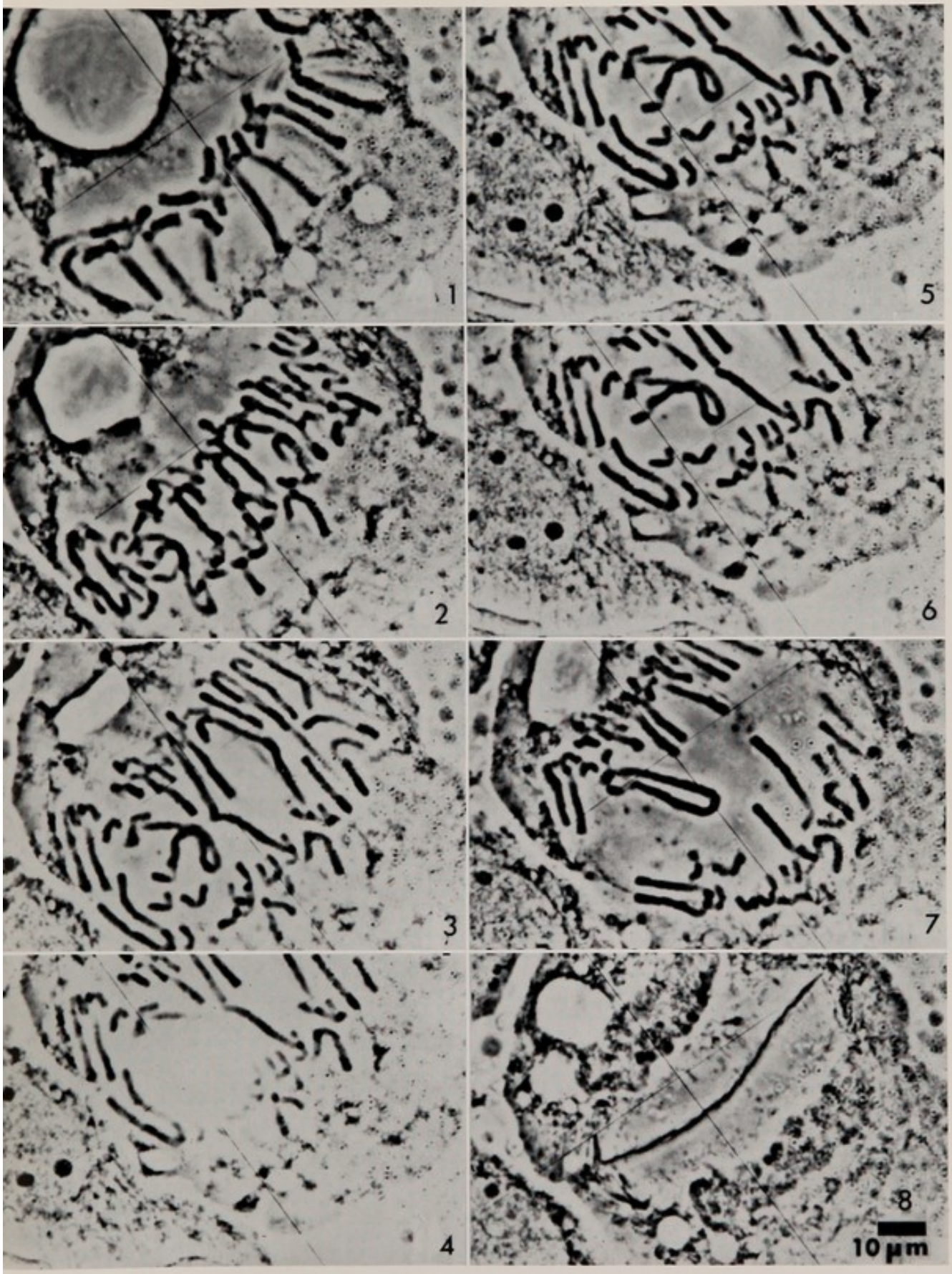
Only results with microslit will be reported here. The work on the effect of the microbeam described above is in progress and the results are not yet known.

The influence on MTs was the main object of these studies. In cells fixed 30 sec.-3 min. after irradiation, pronounced changes are observed. The most common effect is the reduction of the number of MTs in the irradiated region (plate II). In cells fixed 2-3 min. after irradiation fragments of MTs are arranged irregularly in all directions. It should be stressed that only in exceptional cases are sharp " cuts " of MTs found (plate III). This was observed more often in the interzonal region than in the half-spindle. Clear effects on MTs are often seen only at a certain depth in the spindle and not through the whole irradiated region. MTs in the surface region of the spindle are often less disturbed. This is often especially clear if through focus technique of irradiation is not used. With the technique used no difference was found in response of continuous and kinetochore MTs to UV.

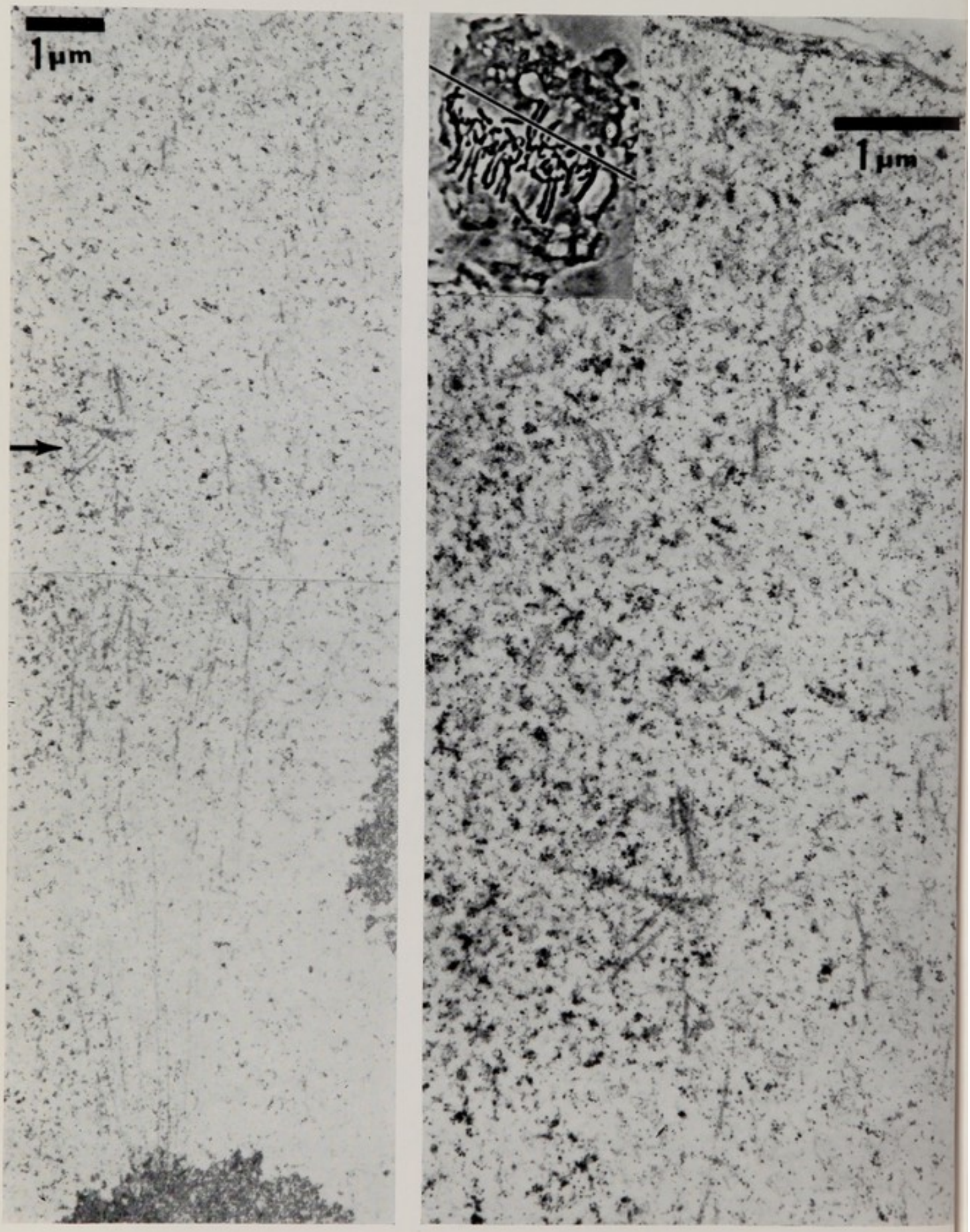
5. Discussion

Experiments reported here should be compared with some data known from the literature. Present data show clearly that UV disorganises MTs in less than 1 min. and probably only within several sec. The details of how this disorganisation occurs are not known however. One of several explanations is that MTs break into shorter pieces which after some time are no longer detectable in the irradiated region, *i.e.* they are altered or eliminated from the spindle. The experiments on combined UV microbeam and polarised light (Sato, Molè-Bajer and Bajer, unpublished) show remarkable agreement in the degree to which there is a reduction in birefringence and the decrease in the number of MTs, contrary to speculations of Forer (1969). Present data support the conclusions of Sato (1969) that MTs are the major components responsible for the birefringence of the spindle.

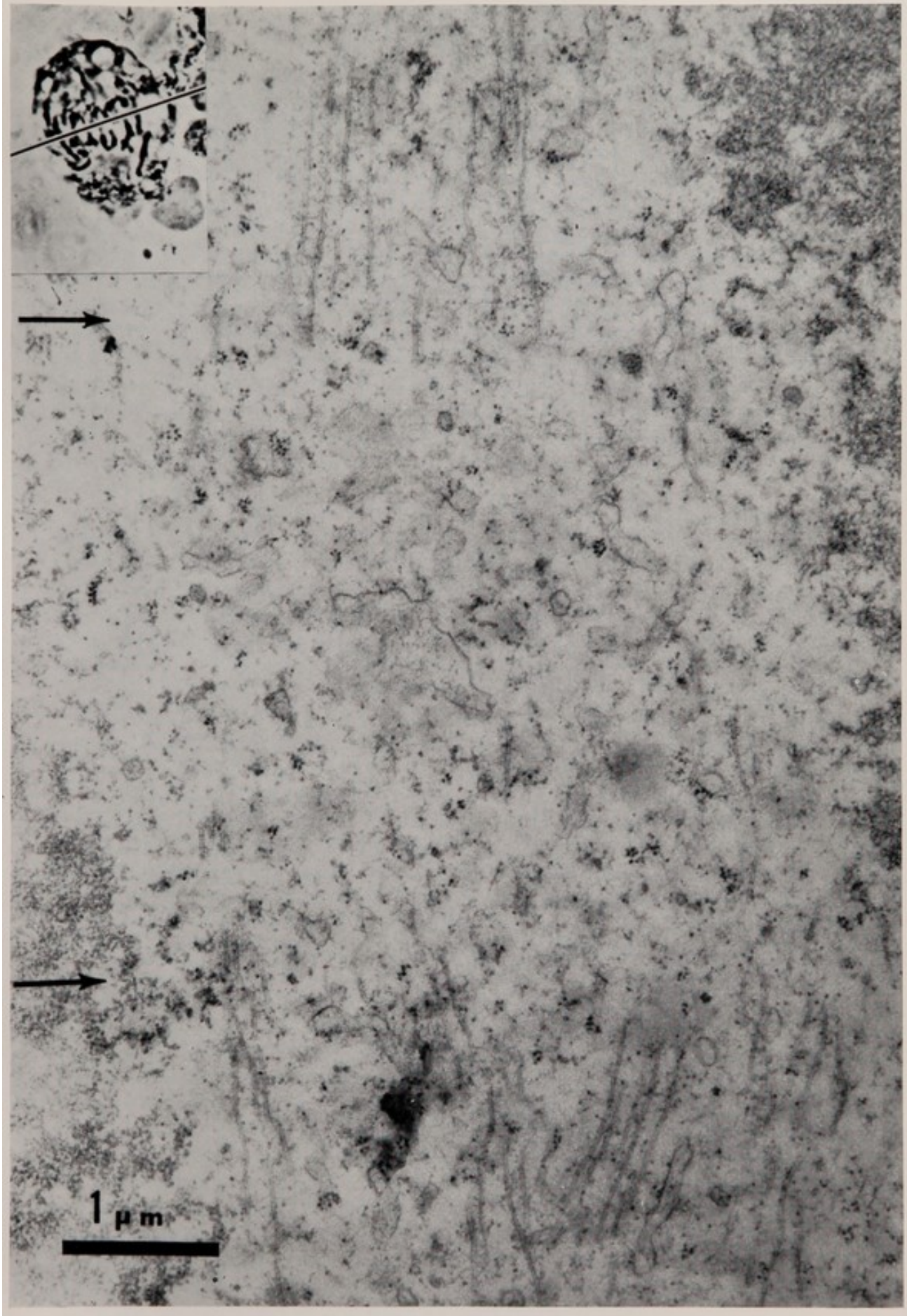
The cells where chromosome movements were followed in detail for a longer time after irradiation do not reveal any clear differences at the fine structure level, when compared with non-irradiated cells. In the cells irradiated with the dose which is adequate to slow down or stop chromosome movements (in control cells) and fixed a short time after irradiation, MTs



UV microbeam irradiation of kinetochore fibres in anaphase. Three chromosome arms were irradiated in prometaphase (2·5 µm. microbeam 5 sec. each). One long chromosome arm was hit in two regions and two bridges (one terminal) are well visible in 3-7. Kinetochore fibre of one of the sister kinetochores joined by the bridge was irradiated for 15 sec. in mid-anaphase with 5 µm. microbeam. 4—micrograph taken during irradiation—cross (two perpendicular lines) indicates precisely the irradiated spot. Kinetochore with irradiated kinetochore fibre moved rapidly towards the opposite unirradiated pole (5-6) and was included in one of the sister nuclei (7), *i.e.* irradiation resulted in non-disjunction. Micrographs from 16 mm. time-lapse film. Time after 1, 2—25′ 24″, 3—38′ 24″, 4—39′ 12″, 5—39′ 36″, 6—40′ 00″, 7—1 hr. 6′ 12″, 8—1 hr. 28′ 48″.



Effect of UV microslit on fine structure of the half-spindle. MTs of kinetochore fibre (note kinetochore—bottom of the micrograph) greatly reduced in number and some of them arranged in irregular direction. Higher magnification (*right*) shows short segments which are interpreted as broken MTs fragments. Arrow indicates lower border of UV microslit. Cell irradiated for 5 sec., fixed 30 sec. after irradiation. LM of this cell shows position of UV microslit. Scale of EM micrographs 12,000 : 1 and 20,000 : 1.



Effect of UV microslit on interzonal region (continuous fibres) in mid-anaphase. LM micrograph of this cell shows position of UV microslit. In irradiated region only short segments of MTs are visible. The width of the region where MTs disappeared is precisely 4 µm. (arrows), *i.e.* the same as the width of the microslit. It should be stressed, however, that in some other sections of this cell, in the irradiated region a few undamaged MTs were found (*cf.* discussion). Cell irradiated 4 sec. and fixed 1 min. after irradiation. Scale of EM micrograph 24,000 : 1.

are greatly reduced in number, or disorganised and only short fragments are detectable. It may be speculated therefore that chromosome movements slow down or stop completely according to the extent of MT disorganisation. The data show also that in cells fixed a long time after irradiation and where the chromosomes resumed normal movements, a reconstruction of MTs took place. It should be stressed, however, that present data experiments do not indicate how MTs are involved in the movement.

Due to the very shallow depth of field of UV microbeam, unless through focus irradiation technique is used, MTs are usually disorganised only in part of the spindle. Therefore birefringence of the spindle in the irradiated region is only reduced and does not disappear completely. Remaining MTs contribute to chromosome movements, but the movements are modified (slowed down, etc.). It is extremely difficult to draw conclusions concerning fine structure from such studies as it is not known to what extent MTs have been damaged. There is no reason to suppose that the response of MTs to UV in crane fly meiosis would differ from that in *Haemanthus* mitosis. Therefore conclusions of Forer (1965, 1966, 1969) drawn from studies of such cells need re-evaluation.

Comparison of the effects of UV microbeam and microslit indicates that the spindle reacts differently if it is irradiated in a small area only (microbeam) or if a considerable region of the spindle is irradiated (microslit). Thus experiments of Inoué (1964) and Forer (1965, 1966) are comparable with those using a short microslit and not a microbeam. In microslit experiments several kinetochore fibres are affected. In experiments on *Haemanthus* reported here the slit was always aligned perpendicularly to the long axis of the spindle. In experiments of Forer (1965, 1966) the slit was aligned at about 45° to the long axis of the spindle. Therefore in his experiments, spindle fibres at different distances from the kinetochores, and of different degrees of organisation, were irradiated. Forer did not use through focus irradiation technique. His results indicate that in most of his experiments only part of the apparatus of chromosome movement was destroyed. This in turn led him to a wrong interpretation of his results (Forer, 1969). He maintains that no correlation exists between birefringence and chromosome traction mechanism and that MTs are not the main birefringent component of the spindle. In *Haemanthus* MTs are greatly responsible for the birefringence of the spindle (Sato, Molè-Bajer and Bajer, in preparation) and chromosome movements. Although Forer has been working on a different material, it is unlikely that the basic mechanism of chromosome movements would be entirely different in crane fly and *Haemanthus*.

Present experiments draw attention also to some properties of the spindle evident from the micromanipulation work of Nicklas and Staehly (1967). They have found that it is difficult to move the chromosome in the spindle in early anaphase, but easy in late anaphase. The behaviour of experimentally formed dicentrics reported here indicates that it is possible to break a traction fibre in early or mid-anaphase, but no effect is seen in late anaphase. This is easily explained: in late anaphase the traction fibre is no longer active and the chromosome bridge is no longer elastic, because of telophase changes. Therefore the bridge at this stage cannot pull the chromosome towards the non-irradiated kinetochore. Therefore, it is understandable that it is easy to move the chromosomes in this stage by micromanipulation.

Behaviour of chromosomes after irradiation in *Haemanthus* can be

explained, if we assume that kinetochore fibres are moving (transported) in anaphase along continuous fibres which form some kind of support (Bajer, 1961, 1968; Bajer and Molè-Bajer, 1969), although the behaviour of the latter is more complicated than initially suggested. UV microslit experiments indicate that two systems of MTs (kinetochore and continuous) are involved in chromosome movement. Therefore chromosome movements can be affected by disorganisation of the half-spindle (kinetochore and continuous fibres), or the interzonal region (continuous fibres alone). The brief backward movements, after irradiation of the half-spindle with the UV microslit, indicate that continuous fibres from one pole extend a considerable distance on the other side of the equator.

It might be concluded therefore that UV microbeam and microslit irradiation is a very useful technique for studies of chromosome movements. Since it disorganises MTs and modifies chromosome movements, it provides convincing evidence that MTs are essential elements for chromosome locomotion.

6. Summary

1. The influence of UV microbeam and microslit irradiation on chromosome movements and the fine structure of the spindle has been studied in anaphase in the endosperm of *Haemanthus katherinae*.

2. Chromosome movements in one of the half-spindles can be slowed down or stopped by mid-anaphase UV microslit irradiation of the kinetochores themselves or the half-spindle close to them.

3. Movements of both chromosome groups are influenced by the irradiation of the interzonal region. Traction fibres in early and mid-anaphase can be inactivated within several seconds of irradiation. In late anaphase, when the chromosomes are ceasing to move, their behaviour, as a rule, is not affected by UV.

4. In cells fixed 30 sec.-1 min. after irradiation, fragments of MTs (microtubules) are detected in the irradiated region or the number of MTs is drastically reduced. In a few cases also clear " cuts " of MTs were observed. Although the MTs are disorganised by the action of UV the mechanism of this process is not known.

5. Some problems of possible errors in UV experiments (depth of field, etc.) are also briefly discussed.

Acknowledgments.—This work has been supported by the following research grants and contracts to the author: PHS Career Development Award No. 5-KO3-GM 34941-04, AEC research contract No. AT(45-1)-2045 and NSF research grant No. GB 24996×.

7. References

BAJER, A. 1961. A note on the behaviour of spindle fibres at mitosis. *Chromosoma, Berl., 12,* 64-71.

BAJER, A. 1968. Behaviour and fine structure of spindle fibres during mitosis in endosperm. *Chromosoma, Berl., 25,* 249-281.

BAJER, A. 1969. Effects of UV microbeam irradiation on chromosome movements and spindle fine structure. *Biophys. J., 9,* A-151.

BAJER, A., AND MOLÈ-BAJER, J. 1969. Formation of spindle fibres, kinetochore orientation and behaviour of the nuclear envelope during mitosis in endosperm. Fine structure and *in vitro* studies. *Chromosoma, Berl., 27,* 448-484.

BLOOM, W., AND ÖZARSLAN, S. 1965. Electron microscopy of UV-irradiated parts of chromosomes. *Proc. Natl. Acad. Sci., 53,* 1294-1302.

FORER, A. 1965. Local reduction of spindle fibre birefringence in living *Nephrotoma suturalis* (Loew) spermatocytes induced by ultraviolet microbeam irradiation. *J. Cell Biol., 24,* 95-117.

FORER, A. 1966. Characterisation of the mitotic traction system, and evidence that birefringent spindle fibres neither produce not transmit force for chromosome movement. *Chromosoma, Berl., 19,* 44-98.

FORER, A. 1969. Chromosome movements during cell division. In *Handbook of Molecular Cytology* (ed. A. Lima-de-Faria), North Holland Co., London.

INOUÉ, S. 1964. Organization and function of the mitotic spindle. In *Primitive Motile Systems in Cell Biology* (eds. R. D. Allen and N. Kamiya), 549-598. Academic Press, New York.

IZUTSU, K. 1961. Effects of ultraviolet microbeam irradiation upon division in grasshopper spermatocytes. II. Results of irradiation during metaphase and anaphase I. *Mie Med. J., 11,* 213-232.

JAGGER, J. 1967. Introduction to research in ultraviolet photobiology. Prentice-Hall, Englewood Cliffs, N.J. Pp. 1-164.

LUYKX, P. 1970. In *Int. Rev. of Cytology.* Cellular mechanisms of chromosome distribution, *2,* 173. Academic Press.

MOLÈ-BAJER, J., AND BAJER, A. 1968. Studies of selected endosperm cells with the light and electron microscope. The technique. *La Cellule.*

NICKLAS, R. B. 1971. Mitosis. In *Advances in Cell Biology II* (ed. D. M. Prescott, L. Goldstein and E. H. McConkey). Appleton-Century Crofts, New York.

NICKLAS, R. B., AND STAEHLY, C. A. 1967. Chromosome manipulation. I. The mechanics of chromosome attachment to the spindle. *Chromosoma, Berl., 21,* 1-16.

SATO, H. 1969. Analysis of the form birefringence in the mitotic spindle. *Am. Zool., 9,* 592.

VOSS, G. 1966. In *V Int. Histological Symp.* (ed. W. Baranow). *Proc. Acad. Sci. Bulgaria, Sofia,* 164-168.

ZIRKLE, R. E. 1964. Rapporteur's Report. In *Recent Progress in Photobiology* (ed. E. J. Bowen), 311-324. *Proc. of V Int. Congr. of Photobiology, Oxford.* Academic Press, New York.

ANAPHASE BEHAVIOUR OF INVERSIONS IN LIVING CRANE-FLY SPERMATOCYTES

ROLAND DIETZ

Max-Planck-Institut für Zellbiologie, 74 Tübingen, Germany

In this study males of *Pales ferruginea* have been used, which were heterozygous for a parakinetic inversion in one of the 3 mediokinetic autosomes. The chromosome mutation was induced by X-raying several generations before the investigation was started.

In inversion heterozygotes of this type a dikinetic chromatid bridge and an akinetic fragment can arise following recombination within the inversion loop. For any particular inversion the length of the fragment, and consequently the structural length of the bridge, is constant and independent of the position of the chiasma (fig. 1, *c* and *d*). Only one inversion stock was used in the present study and thus the structural length of all bridges was the same in all the males examined.

By late anaphase, or sometimes even mid-anaphase, each of the two

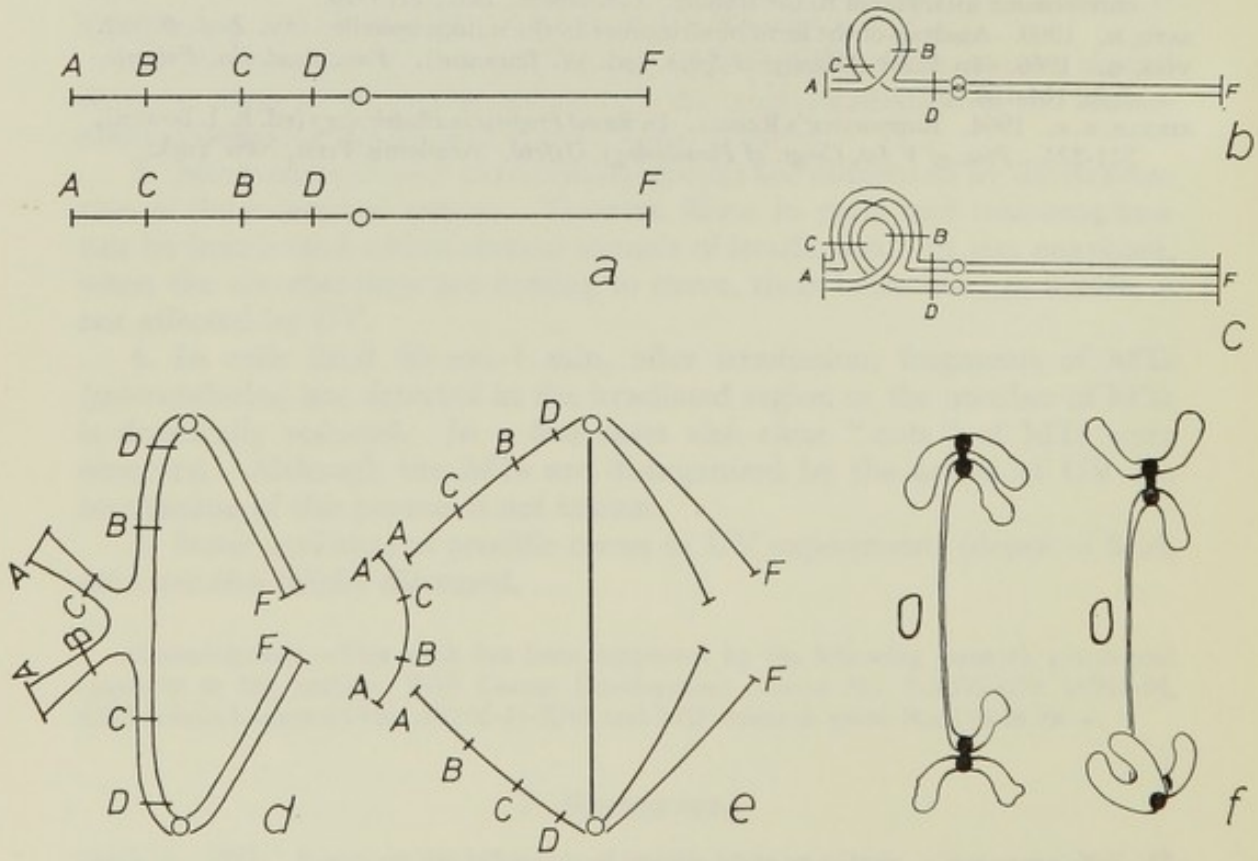


FIG. 1.—Formation of anaphase bridges in inversion heterozygotes. *a*, two single stranded chromosomes with the regular and the inverted sequence of loci. *b*, the single stranded chromosomes after pairing, forming a loop in the inverted region. *c*, the double stranded chromosomes after chiasma formation in the inverted region; note that the length of the fragment is independent of the position of the chiasma. *d*, metaphase. *e*, anaphase, the bridge kinetochores are oriented towards opposite poles. *f*, anaphase bridge and fragment according to nature. Due to the antagonistic strain the chromatid kinetochores are clearly separated; the free chromosome arms often point towards the poles.

kinetochore regions of the bridge prove to be divided into its two constituent chromatid-kinetochores. Those chromatid-kinetochores lag, which are linked by the bridge (fig. 1, *f*). As the free dyads (half bivalents) do not show a comparable separation of chromatids, the disconnection is undoubtedly caused by strong antagonistic strain.

The plate shows different anaphase stages in one of the 32 first spermatocytes on which this study is based. With the beginning of anaphase the syntelic kinetochores moved towards their respective poles. Very soon the kinetochores of the free dyads were ahead of those of the inversion bridge. In all spermatocytes the akinetic fragment remained in the equator for 15-20 min. and only then was it shifted towards one of the two poles. When it moved, the two amphitelic sex-chromosomes remained in the equator, the onset of their segregation varying considerably from cell to cell. Anaphase lasted about an hour.

The 32 spermatocytes were photographed in intervals of two minutes. The distances between the syntelic kinetochores and their poles and the distances between the amphitelic kinetochores of the two sex chromosomes and both poles were measured 6 times independently on positive prints. When the chromatid-kinetochores of a bridge had disconnected, the distance between the small gap separating the chromatid-kinetochores, and the pole was determined. The average values of the 6 measurements were used for all further calculations.

The spindle lengths and the kinetochore-to-pole distances of the cell shown in the plate are given in fig. 2. Here the spindle length decreased in early anaphase, later on it increased slightly, and finally the spindle shortened again. In many other cells the decrease of spindle length in early anaphase was more pronounced while the increase during mid-anaphase was often smaller or totally absent. In nearly all cells the spindle length decreased at the end of anaphase, when the constriction furrow was formed and the spindle apparatus disintegrated.

In 30 anaphase I cells without bridges or other irregularities, the decrease of spindle length in early anaphase was restricted to a few cells and even in these it was not very pronounced. The mid-anaphase increase of spindle length, however, occurred in all these cells and it was generally larger. The average net increase in the regular anaphases amounted to $6{\cdot}7 \pm 0{\cdot}6\ \mu$, while that in anaphases with bridge formation is only $2{\cdot}7 \pm 0{\cdot}5\ \mu$; the difference is highly significant.

Fig. 2 shows that the period of positive acceleration of the free dyads lasted less than one minute. Subsequently the free dyads moved with approximately constant velocities until they reached kinetochore-to-pole distances of 4 to 1 μ. Then their velocity began to decrease gradually; but in general the free dyads did not stop moving until they had reached the spindle poles themselves. In some cells the anaphase velocity of the free dyads was higher, in others it was less than in this example.

The bridge kinetochores had a definitely longer period of positive acceleration during the initial stage of anaphase. After a short interval, in which the velocity was approximately constant, it decreased sharply. The retardation occurred at various times but mostly long before the kinetochores had reached their poles. Thereupon the kinetochore-to-pole distances remained constant or decreased at a much slower rate than before. Owing to simultaneous changes in spindle length the distinction between these phases is

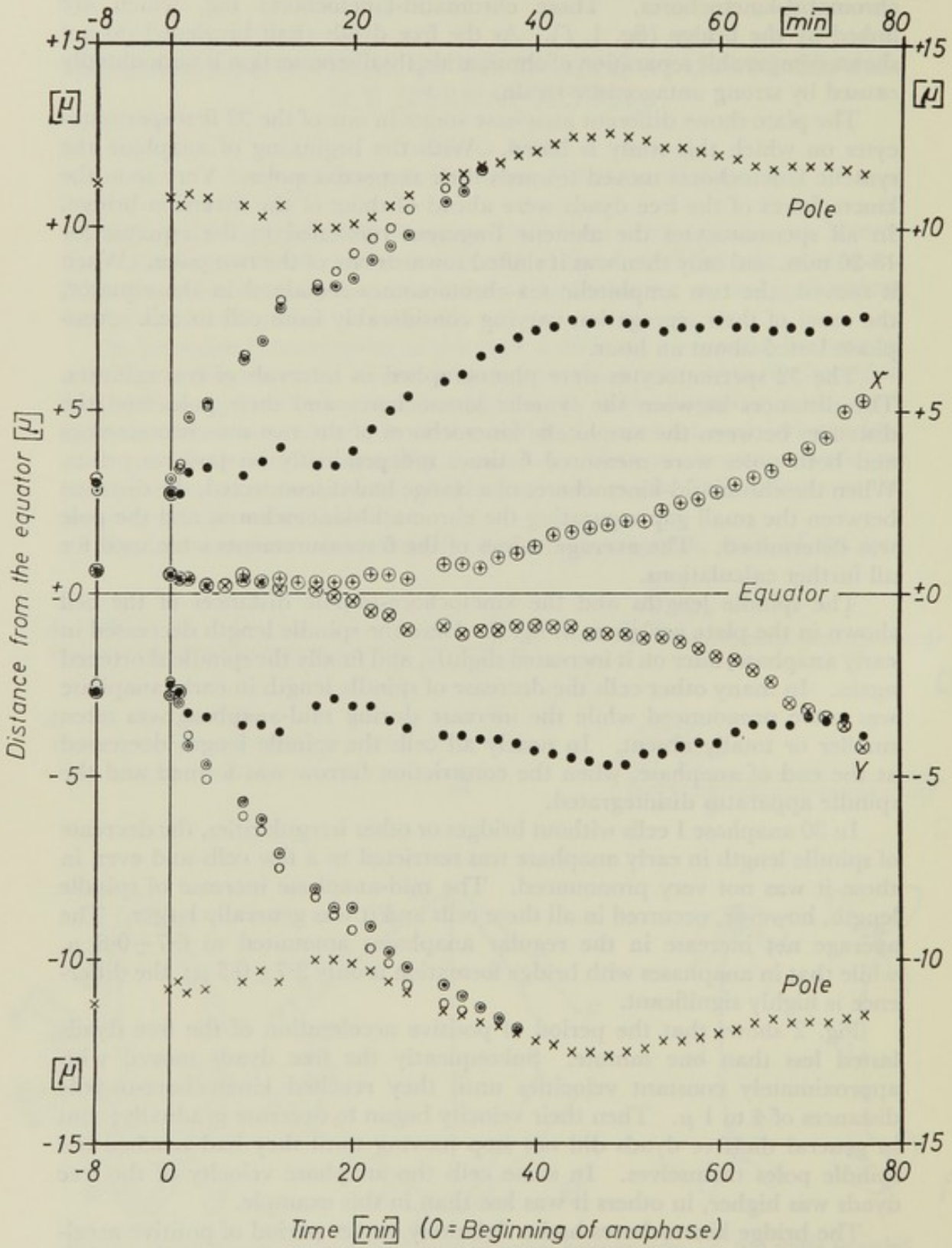


FIG. 2.—Distance-time diagram of the interpolar and the kinetochore-to-pole distances in the spermatocyte shown in the plate. X position of the spindle poles relative to the theoretical equator, O and ⊙ position of the free dyads, ● position of the bridge kinetochores, ⊕ position of the X, ⊗ position of the Y.

obscured in diagrams of the type presented in fig. 2; but when the kinetochore-to-pole distances are plotted against time, in 27 spermatocytes the deflection in the curve was very distinct (see fig. 8).

In some cases (*e.g.* fig. 2), one kinetochore of the inversion bridge approached its pole significantly faster than its partner so that the middle of the bridge was displaced from the equator. In all these cases, one of the two amphitelic sex univalents finally migrated farther from the equator than the other one, and the pole which was approached more closely, was invariably that towards which the bridge had been displaced.

In an analysis of interdependences between the various processes the following abbreviations will be used:

ls_a length of the spindle at the beginning of anaphase

d_a average kinetochore-to-pole distance of the inversion bridge at the beginning of anaphase

lb_a length of the bridge at the beginning of anaphase

$v_{2\text{-}8D}$ average velocity of the kinetochores of the free dyads between the 2nd and the 8th minute of anaphase

$v_{2\text{-}8I}$ average velocity of the kinetochores of the inversion bridge between the 2nd and the 8th minute of anaphase

$\Delta lb_{2\text{-}8}$ difference in length of the inversion bridge between the 2nd and the 8th minute of anaphase

$\Delta ls_{2\text{-}8}$ difference in length of the spindle between the 2nd and the 8th minute of anaphase

t_r time in minutes after the beginning of anaphase when the shortening of the kinetochore-to-pole distance of the bridge retarded sharply

d_r average kinetochore-to-pole distance of the inversion bridge at the beginning of the retarded shortening

lb_r length of the bridge at the beginning of the retarded shortening of its kinetochore-to-pole distances

$\Delta d_{26\text{-}36}$ length difference of the kinetochore-to-pole distances of the bridge between the 26th and the 36th minute of anaphase

$\Delta lb_{26\text{-}36}$ length difference of the bridge between the 26th and the 36th minute of anaphase

$\Delta ls_{26\text{-}36}$ length difference of the spindle axis between the 26th and the 36th minute of anaphase

d_{40} average kinetochore-to-pole distance of the inversion bridge at the 40th minute of anaphase

lb_{40} length of the bridge at the 40th minute of anaphase

The following relationships have been found:

(1) $v_{2\text{-}8D}/v_{2\text{-}8I}$

$r = 0{\cdot}55$ $b_y = 0{\cdot}56$ $b_x = 0{\cdot}55$

$t_{30} = 3{\cdot}6$ $P < 0{\cdot}002$

The velocities with which the kinetochores of the bridge approach their poles in early anaphase are positively correlated with the velocities of the free dyads during the same period. The regression coefficient r is a measure of the closeness of the relationship between the two varietal characters, 1·0 standing for complete interdependence, 0·0 for no connection. The regression coefficient b_y indicates the extent to which the varietal character Y (here $v_{2\text{-}8I}$) increases, when the varietal character X (here $v_{2\text{-}8D}$) increases by 1 unit. In this particular case it is saying: When $v_{2\text{-}8D}$ increases by

1 μ/min., the most probable increase of $v_{2\text{-}8I}$ is 0·56 μ/min. The regression coefficient b_x gives the corresponding information, when conclusions are inferred from the varietal character Y towards X. Using the average values and the regression coefficients, regression lines can be calculated (fig. 3).

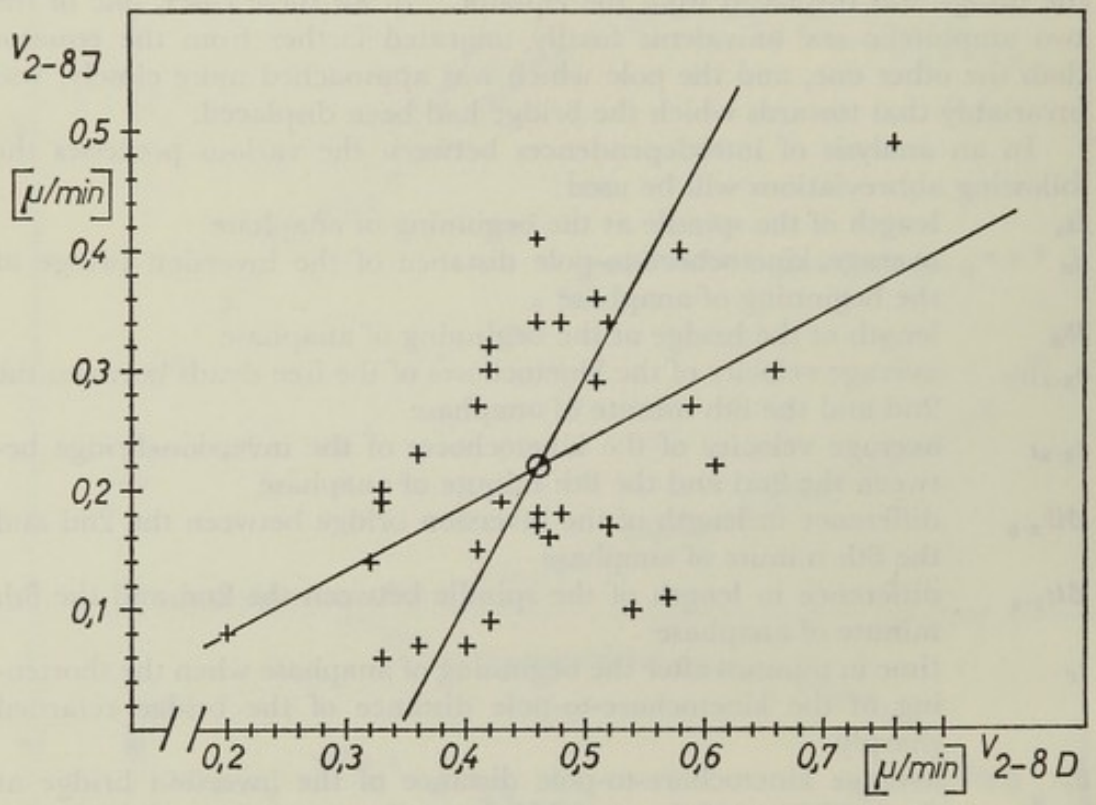


FIG. 3.—Correlation diagram of the average velocity with which the free dyads approach their poles between the 2nd and the 8th min. of anaphase, $v_{2\text{-}8D}$, (abscissa) versus the average velocity with which the bridge kinetochores approach their poles during the same period, $v_{2\text{-}8I}$, (ordinate) in 32 spermatocytes *I* of *Pales ferruginea*.

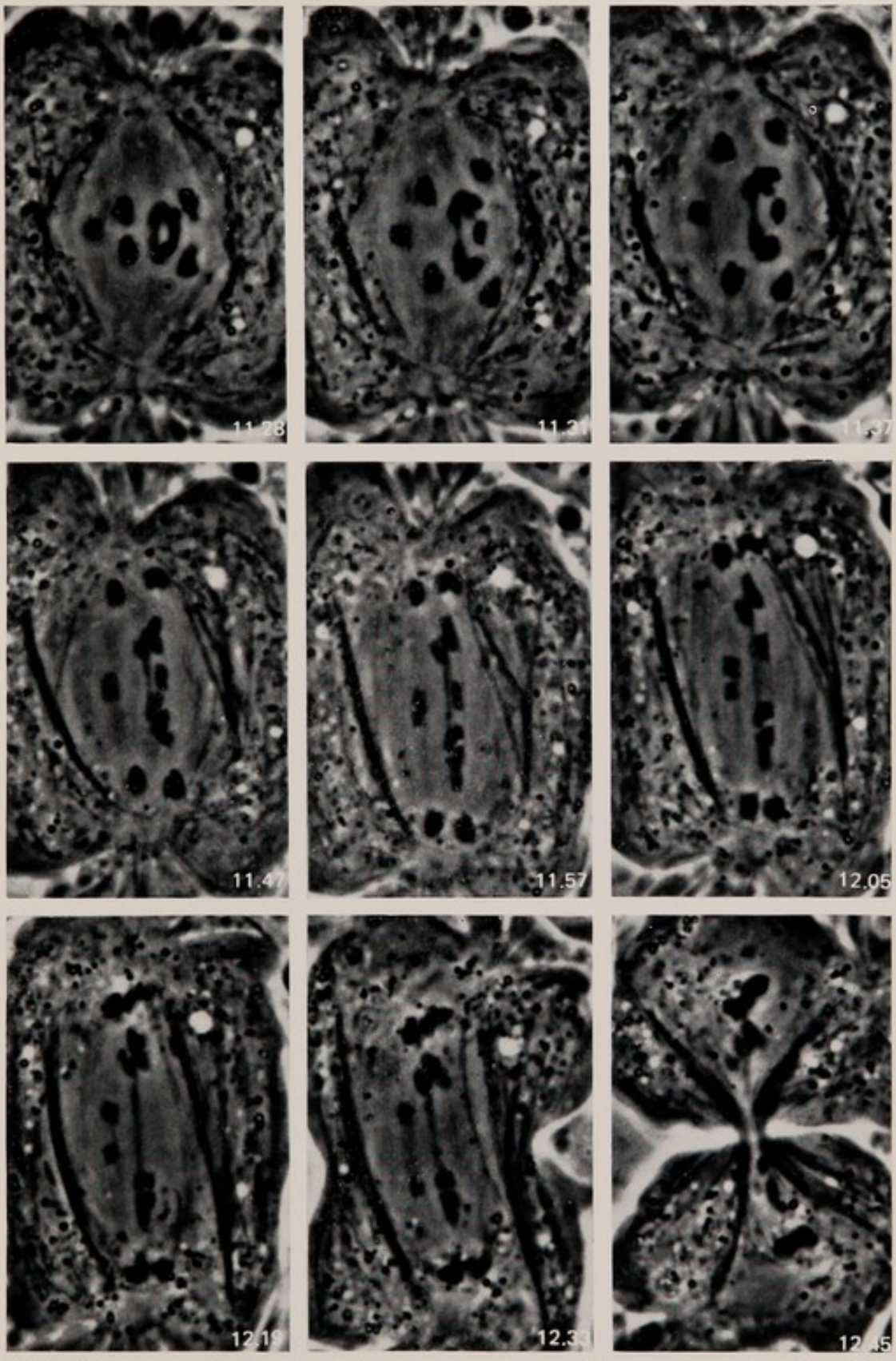
The diagram shows that in cells in which the free dyads move with a velocity of 0·2 μ/min., the most probable velocity of the kinetochores of the inversion bridge is 0·08 μ/min.; and that in cells in which the free dyads move with an average velocity of 0·8 μ/min., a velocity of 0·4 μ/min. can be expected for the kinetochores of the inversion bridge. As a rough approximation it can be said: Between the 2nd and the 8th min. of anaphase the resistance of a chromatid bridge which is increasingly extended between its two kinetochores, causes these kinetochores to move only half as fast as in regular anaphase, *i.e.* when free chromosomes are transported.

(2) $v_{2\text{-}8I}/lb_{40}$

$r = 0{\cdot}56$ $\quad b_x = 0{\cdot}016$ $\quad b_y = 19{\cdot}11$

$t_{30} = 3{\cdot}7$ $\quad P < 0{\cdot}001$

The higher the velocity of the kinetochores of an inversions bridge in early anaphase, the longer is the bridge at the 40th min. of anaphase. Fig. 4 shows that in a cell in which the bridge-kinetochores approach their poles with an average velocity of 0·05 μ/min. during the 2nd and the 8th min. of anaphase, the bridge will have reached a most probable length of 10·3 μ by the 40th min. of anaphase, whereas in a cell in which the two bridge kinetochores approach their poles with a velocity of 0·5 μ/min., the bridge will



Successive stages of first anaphase in a spermatocyte of *Pales ferruginea* with an inversion bridge and a fragment.

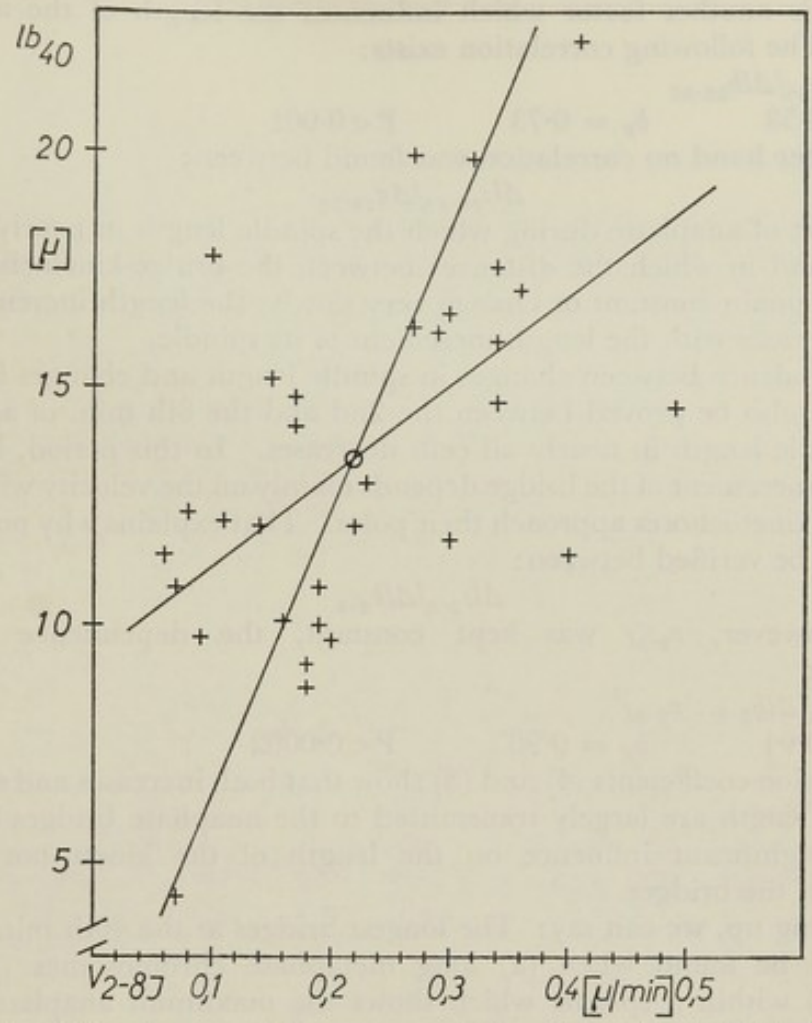


FIG. 4.—Correlation diagram of the average velocity with which the bridge kinetochores approach their poles between the 2nd and the 8th min. of anaphase, $v_{2\text{-}8I}$, (abscissa) versus the length of the bridge at the 40th min. of anaphase, lb_{40}, (ordinate) in 32 spermatocytes *I* of *Pales ferruginea*.

have reached a length of 18·9 μ by the same time. It should be emphasised that this positive correlation is not self-evident.

(*a*) As mentioned before and summarised in fig. 8, the early anaphase velocity of the bridge kinetochores is strongly retarded at various times, and if fast moving kinetochores stop early while slow ones move late, no correlation or even a negative one would be expected between $v_{2\text{-}8I}$ and lb_{40}.

(*b*) There are other factors which influence lb_{40}. For instance, the degree of spiralisation of metaphase chromosomes has an influence. This, at least, can be concluded from the following correlation:

(3) lb_a/lb_{40}

$r = 0{\cdot}62$ $\quad b_x = 0{\cdot}29$ $\quad b_y = 1{\cdot}34$

$t_{30} = 4{\cdot}4$ $\quad P < 0{\cdot}0002$

i.e. The more contracted the inversion chromosome at metaphase, the smaller the inversion bridge 40 min. after the beginning of anaphase. No correlation could be verified between:

$$lb_a/v_{2\text{-}8I}$$

Presumably the variability of $v_{2\text{-}8I}$ is affected to such an extent by other (still unknown) factors that the influence of lb_a cannot be detected.

There is another factor which influences the length of the anaphase bridges. The following correlation exists:

(4) $\Delta ls_{26\text{-}36}/\Delta lb_{26\text{-}36}$
$r = 0{\cdot}58 \qquad b_y = 0{\cdot}73 \qquad P < 0{\cdot}001$

On the other hand no correlation was found between:

$$\Delta ls_{26\text{-}36}/\Delta d_{26\text{-}36}$$

In that part of anaphase during which the spindle length in nearly all cells increases and in which the distances between the bridge-kinetochores and the poles remain constant or change very slowly, the length increment of a bridge increases with the length increment of its spindle.

A dependence between changes in spindle length and changes in bridge length can also be proved between the 2nd and the 8th min. of anaphase, when spindle length in nearly all cells decreases. In this period, however, the length increment of the bridge depends mainly on the velocity with which the bridge kinetochores approach their poles. That explains why no correlation could be verified between:

$$\Delta ls_{2\text{-}8}/\Delta lb_{2\text{-}8}$$

When, however, $v_{2\text{-}8I}$ was kept constant, the dependence became significant:

(5) $\Delta ls_{2\text{-}8}/\Delta lb_{2\text{-}8} \cdot v_{2\text{-}8I}$
$r = 0{\cdot}94 \qquad b_y = 0{\cdot}90 \qquad P < 0{\cdot}0002$

The regression coefficients (4) and (5) show that both increases and decreases of spindle length are largely transmitted to the anaphase bridges but they have no significant influence on the length of the kinetochore-to-pole distances of the bridges.

Summing up, we can say: The longest bridges at the 40th min. of anaphase will be found when (*a*) long metaphase chromosomes (*b*) move quickly (*c*) within a spindle which shows the maximum anaphase length increase. The three factors very independently of each other.

The correlations quoted so far show no evidence that those bridge-kinetochores which move most rapidly at early anaphase are those which later approach their poles most closely. A positive correlation between $v_{2\text{-}8I}/lb_{40}$ would result also, if the velocity of the chromosomes in early anaphase was positively correlated with the length increment of the spindles in mid-anaphase. This objection can be dismissed because there is no correlation between:

$$v_{2\text{-}8}/ls_{26\text{-}36}$$

whereas the following correlations could be verified:

(6) $v_{2\text{-}8I}/d_{40}$
$r = -0{\cdot}57 \qquad b_x = -0{\cdot}03 \qquad b_y = -11{\cdot}70$
$t_{30} = 3{\cdot}8 \qquad P < 0{\cdot}001$

(7) $v_{2\text{-}8I}/d_r$
$r = -0{\cdot}60 \qquad b_x = -0{\cdot}04 \qquad b_y = -10{\cdot}07$
$t_{25} = 3{\cdot}5 \qquad P < 0{\cdot}001$

The most probable relationship between $v_{2\text{-}8I}$ and d_{40} is illustrated in fig. 5, that between $v_{2\text{-}8I}$ and d_r is given in fig. 6. In a spindle in which the bridge-kinetochores each approach their poles with a velocity of 0·5 μ/min., they will most probably slow down or stop moving at a kinetochore-to-pole distance of 2·7 μ. On the other hand, two bridge kinetochores which approach their poles with a velocity of 0·05 μ/min. each, will retard or stop moving at a kinetochore-to-pole distance of 6·9 μ. Taking into account

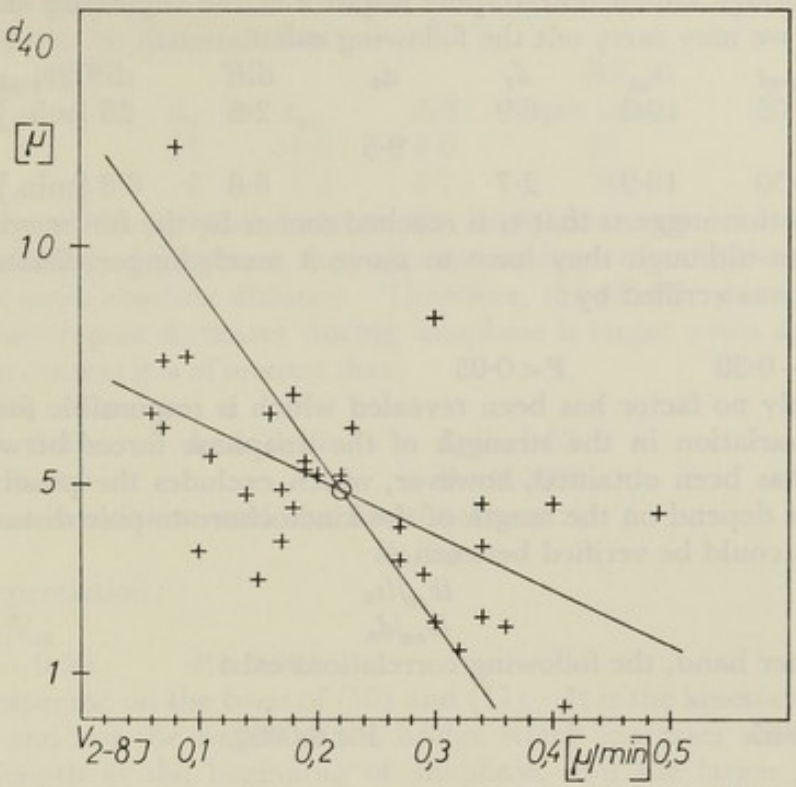


FIG. 5.—Correlation diagram of the average velocity with which the bridge kinetochores approach their poles between the 2nd and the 8th min. of anaphase, $v_{2\text{-}8I}$, (abscissa) versus the average kinetochore-to-pole distance of the bridge at the 40th min. of anaphase, d_{40}, (ordinate) in 32 spermatocytes *I* of *Pales ferruginea*.

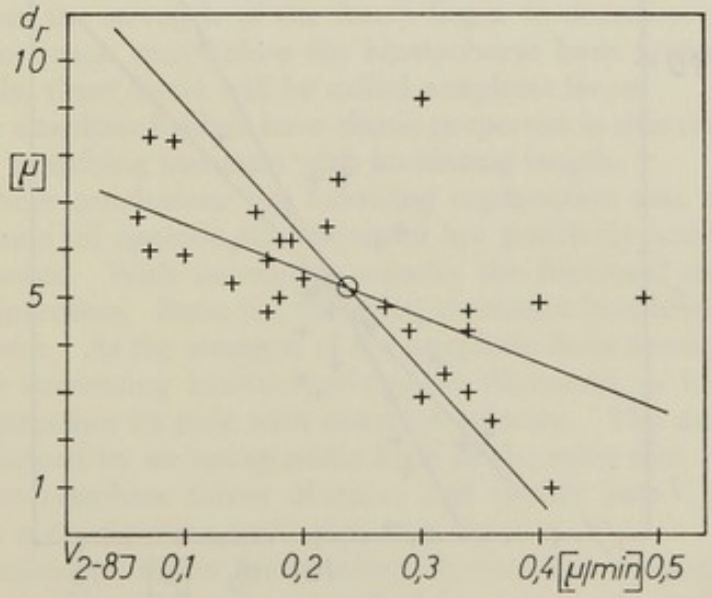


FIG. 6.—Correlation diagram of the average velocity with which the bridge kinetochores approach their poles between the 2nd and the 8th min. of anaphase, $v_{2\text{-}8I}$, (abscissa) versus the average kinetochore-to-pole distance of the bridge at which the pole approach stops or is strongly retarded, d_r, (ordinate) in 27 spermatocytes *I* of *Pales ferruginea*.

that the average kinetochore-to-pole distance at the beginning of anaphase was 9·5 μ, we may carry out the following calculation:

$v_{2\text{-}8I}$	lb_{40}	d_r	d_a	diff	diff/$2v_{2\text{-}8I}$
0·05	10·3	6·9		2·6	26 [min.]
			9·5		
0·50	18·9	2·7		6·8	6·8 [min.]

The calculation suggests that t_r is reached sooner by the fast moving bridge kinetochores although they have to move a much longer distance. This suggestion was verified by:

(8) d_r/t_r

$r = -0{\cdot}38$ $\quad$ $P < 0{\cdot}05$

In this study no factor has been revealed which is responsible for the considerable variation in the strength of the anaphase forces between cells. Evidence has been obtained, however, which excludes the possibility that these forces depend on the length of the kinetochore-to-pole distances. No correlation could be verified between:

lb_{40}/ls_a

lb_{40}/d_a

On the other hand, the following correlations exist:

(9) d_a/d_r

$r = 0{\cdot}65$ $\quad$ $P < 0{\cdot}0002$

(10) d_a/d_{40}

$r = 0{\cdot}75$ $\quad$ $b_x = 0{\cdot}52$ $\quad$ $b_y = 1{\cdot}08$

$t_{30} = 6{\cdot}3$ $\quad$ $P < 0{\cdot}0002$

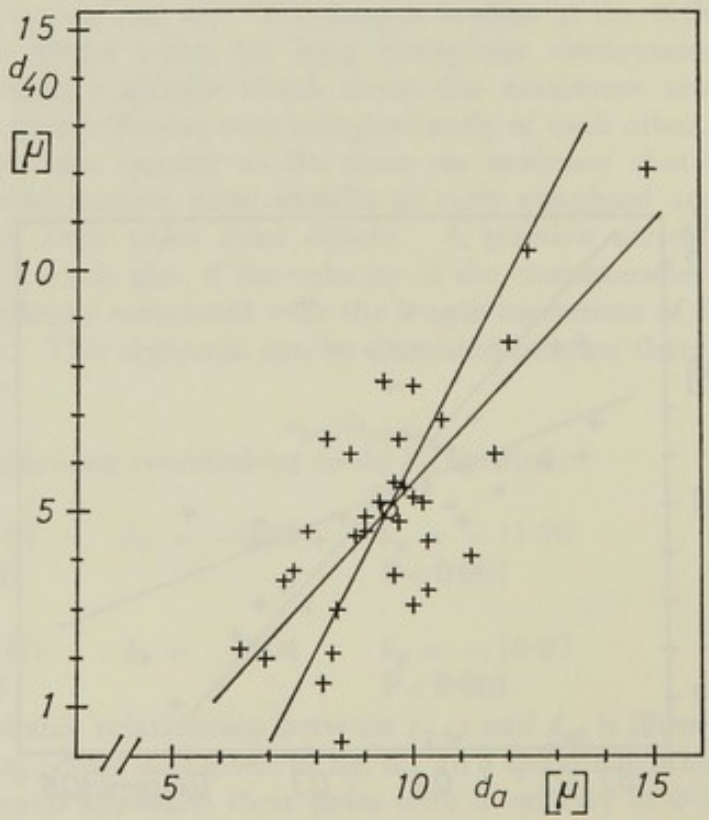


FIG. 7.—Correlation diagram of the average kinetochore-to-pole distance of the bridge chromosome at the beginning of anaphase, d_a, (abscissa) versus the average kinetochore-to-pole distance of the bridge at the 40th min. of anaphase, d_{40}, (ordinate) in 32 spermatocytes *I* of *Pales ferruginea*.

The relationship between d_a and d_{40} is illustrated in fig. 7. From the regression line it can be gathered that:

d_a	d_{40}	diff	diff per cent.
15	11·0	4·0	27
6	1·3	4·7	78

Irrespective of the distance between the bridge-kinetochores and the poles at the beginning of anaphase, they all stop moving when they have traversed about the same absolute distance. Therefore, the relative shortening of the kinetochore-to-pole distances during anaphase is larger when d_a is smaller.

In this context it is of interest that:

(11) d_a/ls_a

$r = 0{\cdot}87$ $\quad b_x = 0{\cdot}46$ $\quad b_y = 1{\cdot}63$

$t_{30} = 9{\cdot}6$ $\quad P < 0{\cdot}0002$

whereas no correlation could be found between:

lb_a/ls_a

The correlation:

(12) ls_a/d_{40}

$r = 0{\cdot}56$ $\quad P < 0{\cdot}001$

is to be expected on the basis of (10) and (11). It is the kinetochore-to-pole distance and not the length of the bridge which increases with increasing spindle length at the beginning of anaphase, and the larger the spindle length at the beginning of anaphase, the larger the kinetochore-to-pole distance of the bridge at the 40th min. of anaphase.

The family of curves presented in fig. 8 has been constructed using the regressions stated under (1), (6), (7), (9) and (10). From this family of curves it can be concluded that:

(i) During small time intervals, forces are generated which draw the kinetochores towards the poles to which they are oriented. The strength of these forces varies from cell to cell. Over a wide range it is independent of the kinetochore-to-pole distance; only at kinetochore-to-pole distances of 4 to 1 μ does the strength of the forces begin to decrease. Generally the forces do not reach zero before the kinetochores have arrived at the pole. Subsequently, these forces will be called anaphase forces.

(ii) The anaphase bridges have elastic properties in that resistance against any further stretching increases with increasing length.

Using these conclusions the following explanation can be offered: In early anaphase all syntelic chromosomes are positively accelerated by the anaphase forces. With increasing velocity the frictional resistance of the free dyads increases. Soon the frictional resistance becomes as large as the anaphase force. As the strength of the anaphase force remains constant (in spite of the decreasing kinetochore-to-pole distances), a free dyad subsequently approaches its pole with constant velocity. The anaphase force is counter-balanced by an antagonistic force of the same size. Only near the poles do the anaphase forces decrease and finally stop. The dyads are subjected to a deceleration until they stop moving.

The acceleration of the bridge kinetochores in early anaphase is limited not only by the frictional resistance which increases with increasing velocity, but by the resistance which is caused by the extension of the bridge. Therefore, $v_{2\text{-}8I}$ is smaller than $v_{2\text{-}8D}$. The velocity of the bridge kinetochore begins to decrease, as soon as the frictional resistance plus the resistance

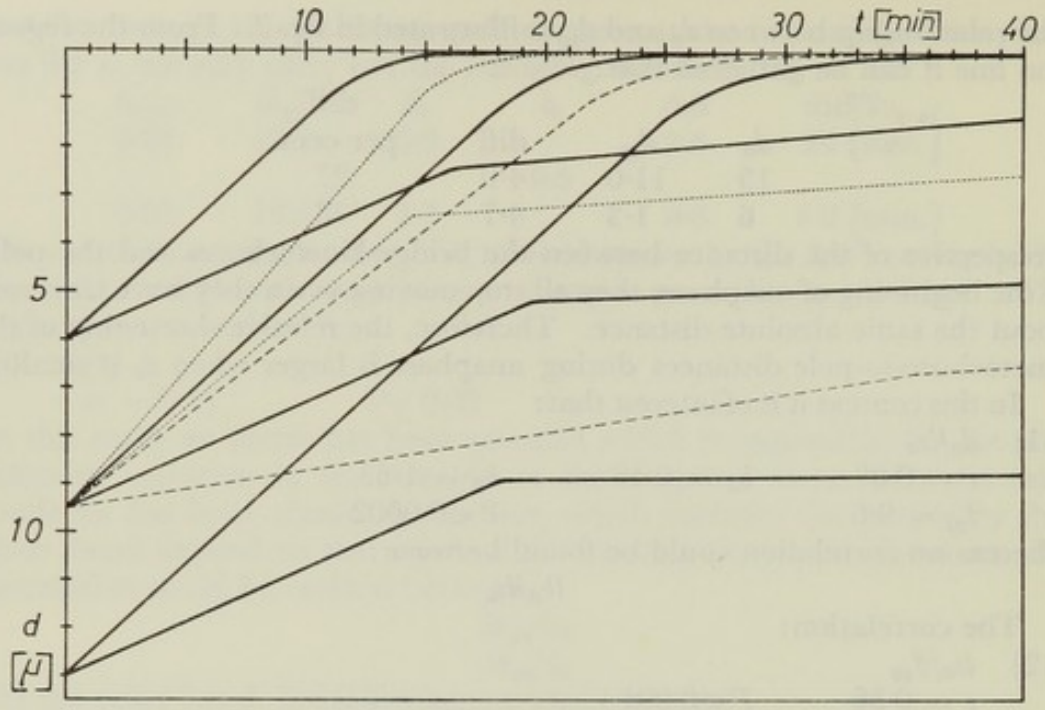


FIG. 8.—Schematic presentation of the anaphasic pole approach of the kinetochores of free dyads and of inversion bridges, based on the correlations quoted under (1), (6), (7) and (10). Continuous lines: three pairs of curves, consisting in each case of a steep one, which represents the kinetochores of the free dyads and a less steep one, which represents the bridge-kinetochores. One of the curve pairs starts at the average kinetochore-to-pole distance (9·5 μ), the others at extremely short (6·0 μ) and long (13·0 μ) kinetochore-to-pole distances respectively. To indicate that the anaphase velocity is independent of the kinetochore-to-pole distance at the beginning of anaphase, in all three pairs the same slope was chosen for the corresponding curves. The kinetochores of the free dyads and of the bridges differ not only with regard to their velocity. Whereas, irrespective of their kinetochore-to-pole distance at the beginning of anaphase, the free dyads move with constant velocity until they have nearly reached their poles, all bridge kinetochores, moving with a definite velocity, stop approaching their poles, or retard considerably, when they have traversed approximately the same distance. The kinetochore-to-pole distances at which they stop are positively correlated with the kinetochore-to-pole distances at the beginning of anaphase. Dotted lines: pair of curves, representing the kinetochores of the free dyads and of the bridge in a cell in which anaphase velocity is extremely high and in which the kinetochore-to-pole distance at the beginning of anaphase was chosen to coincide with the average value. Dashed lines: pair of curves, representing the kinetochores of the free dyads and of the bridge in a cell in which anaphase velocity is extremely low and in which the kinetochore-to-pole distance at the beginning of anaphase was chosen to coincide with the average value. Again the free dyads move with constant velocity until they have nearly reached their poles. The bridge-kinetochores move the faster, the faster the free dyads approach their poles, and the faster the bridge-kinetochores move, the sooner they stop and the closer they approach their poles.

caused by the elasticity of the bridge becomes larger than the anaphase force. The kinetochores are subjected to deceleration until they finally stop. During this period the frictional resistance decreases to zero. Afterwards the anaphase force is counterbalanced by the elastic tension of the bridge. As the strength of the anaphase force has not changed, the free dyads continue to approach their poles with constant velocity at the same time. The length of the bridge depends on the strength of the anaphase force, which varies from cell to cell. Therefore, the bridges will grow the longer, the faster the free dyads move.

The conclusion that, within limits, the strength of the anaphase forces is independent of the kinetochore-to-pole distance offers the simplest explanation for the constant velocity of free dyads during the greater part of their polar migration and for the generation of anaphase forces of equal average strength in both large and small spindles. This implies that, in all spindles, the bridges will be stretched until, at an average length, the average elastic resistance of the bridges counterbalances the average anaphase force. When, however, in large and small spindles the bridges are stretched to an average length, the final kinetochore-to-pole distances must be larger with larger spindles.

In recent hypotheses of chromosome movement two principal models of spindle organisation are discussed. When Östergren, Molè-Bajer and Bajer (1960) published their sailing-boat hypothesis, they were the first to suggest that the shortening of the chromosomal spindle fibre in anaphase is the consequence and not the cause of the polar migration of chromosomes. They assumed that within the spindle some molecular pumping activity causes the chromosomal spindle fibres (and with them the chromosomes) to be shifted towards the poles, and they compared a chromosomal spindle fibre with a sail which utilises a force (the " molecular wind ") without generating it. At the poles the fibres are depolymerised.

The depolymerisation prevents them from sticking out of the spindle, but it does not generate the force responsible for the anaphase movement of chromosomes (fig. 9, *a*). On this view the chromosomal fibres do not necessarily connect the kinetochores with the poles, the fibres may be much shorter. Bajer (1968), retaining the idea of a passive chromosomal fibre, attempted to include the results which electron microscopy has contributed to our knowledge of spindle organisation: In anaphase the microtubules, which constitute the continuous spindle fibres, grow by polymerisation. Simultaneously the microtubules which constitute the chromosomal spindle fibres slide over the growing continuous microtubuli. The force is thought to be generated by the amorphous material between the two types of microtubuli. McIntosh, Hepler and Van Wie (1969) and Hepler, McIntosh and Cleland (1970) suggest a similar model. They find bridges between microtubules which lie side by side and they suppose that these bridges generate the force which makes the two systems slide relative to one another. Subirana (1968) has suggested two other models of force generation: (i) Between the protein of the chromosomal fibres and the protein of the surrounding matrix interactions may occur, comparable to those between actin and myosin. (ii) A chromosomal fibre may propel itself due to rhythmical conformational changes of its molecular subunits. In this case the chromosomal fibre parallels a rowing-boat in function rather than a sail, but in all cases the whole fibre is shifted.

The other model of the organisation of the spindle apparatus has been propagated by Franz Schrader. The chromosomal fibres connect the chromosomes with the spindle poles. The poles are interconnected by continuous fibres. The elongation or shortening of the continuous fibres causes the pole-to-pole distance to change, and by means of the chromosomal fibres these changes are transmitted to the chromosomes. In addition, the chromosomal fibres themselves change their length and thus generate forces which cause the chromosomes to move. Dietz (1963, 1966, 1969) has developed this model: the mitotic apparatus is a composite structure, its unit is

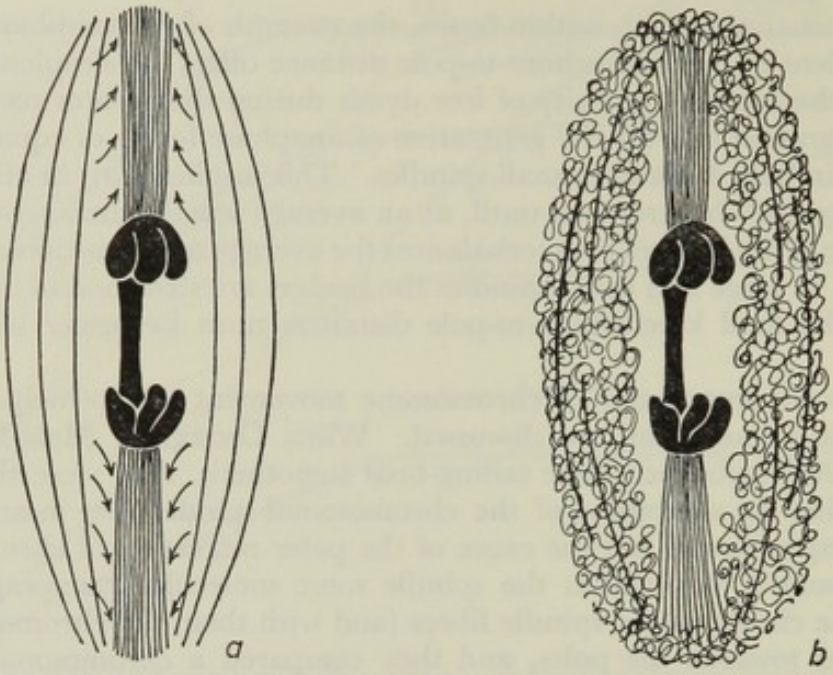


FIG. 9.—Hypothetical models of spindle organisation and anaphasic chromosome transport. *a*, The whole chromosomal fibre is supposed to be shifted towards the poles, *i.e.* in any time interval during anaphase the kinetochore and all points along its chromosomal fibre move the same distance. The shortening of the fibre is a consequence of its displacement, but does not cause it. A variety of forces and mechanisms have been suggested, to explain the generation of the assumed forces. *b*, The pole ends of the chromosomal fibres are supposed to be firmly anchored in the unoriented or only partially oriented peripheral spindle gel. In anaphase the chromosomal fibres and their elements are supposed to shorten by depolymerisation; the pole approach of the chromosomes is assumed to be caused by the shortening of the chromosomal fibres, which is brought about by the energy liberated by depolymerisation. The peripheral spindle gel counteracts the tension of the chromosomal fibres. Changes in its length add to the forces generated by the chromosomal fibres. The finding that all changes of spindle length cause the length of the bridge to change in the same way and nearly the same degree can be expected on the basis of the model pictured in *b*; on the basis of the model pictured in *a*, the additional assumption has to be made that changes in spindle length are caused (almost) exclusively by changes in the interzonal region. The finding that the strength of the force acting on a kinetochore is independent of the pole-distance of the kinetochore is comprehensible on the basis of the model pictured in *b*, because the force is likely to be a function of the diameter of the chromosomal fibre which may vary independently from the spindle length. On the other hand, this finding is a strong argument against the spindle model pictured in *a*, because the anaphase force is likely to increase with increasing length of the chromosomal fibre, an expectation which has been disproven.

the chromosomal spindle. A bipolar chromosomal spindle consists of two antagonistic, birefringent chromosomal fibres in the central axis, surrounded by a weakly birefringent or even isotropic peripheral spindle gel. In the pole regions these two elements of the chromosomal spindle are firmly connected. The whole peripheral spindle gel, *i.e.* the isotropic fraction of the spindle apparatus plus the microtubules which are inserted in this fraction (and which presumably are responsible for the appearance of continuous fibres in light microscopy), determine the pole-to-pole distance and counteract the tension exerted by the chromosomal spindle fibres (fig. 9, *b*). A hypothesis of chromosome movement based on this spindle model has been suggested (Dietz, 1969). During anaphase the spindle becomes progressively hydrated until it finally dissolves. The more or less isotropic peripheral spindle gel

and the anisotropic chromosomal spindle fibres react in a different manner. Partial hydration of the building blocks of the isotropic peripheral spindle gel is believed to mean a decrease of free energy ($-\Delta G$). Therefore, within any time interval a certain number of intermolecular bindings will be opened by hydration, and a smaller number will be reformed. The opening and the reformation of bindings happen at random. Because of this the concentration of bindings decreases (almost) equally in all parts of the peripheral spindle gel. Generally it takes a long time for a building block to diffuse away. Because whenever a number of bindings have been opened, other bindings will have reformed; at any time most building blocks will be attached to others, only the number of bindings decreases randomly. The more bindings are opened, the more the gel will swell. Finally, in late anaphase, it disintegrates into larger pieces and loses its rigidity.

On the other hand, a progressive hydration of the building blocks of isometric microtubules is believed to mean an increase of free energy ($+\Delta G$). The reason is unknown but a possible explanation is that when partially hydrated, the building blocks are stil held in the highly ordered lattice and do not gain enough entropy to compensate for the increase of enthalpy due to the hydration of bindings. Therefore, a tubule consisting of many partially hydrated building blocks is unstable. This situation is instantly removed by the complete hydration of a few building blocks, a process which is made possible by the simultaneous reformation of a compensating number of bindings between the other partially hydrated building blocks. Holding a minimum number of building blocks highly ordered and tightly linked, the system gains a maximum of free energy, because it allows a small but maximum number of building blocks to gain full entropy by going into solution. The ability of the dissolved protein to reform microtubules by a self assembly process (Stephens, 1969) may depend on the very same properties of the system. Whenever a building block leaves a chromosomal fibre, or one of its presumed elements, a microtubule, it is instantly replaced by one of its neighbours. The more material goes into solution the more the chromosomal spindle fibre shortens. It shortens without obvious changes of diameter or birefringence and until it has vanished completely. The strength of the anaphase force depends on the number of additive reaction sites, which is approximately equivalent to the diameter of the chromosomal fibre, or the number of microtubules within the bundle that constitutes the birefringent fibre. The rate of force generation depends on that of the hydratation of the spindle.

Conditions are different in microtubules which cannot shorten. The building blocks in these microtubules cannot reform a compensating number of bindings and, therefore, they cannot allow other building blocks of the same tubule to be totally hydrated and diffuse away. This applies to the microtubules in the peripheral spindle gel and to those of (at least) one chromosomal fibre of each amphitelically oriented chromosome. Under these circumstances, the microtubules initially exert strain; they just tend to shorten. The strain is demonstrated by the deformation of sex univalents and by the fact that bridges are held in maximal extension. Furthermore, the strain may well be responsible for the shortening of the spindle in early anaphase. Later on, a certain fraction of the microtubules which has become highly unstable will disintegrate. Here again, maximum free energy is gained when a certain number of other microtubules is partially or fully reorganised, *i.e.* when supplied with a maximum of reformed bindings. This

allows a maximum number of building blocks from other microtubules to be totally hydrated and to gain full entropy. A high number of disintegrating microtubules may actually force a small number of others to grow in length (Dietz, 1969 and in press).

The main motivation of this hypothesis is derived from the fact that during anaphase interactions exist between all chromosomes, especially between those which are amphioriented. These interactions can uniformly be explained on the assumption that the rate of dissolution of a chromosomal fibre is reduced when, owing to an earlier or more vigorous dissolution of a neighbouring chromosomal fibre, the concentration of dissolved building blocks in its surroundings is high. Motivated by studies of the spindle birefringence, Inoué and Sato (1967) also came to the conclusion that the anaphase movement of chromosomes is caused by the dissolution of the spindle apparatus.

A study of the behaviour of anaphase bridges provides an opportunity for testing hypotheses concerning spindle structure and chromosome movement. In so doing, it can be taken for granted that in *Pales ferruginea* the birefringent chromosomal fibres extend all the way from the kinetochore to the pole (Dietz, 1963).

It has been proved that when the spindle length increases or decreases by a certain amount, the length of the bridge is caused to change by (almost) the same amount. Knowing from the micromanipulation studies of Nicklas and Staehly (1967) that (with the exception of late anaphase) there is little or no increase of the kinetochore-to-pole distance when a chromosome is stretched with a microneedle, the behaviour of the bridge is that expected on the basis of the spindle model pictured in fig. 9, *b*. The spindle model pictured in fig. 9, *a* demands the auxiliary assumption that almost all the changes in spindle length are limited to the interzonal region.

It has been proved that the average maximal length of the anaphase bridges is the same in long and in small spindles, *i.e.* in both the correspondingly long and short kinetochore-to-pole distances of the bridges shorten by about the same absolute length during anaphase. On the basis of the spindle model pictured in fig. 9, *a* it should be expected that the strength of the anaphase force is a function of the length of the chromosomal fibre, and the number of its microtubular elements (which will be approximately proportional to its diameter), because the strength depends on the size of the interacting surfaces. On the basis of the spindle model pictured in fig. 9, *b* the strength of the anaphase force is expected to be a function of the diameter of the chromosomal fibre alone. Provided that in the first spermatocyte divisions of *Pales ferruginea* the diameter of the chromosomal fibre varies independently of its length—and preliminary studies with the polarising microscope suggest this—only model 9, *b* agrees with the data; because in spindles of all sizes the chromosomal fibres will generate an average anaphase force, and therefore stretch the bridges to an average length. On the basis of the model pictured in fig. 9, *a* the length increment of the bridges should increase with the length of their chromosomal fibres. That this is not the case, that all bridges are stretched to the same average degree, can only be explained when the highly improbable assumption is made that the anaphase diameter of a chromosomal fibre is negatively correlated with its metaphase length. Polarisation microscope studies are planned which ought to give a definite answer.

Summary

1. Chromosome movement at first meiotic anaphase has been studied in 32 cells of an X-ray induced mutant of *Pales ferruginea* ($2n = 6+XY$) heterozygous for an autosomal paracentric inversion.

2. Free half-bivalents approach the poles at nearly constant velocity until the kinetochore-to-pole distance is reduced to 1-4 μ.

3. The anaphase movement of dicentric bridge kinetochores generally stops or is retarded at much greater distances from the poles but, prior to this, their velocity is positively correlated with that of the free-half-bivalents.

4. The anaphase length increase of chromatid bridges is greater:

(*a*) the lower the degree of metaphase chromosome contraction (*i.e.* the longer the intercentric distance at metaphase),

(*b*) the longer the length increase of the spindle, and

(*c*) the faster the free dyads approach the poles.

5. The polar approach of the bridge kinetochores ceases when the bridges have reached the lengths expected on the basis of the above correlations irrespective of the kinetochore-to-pole distance at that time. The residual distances differ considerably, depending on spindle length at the beginning of anaphase.

6. Excepting distances smaller than 4 μ, the strength of the anaphase force is independent of the kinetochore-to-pole distance. In the case of crane-fly spermatocytes, at least, this means that within the range mentioned the anaphase force is independent of the length of the chromosomal fibre. This finding argues strongly against certain hypotheses of chromosome movement, and is conceivable on the basis of others.

Acknowledgments.—The capable help of Miss G. Müller and Miss B. Hornung is gratefully acknowledged.

References

BAJER, A. 1968. Behavior and fine structure of spindle fibers during mitosis in endosperm. *Chromosoma, Berl., 25*, 249-281.

DIETZ, R. 1963. Polarisationsmikroskopische Befunde zur chromosomeninduzierten Spindelbildung bei der Tipulide Pales crocata (Nematocera). *Zool. Anz., Leipzig, Suppl., 26*, 131-138.

DIETZ, R. 1966. The dispensability of the centrioles in the spermatocyte divisions of *Pales ferruginea* (Nematocera). *Chromosomes Today I, Suppl. Heredity, Edinb., 19*, 161-166.

DIETZ, R. 1969. Bau und Funktion des Spindelapparats. *Naturwissenschaften, Berl., 56*, 237-248.

HEPLER, P. K., MCINTOSH, J. R., AND CLELAND, S. 1970. Intermicrotubule bridges in mitotic spindle apparatus. *J. Cell Biol., N.Y., 45*, 438-444.

INOUÉ, S., AND SATO, H. 1967. Cell motility by labile association of molecules. The nature of mitotic spindle fibers and their role in chromosome movement. *J. gen. Physiol., N.Y., 50*, Part 2, 259-292.

MCINTOSH, J. R., HEPLER, P. K., AND WIE, D. G. VAN. 1969. Model for mitosis. *Nature, Lond., 224*, 659-663.

NICKLAS, R. B., AND STAEHLY, C. A. 1967. Chromosome micromanipulation. I. The mechanics of chromosome attachment to the spindle. *Chromosoma, Berl., 21*, 1-16.

ÖSTERGREN, G., MOLÈ-BAJER, J., AND BAJER, A. 1960. An interpretation of transport phenomena at mitosis. *Ann. N.Y. Acad. Sci., N.Y., 90*, 381-408.

STEPHENS, R. E. 1969. Factors influencing the polymerization of outer fibre microtubule protein. *Quart. Rev. Biophys., Lond., 1*, 377-390.

SUBIRANA, J. A. 1968. Role of spindle microtubules in mitosis. *J. theoret. Biol., Lond., 20*, 117-123.

MODE OF ACTION OF THE PAIRING-LIMITATION GENE IN WHEAT (*TRITICUM AESTIVUM*, 6x)

C. J. DRISCOLL, N. L. DARVEY and H. NEWTON BARBER
School of Botany, University of New South Wales

1. Introduction

Hexaploid wheat offers unique opportunities for studying the mechanism of chromosome pairing because of the presence of homoeologous as well as homologous chromosomes, the ready manipulation of a major gene or group of genes that affects pairing and the availability of many aneuploid lines that can be used in these investigations.

The relationships between the chromosomes of hexaploid wheat have been determined by Sears (1965). Each of the seven chromosomes of each genome has a related or homoeologous chromosome in each of the other two genomes. Despite close genetic relationships between homoeologues, as evidenced by compensations in pertinent nullisomic-tetrasomic lines (Sears, *loc. cit.*), pairing at meiosis is normally limited to true homologues.

That this limitation is under specific genetic control was detected by Sears and Okamoto (1958) and Riley and Chapman (1958). This control which is principally determined by a gene(s) on the long arm of chromosome 5B has been extensively analysed by Riley and co-workers (Riley, 1960*a*; Riley and Kempanna, 1963). In the absence of $5B^L$ homoeologues as well as homologues pair.

Considerable impetus to the study of the mode of action of the $5B^L$ gene(s) was given by Feldman's (1966) study of the effect of increased dosage of $5B^L$, when, despite a considerable reduction in total pairing, he showed that homoeologues as well as homologues can pair. Interlocking of bivalents was also observed under these conditions.

Feldman postulated that chromosomes enter into a " premeiotic association " as a prerequisite to synapsis and it is this earlier stage that is affected by the dosage of the $5B^L$ gene(s). Further, it was postulated that " premeiotic association " is principally an extension of a more general association referred to as somatic association, present in all cells. He obtained evidence for somatic association from observations on the distribution of marked chromosomes at mitotic metaphase in root tip cells. Homologous telocentric chromosomes were found to be closer to each other than non-homologues, the latter conforming to one model of a random distribution (Feldman, Mello-Sampayo and Sears, 1966).

Our observations provide evidence for a pre-synaptic association of chromosomes. However, we find no evidence for somatic association in root tip tissue. It is also believed that $5B^L$ affects processes after the chromosomes are associated.

2. Materials and methods

The various aneuploids used in these investigations were of the wheat variety Chinese Spring.

Colchicine was applied to plants by injecting approximately 0·25 ml. of

an aqueous solution into the uppermost leaf sheath. Concentrations of colchicine successfully used have ranged from 0·03 per cent. to 2·5 per cent. Spikes were fixed from two to seven days later in Carnoy's 6 : 3 : 1 and anthers stained in aceto-carmine.

For observations on the number of nucleoli per nucleus, root tips were prepared according to the method of Rattenbury (1952), *i.e.* after fixation in 6 formalin : 13 alcohol (95 per cent.) : 1 acetic acid (glacial), they were hydrolysed for 2 hours in IN HCl at 60° C. and squashed in aceto-carmine.

Volumes of nucleoli and nuclei were obtained from fresh material, sectioned without freezing, unsquashed and examined by interference microscopy.

Studies of distances between chromosomes at mitotic metaphase were carried out by the method used by Feldman, Mello-Sampayo and Sears (1966). Root tips were pretreated in 0° C. water, fixed in acetic acid for 2-3 hours, hydrolysed in IN HCl for 12 min., stained with Feulgen and mounted in aceto-carmine. Distances between marked chromosomes and between the two furthest chromosomes in squashed metaphase cells were measured. Cumulative frequencies of distances, converted to the unit circle, were used in comparisons between sets of observations and between each set of observations and the theoretical distribution of the cumulative frequency of distances between random points in a circle.

3. Results and discussion

Two of the cytological abnormalities observed by Feldman (1966) with excess $5B^L$, viz. asynapsis and interlocking of bivalents, had been observed by Barber (1942) in *Fritillaria meleagris* following treatment with colchicine before meiosis. Homoeologous pairing could not have occurred in this material, *F. meleagris* being a diploid. Following application of colchicine to hexaploid wheat, Driscoll, Darvey and Barber (1967) and Driscoll (1968), reported induction of marked asynapsis and homoeologous pairing. Interlocking of bivalents is not observed in this material and it now appears that the rare multivalents previously reported may in fact only involve stickiness between chromosomes in highly asynaptic cells. Thus the only definite similarity between excess $5B^L$ and colchicine application in hexaploid wheat is the occurrence of univalents.

Conclusive evidence that colchicine affects association of related chromosomes and not synapsis and chiasma formation *per se* was provided by Driscoll and Darvey (1970) following observations on the effect of colchicine on hexaploid wheat monoisosomic for one of the 21 chromosomes. In this case chiasma formation in the isochromosome was not affected whereas chiasma formation in the conventional pairs of chromosomes was greatly reduced. The immunity of the isochromosome to colchicine stems from the fact that the two homologous arms of an isochromosome, being attached to a common centromere are always close together, especially in regions adjacent to the centromere. Thus colchicine does not affect synapsis and chiasma formation if homologous chromosome arms are held close together. This conclusion agrees with the observation of Driscoll, Darvey and Barber (1967), that cells undergoing the last premeiotic mitosis at the time of colchicine treatment form up to 42 bivalents if treated before anaphase.

Thus association of homologues is a separable stage in the overall process

of chromosome pairing. If homologues do not actively come into close proximity to each other the synaptic attraction forces, which operate later in the process of chromosome pairing, are ineffective.

Our data indicate that this association of homologous chromosomes is unique to meiosis. It does not occur in all somatic tissue. This is contrary to previously published data which has supported the concept of somatic association. Our data, the full details of which will be found elsewhere (Darvey and Driscoll, unpublished), involve two different types of observations. The first involves fusion of nucleoli and the second, distributions of marked chromosomes at mitotic metaphase.

Hexaploid wheat possesses two pairs of major nucleolar organising regions (N.O.Rs) located on the obviously satellited short arms of chromosome pairs 1B and 6B. Other chromosomes, for example, 5D, infrequently produce smaller nucleoli; these, however, will be ignored in this paper.

At late mitotic anaphase four developing nucleoli are invariably formed in each daughter nucleus. At interphase, however, nuclei possess either one, two, three or four nucleoli. The occurrences of less than four nucleoli result from fusions of developing nucleoli. Chromosomes undergo little movement in the nucleus during mitotic interphase (Evans and Bigger, 1961; Darvey and Driscoll, unpublished). Thus, instances of fusion of nucleoli must be the result of chance close proximity of developing nucleoli.

Direct evidence for this was obtained by observing the amount of nucleolar fusion in monoisosomic $6B^s$ compared with ditelosomic $6B^s$. Both stocks possess a maximum of four nucleoli. However, in the former, two of the nucleolar organising arms are connected to the same centromere, thus keeping the N.O.Rs together. The amount of fusion in the monoisosomic was significantly greater than in the ditelosomic. (Distributions of numbers of nucleoli per nucleus significantly different, $P < 0{\cdot}001$; mean number of nucleoli : monoisosomic $6B^s = 1{\cdot}73$; ditelosomic $6B^s = 1{\cdot}94$.) This was more obvious in the comparison of the amount of fusion in diiso $6B^s$ and nullisomic 1D tetrasomic 1B, each of which possesses six N.O.Rs. (Distributions of numbers of nucleoli per nucleus significantly different, $P < 0{\cdot}001$; mean number of nucleoli : diisosomic $6B^s = 1{\cdot}77$; nullisomic 1D tetrasomic $1B = 2{\cdot}12$.)

These results confirm the absence of extensive movement of interphase chromosomes. Instances of fusion of nucleoli demonstrate an association of N.O.Rs. Such association need not be intimate, but could be up to twice the radius of a nucleolus. Such association of N.O.Rs does not imply association of the nucleolus organising chromosomes throughout their entire length.

Individuals deficient for an homologous pair of nucleolus organising arms, viz. ditelocentric long arm 1B and ditelocentric long arm 6B, have either one or two nucleoli per interphase nucleus. The frequency of nuclei with one nucleolus was 0·59 in both of these stocks. The fact that nucleoli organised on two homologous N.O.Rs only fuse in 59 per cent. of nuclei is evidence for the fact that homologous N.O.Rs are not intimately associated. Intimate association would result in fusion frequencies approaching 100 per cent.

The intercross of ditelocentric $1B^L \times$ ditelocentric $6B^L$ also possesses two nucleoli. However, they are organised by non-homologous chromosomes. The degree of fusion in this hybrid, 57 per cent., is not statistically different

from that observed in its parents and clearly demonstrates that non-homologous N.O.Rs lie on the average as close to each other as homologous N.O.Rs.

Thus we conclude that there is no somatic association of homologous N.O.Rs in somatic interphase nuclei.

Observations, such as these, at interphase are most valuable as it is at this stage that the proponents of somatic association claim strongest association.

It was of interest to determine whether or not the amount of fusion observed conformed with that expected on the basis of randomness of positions of N.O.Rs in the nucleus. The model used for determining expected frequencies of fusion was as follows: nucleoli originate as points and enlarge into spheres; these points do not move unless they are located at a distance less than the final radius of a nucleolus from the nuclear membrane, at which time they will move along a radius of the nucleus until at a distance of the radius of a nucleolus from the nuclear membrane; two nucleoli will fuse if the final positions of their points of origin are equal to or less than twice the radius of a nucleolus. This complex geometrical probability problem was solved by Professor A. M. Hasofer, Department of Statistics, of this University, who determined the expected frequency of fusion of two nucleoli for various ratios of radius of a nucleolus to radius of nucleus (Hasofer, unpublished).

The actual ratios of the radii in ditelocentric $1B^L$, ditelocentric $6B^L$ and the intercross were determined by interference microscopy on sections of living material, mounted in solutions of various osmotic pressures. These data were homogeneous, the ratios being between 0·36 and 0·37. The expected amount of fusion on the basis of randomness is 37-40 per cent. which is significantly less than the observed frequency of 57-59 per cent.

This excess in the observed frequency of fusion is due to the fact that N.O.Rs do lie closer than random in the nucleus because of the anaphase orientation of mitotic chromosomes, accentuated in this case because all N.O.Rs are at about the same distance from their centromeres.

Thus the conclusions drawn from the studies on nucleoli are firstly, more fusion of nucleoli occurs because N.O.Rs lie closer to each other than at random, and secondly, this proximity does not have homology as its basis, since non-homologous N.O.Rs lie as close to each other as homologous N.O.Rs; the proximity is a non-specific legacy of orientation of anaphase chromosomes. These observations negate the concept of somatic association in hexaploid wheat.

Our second search for evidence for somatic association involved using the method of Feldman, Mello-Sampayo and Sears (1966).

In one set of observations on the distances between two $2A^S$ telocentrics and telocentrics $7B^S$ and $2A^S$, special precautions were taken to eliminate personal errors. In these cases independent observations were made on each cell by three observers after the slides had been coded by a fourth person. After each cell had been measured independently by the observers, measurements were compared and only cells in which agreement existed or could by further observation and argument be reached were retained. Rejected cells included those which possessed less than 42 chromosomes and those in which there was doubt regarding the recognition of the two telocentrics. Non-circular arrangements were not excluded. Unanimous agreement by the three observers was necessary for retention of measurements. The

rejection rate was 64·3 per cent. After decoding it was found that there were 72 measurements of distances between homologous telocentrics and 64 measurements of distances between non-homologous telocentrics. From the Kolmogorov-Smirnov two-sample, two-tail test, no significant difference was found between these two samples. Also, neither sample agreed with the distribution of distances between two random points in a circle, both pairs of telocentrics being closer than expected on *that* theoretical model. The mean distance between the homologous pairs was 0·33 and the mean distance between the non-homologous pairs was 0·30.

In order to check whether the high percentage of rejection was introducing any bias in the measurements, all measurements made on the rejected cells whether by one, two or three observers, were analysed separately. The results are in general agreement with those from the accepted cells.

From these observations we conclude that homologous telocentrics do not lie closer to each other than non-homologous telocentrics and that both pairs lie closer to each other than expected on the basis of the particular theoretical distribution used.

Observations made on a number of other pairs of telocentric chromosomes lead to the same conclusion.

In stocks involving aneuploidy for the satellited chromosomes, measurements were made of the distances between homologous N.O.Rs and non-homologous N.O.Rs. Again no significant differences exist between these distances (Darvey and Driscoll, unpublished).

Mention should also be made of the theoretical distribution used in this type of analysis. Our data which are being prepared for detailed publication, involve a number of means all of which are less than the mean distance between random points of a circle. If random points in a circle were the correct model approximately half the number of means would be expected to be greater because of non-significant chance fluctuation. Thus this may be the wrong theoretical model. Perhaps cold water treatment results in the condensed chromosomes being arranged randomly in a sphere which is squashed to an oblate sphere or disc. This distortion could result in a mean distance less than the mean distance of random points in a circle. This latter point has not, however, been examined mathematically.

The conclusions from our mitotic metaphase observations are firstly, homologous chromosomes lie no closer to each other than non-homologous chromosomes, and secondly, the mean distance between any two chromosomes in a squashed metaphase cell is closer than the mean distance between two random points in a circle.

Thus, both types of observations on root tip tissue indicate homologous chromosomes are not somatically associated in hexaploid wheat. Thus the pre-synaptic association of chromosomes is peculiar to meiosis. Recent observations on numbers of nucleoli at various stages between the pre-meiotic interphase and pachytene indicate that chromosome association takes place just prior to synapsis (Darvey and Driscoll, unpublished).

As to whether the $5B^L$ gene(s) affect the process of chromosome association is open to some doubt. The effects of colchicine, which definitely affects chromosome association, and the effects of excess dosage of $5B^L$ are different in a number of details. With colchicine there is a greater ratio of ring : rod bivalents, and multivalents are rare or absent. Thus it appears likely that association of chromosomes, which *a priori* is less precise than detailed syn-

apsis, involves homoeologues and homologues such that seven groups of six chromosomes form prior to initiation of synapisis. The dosage of $5B^L$ may then determine which chromosomes will proceed into zygotene. This postulated mechanism is similar to that proposed by Riley (1960*b*); the difference being that the close spatial proximity of the six homoeologues comes about prior to synapsis. This mechanism is compatible with secondary association of bivalents, as demonstrated by Riley (*loc. cit.*) and Kempanna and Riley (1964). This indicates that the $5B^L$ gene(s) act on the chromosomes themselves rather than say on microtubules by which chromosomes approach each other. Different dosages of $5B^L$ could determine whether homoeologues, homologues or neither have sufficient recognition points available for initiation of synapsis.

Pairing of chromosomes at meiosis may involve three phases:

(i) association of homologues and homoeologues prior to detailed synapsis. This process is inhibited by colchicine;

(ii) recognition of true homologues which is thought to involve monitoring of initiation points by $5B^L$;

(iii) completion of synapsis.

4. Summary

1. Synapsis of chromosomes at meiosis is dependent upon earlier association of potential partners. This earlier process of association is suppressed by colchicine; the later processes are immune to colchicine.

2. Our observations indicate an absence of somatic association of homologous chromosomes in hexaploid wheat. This conclusion is reached from

(*a*) homologous nucleolar organising regions are no closer to each other than non-homologous nucleolar organising regions at mitotic interphase,

(*b*) homologous telocentrics are no closer to each other than non-homologous telocentrics at mitotic metaphase. Similarly homologous N.O.Rs are no closer to each other than non-homologous N.O.Rs. Thus association of chromosomes in this species appears to be peculiar to meiosis.

3. The pairing-limitation gene(s) on chromosome $5B^L$ appear to act after the association of the six homoeologues is completed by determining which of the associated chromosomes can proceed into synapsis.

Acknowledgments.—These investigations were supported by the Wheat Industry Research Council, Rural Credits Development Fund of the Reserve Bank, and Australian Research Grants Committee.

5. References

BARBER, H. N. 1942. The experimental control of chromosome pairing in *Fritillaria*. *J. of Genetics*, *43*, 359-374.

DRISCOLL, C. J. 1968. Alien transfer by irradiation and meiotic control. *Proc. 3rd Int. Wheat Genet. Symp. Canberra*, Aust. Acad, Sci., Canberra, 196-203.

DRISCOLL, C. J., AND DARVEY, N. L. 1970. Chromosome pairing: effect of colchicine on an isochromosome. *Science*, *169*, 290-291.

DRISCOLL, C. J., DARVEY, N. L., AND BARBER, H. N. 1967. Effect of colchicine on meiosis of hexaploid wheat. *Nature*, *216*, 687-688.

EVANS, H., AND BIGGER, T. R. L. 1961. Chromatid aberrations induced by gamma irradiation. II. Non randomness in the distribution of chromatid aberrations in relation to chromosome length in Vicia faba root-tip cells. *Genetics, 46,* 277-289.

FELDMAN, M. 1966. The effect of chromosome 5B, 5D, and 5A on chromosomal pairing in *Triticum aestivum. Proc. Nat. Acad. Sci. U.S., 55,* 1447-1453.

FELDMAN, M., MELLO-SAMPAYO, T., AND SEARS, E. R. 1966. Somatic association in *Triticum aestivum. Proc. Nat. Acad. Sci. U.S., 56,* 1192-1199.

KEMPANNA, C., AND RILEY, R. 1964. Secondary association between genetically equivalent bivalents. *Heredity, 19,* 289-299.

RATTENBURY, J. A. 1952. Specific staining of nucleolar substance with aceto-carmine. *Stain Technology, 27,* 113.

RILEY, R. 1960*a*. The diploidisation of polyploid wheat. *Heredity, 15,* 407-429.

RILEY, R. 1960*b*. The secondary pairing of bivalents with genetically similar chromosomes. *Nature, 185,* 751-752.

RILEY, R., AND CHAPMAN, V. 1958. Genetic control of the cytologically diploid behaviour of hexaploid wheat. *Nature, 182,* 713-715.

RILEY, R., AND KEMPANNA, C. 1963. The homoeologous nature of the non-homologous meiotic pairing in *Triticum aestivum* deficient for chromosome V(5B). *Heredity, 18,* 287-306.

SEARS, E. R. 1965. Nullisomic-tetrasomic combinations in hexaploid wheat. In *Chromosome Manipulations and Plant Genetics* (eds. R. Riley and K. R. Lewis). Oliver and Boyd, Edinburgh.

SEARS, E. R., AND OKAMOTO, M. 1958. Intergenomic chromosome relationships in hexaploid wheat. *Proc. Xth Int. Cong. Genet. Montreal, 2,* 258-259.

GENETIC CONTROL OF THE POST-SYNAPTIC PHASE OF MEIOSIS IN RYEGRASS

B. S. AHLOOWALIA
Agricultural Institute, Oak Park Research Centre, Carlow, Eire

1. Introduction

The study of spontaneous and induced mutants, which are aberrant in one or more cytologically recognisable stages of meiosis has shown that the various stages of meiosis in the eukaryotes are under genetic control. Asynaptic mutants, in which homologous chromosomes fail to pair, have been reported in *Zea* (Beadle, 1930; Miller, 1963), *Sorghum* (Ross *et al.*, 1960; Pritchard, 1965), and *Triticum* (Martini and Bozzini, 1966). Desynaptic mutants, in which homologous chromosomes pair but separate precociously have been described in *Matthiola* (Mann-Lesley and Frost, 1927), *Secale* (Prakken, 1943), *Triticum* (Li, Pao and Li, 1945), *Lycopersicon* (Soost, 1951; Moens, 1969), *Hordeum* (Enns and Larter, 1960), *Oryza* (Wang *et al.*, 1965), *Pisum* (Gottschalk and Jahn, 1964; Gottschalk and Villalobos-Pietrini, 1965), *Avena* (Thomas and Rajhathy, 1966), and *Pennisetum* (Jauhar and Singh, 1970) (see Riley and Law, 1965 for more examples). Some of the reported mutants are equivocally desynaptic since pachytene analysis was not possible. In others, a genetic control was presumed without any experimental evidence. The asynaptic mutant of maize (Miller, *loc. cit.*) and desynaptic mutants of tomato (Moens, *loc. cit.*) were shown to be determined by single recessive genes designated *as* and *ds* respectively.

In a previous report (Ahloowalia, 1969*a*), a desynaptic mutant was described in which chromosomes paired at zygotene but separated precociously so that some or all homologous chromosomes were present as univalents at first metaphase. Further, this mutant was shown to be thermosensitive since a relatively higher temperature increased desynapsis (Ahloowalia, 1969*b*). This report presents evidence on the genetic control of the desynaptic phenotype as studied in the selfed, F_1 and F_2 progenies.

2. Materials and methods

Desynapsis was found in a mixoploid ($2n = 14$ and 28) plant produced by colchicine treatment (Ahloowalia, 1967). The plant was repeatedly cloned till pure diploid and tetraploid sectors were isolated. The selfed progeny was obtained by controlled crossing of diploid clones. The F_1 was the progeny of a single diploid desynaptic clone open-pollinated with normal diploids. Two F_1 plants were crossed to give F_2 progeny. The florets were not emasculated in any of these crossings since perennial ryegrass is highly self-incompatible.

All plants were grown in a glasshouse, where temperatures fluctuated widely. The dissected spikes were fixed in Carnoy 6 : 3 : 1 mordanted with a saturated solution of ferric chloride in 45 per cent. acetic acid. The pollen mother cells (*p.m.c.*'s) were examined after staining with aceto-carmine as described previously (Ahloowalia, 1968). In most plants, a minimum of 25 *p.m.c.*'s were scored for chiasma number at first metaphase.

3. Results

(i) *Inbred progeny*

The inbred seed showed about 50 per cent. germination. Of the 36 seedlings obtained, four were albino, eight were chlorotic and one was dwarf. The aberrant seedlings and two others did not survive.

All the 14 plants examined for chromosome number were diploid and desynaptic. The chromosome association at metaphase I varied from 0-7 bivalents per cell. Individual plants showed a wide variation in the degree of desynapsis as indicated by the mean bivalent number per cell (table 1).

Table 1

Number of bivalents, chiasmata, and fertility of the inbred progeny

Plant no.	*Mean no. of bivalents/ cell	S.E.	*Mean no. of chiasmata/ bivalent	Mean no. of chiasmata/ cell	S.E.	No. of seeds/ plant
1	6·48	0·14	1·90	12·28	0·39	38
14	6·20	0·21	1·95	12·08	0·48	68
26	6·00	0·22	1·78	10·68	0·56	64
11	5·88	0·25	1·46	8·60	0·35	52
10	5·76	0·22	1·98	11·40	0·53	13
12	5·52	0·36	1·62	8·96	0·67	3
29	5·24	0·29	1·57	8·24	0·58	46
2	5·24	0·29	1·33	6·96	0 41	14
33	4·24	0·43	1·77	7·52	0·81	—
25	3·68	0·36	1·46	5·36	0·61	4
6	3·48	0·33	1·43	4·96	0·52	2
9	3·28	0·34	1·24	4·08	0·51	10
32	2·60	0·34	1·26	3·28	0·50	1
21	1·32	0·21	1·03	1·36	0·25	—
Mean	4·63		1·63	7·55		26

* Based on 25 cells.

Table 2

Analysis of variance of bivalent number per cell between plants in the inbred progeny

Variance due to	D.F.	Sum of squares	Mean square	F
Between genotypes	13	785·40	60·415	27·60***
Within genotypes (error)	336	735·54	2·189	
Total	349	1520·94		

*** Exceeds the 0·1 per cent. level.

An analysis of variance (F test) revealed a highly significant ($P < 0{\cdot}001$) difference between plants in mean bivalent number (table 2). The cells with univalents increased with the degree of desynapsis (fig. 1). Similarly, the coefficient of variability (C.V.) within plants increased with an increase in desynapsis (fig. 2).

The number of chiasmata ranged from 0 to 17 per cell and decreased with a reduction in bivalent number (table 1). There was a highly significant ($P < 0{\cdot}001$) difference in chiasma number between plants (table 3) and coefficient of variability increased with an increase in desynapsis (fig. 2).

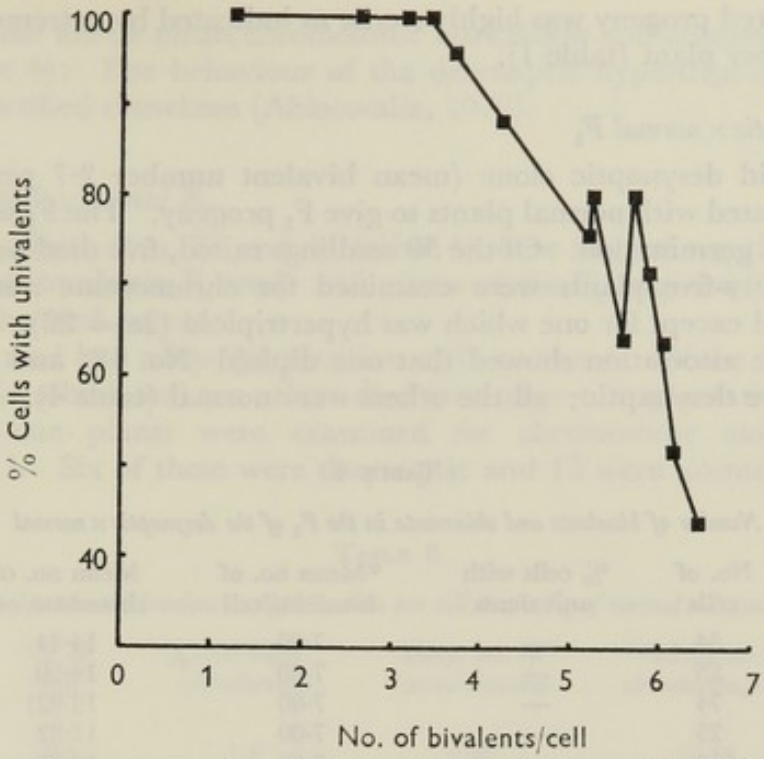


FIG. 1.—Inter-relation of cells with univalents and extent of desynapsis in the inbred progeny.

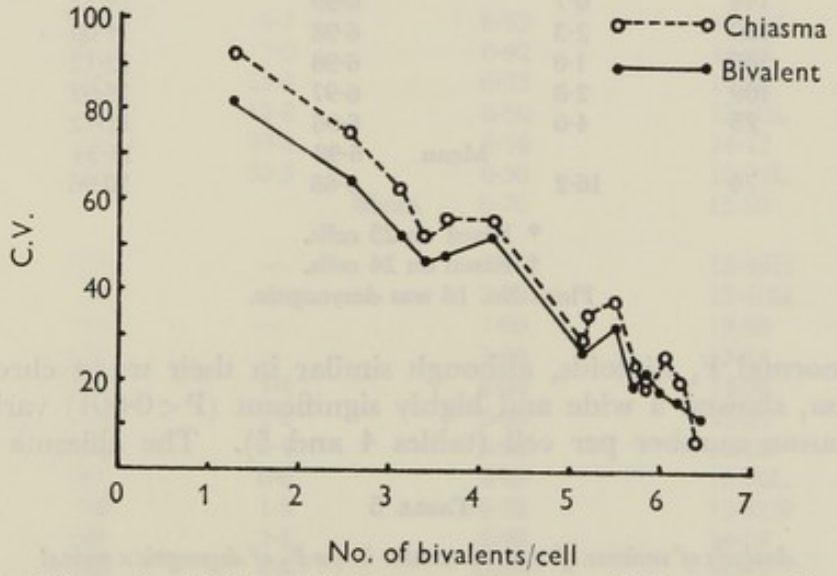


FIG. 2.—Coefficient of variability (C.V.) in mean bivalent and chiasma number per cell in the inbred progeny.

TABLE 3

Analysis of variance of chiasma number per cell between plants in the inbred progeny

Variance due to	D.F.	Sum of squares	Mean square	F
Between genotypes	13	3788·07	291·39	41·66***
Within genotypes (error)	336	2349·92	6·99	
Total	349	6137·99		

*** Exceeds the 0·1 per cent. level.

The inbred progeny was highly sterile as indicated by extreme reduction in seed set per plant (table 1).

(ii) *Desynaptic × normal F_1*

A diploid desynaptic clone (mean bivalent number 2·7 per cell) was open-pollinated with normal plants to give F_1 progeny. The F_1 seed showed 56 per cent. germination. Of the 50 seedlings raised, five died before maturity. Twenty-five plants were examined for chromosome number; all were diploid except for one which was hypertriploid ($2n = 22$). A study of chromosome association showed that one diploid (No. 18) and the hypertriploid were desynaptic; all the others were normal (table 4).

TABLE 4

Number of bivalents and chiasmata in the F_1 of the desynaptic × normal

Plant no.	No. of cells	% cells with univalents	*Mean no. of bivalents/cell	Mean no. of chiasmata/cell	S.E.
4	34	—	7·00	14·44	0·36
30	25	—	7·00	14·00	0·24
48	74	—	7·00	13·92†	0·28
28	25	—	7·00	13·32	0·36
2	112	—	7·00	13·28	0·36
38	113	—	7·00	12·88	0·32
42	25	—	7·00	12·80	0·33
40	144	0·7	6·99	14·32	0·24
10	130	2·3	6·98	14·00	0·31
27	108	1·8	6·98	12·12	0·35
44	109	2·8	6·97	13·92	0·27
11	25	4·0	6·96	11·12	0·20
		Mean	6·98	13·34	
18	74	16·2	6·68	10·96	0·50

* Based on 25 cells.
† Based on 24 cells.
Plant No. 18 was desynaptic.

The normal F_1 diploids, although similar in their mean chromosome association, showed a wide and highly significant ($P < 0·001$) variation in their chiasma number per cell (tables 4 and 5). The chiasma number

TABLE 5

Analysis of variance of chiasma number in the F_1 of desynaptic × normal

Variance due to	D.F.	Sum of squares	Mean square	F
Between genotypes	11	265·53	24·14	10·92***
Within genotypes	287	635·67	2·21	
Total	298	901·20		

*** Exceeds the 0·1 per cent. level.

ranged from 8 to 20 per cell, and in two plants, Nos. 27 and 11, was significantly lower ($P < 0·05$) and significantly higher ($P < 0·05$) than that of the mean for F_1. The female parent had a mean of 3·72 chiasmata per cell.

The single diploid desynaptic plant was produced, perhaps, by selfing rather than crossing, since the female parent was not emasculated. This

plant had the lowest mean chromosome association and chiasmata per cell in F_1 (table 4). The behaviour of the desynaptic hypertriploid has been already described elsewhere (Ahloowalia, 1970).

(iii) *Desynaptic × normal F_2*

Two F_1 plants Nos. 27 and 44 (table 4) were bagged together without emasculation to obtain F_2 seed. All plants of the F_2 originated from plant No. 27 as the seed parent.

The F_2 seed had a low germination of 75 per cent. Of the 75 seedlings obtained, six albino and ten others died soon after transplantation.

Twenty-one plants were examined for chromosome association at metaphase I. Six of these were desynaptic and 15 were normal (table 6).

TABLE 6

Mean number of bivalents and chiasmata per cell in the F_2 of normal × desynaptic

Plant no.	No. of cells	% cell with univalents	Mean no. of bivalents/cell	**Mean no. of chiasmata/cell	S.E.
F_1					
−27	108	1·8	6·98	12·12	0·35
−44	109	2·8	6·97	13·92	0·27
F_2					
desynaptic					
61	43	4·7	6·93	12·48L	0·38
42	114	7·0	6·92	14·28	0·36
51	85	15·3	6·82	14·28	0·63
64	86	12·8	6·80	12·68L	0·26
63	104	30·8	6·56	14·12	0·52
16	76	30·3	6·50	12·24L	0·36
		Mean	6·76	13·35	
Normal					
37	136	—	7·00	15·48H	0·31
46	54	—	7·00	15·40H	0·26
10	55	—	7·00	13·90	0·31*
39	100	—	7·00	13·24	0·19
24	140	1·4	6·99	14·56	0·34
60	75	1·3	6·99	13·84	0·42
65	141	1·4	6·99	13·12	0·33
12	126	0·8	6·99	12·76L	0·40
18	66	1·5	6·98	15·32H	0·31
3	142	2·1	6·98	14·08	0·35
43	113	1·8	6·98	13·16	0·32
58	146	3·4	6·97	12·80L	0·26
22	108	3·7	6·96	14·96	0·36
49	99	4·1	6·96	13·64	0·28
66	25	4·0	6·96	—	—
		Mean	6·98	14·07	
			LSD	0·999	

* Based on 10 cells.
** Based on 25 cells.
L = significantly lower than the mean and
H = significantly higher than the mean.

A comparison of the mean bivalent number of desynaptic and normals showed a highly significant difference ($P < 0·01$, $t = 4·9956$, $N = 19$) between the two. Thus, the F_2 segregated in a ratio of 3 : 1 for normals: desynaptics ($\chi_2 = 0·143$, $P = 0·9$-$0·8$).

The desynaptic and normal F_2 plants also differed in their chiasma number per cell (table 6). Analysis of variance showed a highly significant difference ($P < 0{\cdot}01$) between as well as within the desynaptic and the normal groups (table 7).

TABLE 7

Analysis of variance of chiasma number per cell between the normal and desynaptic plants in F_2

Variation source	D.F.	Sum of squares	Mean square	F
Normals *vs* desynaptics	1	47·60	47·60	14·66**
Residual within groups	17	426·88	25·11	7·73**
Within plants (error)	456	1480·84	3·25	—
Total	474	1955·32		

** Exceeds the 1 per cent. level.

The F_1 plants Nos. 27 and 44 had a relatively low $12{\cdot}12 \pm 0{\cdot}35$ and a high $13{\cdot}92 \pm 0{\cdot}27$ chiasma number per cell respectively. These two plants differed significantly ($P = 0{\cdot}01$, $t = 4{\cdot}05$, $N = 48$) in their mean chiasma number, although both had an equal number of bivalents per cell. High chiasma frequency was apparently a dominant character, since most of the F_2 plants had a chiasma number higher than the expected mean of the F_1 parents ($12{\cdot}12 + 13{\cdot}92/2 = 13{\cdot}02$). However, within both the normals and desynaptics, plants with a relatively low and relatively high chiasma number were present (fig. 3). Thus segregation for chiasma number in the

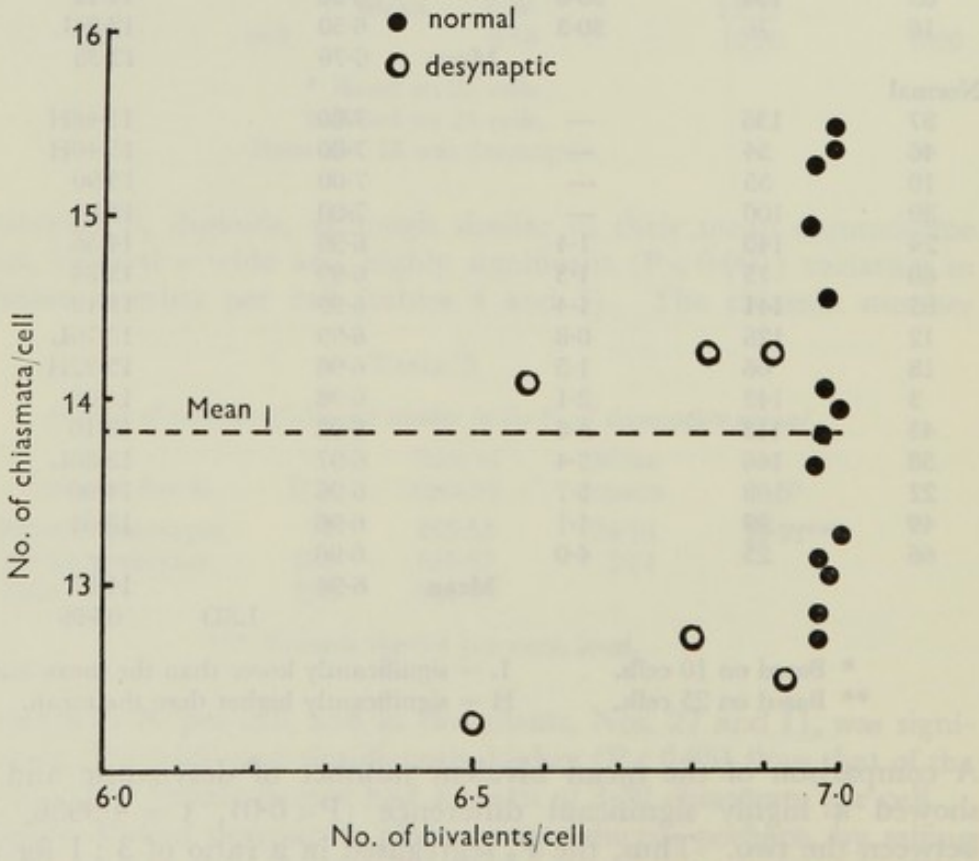


FIG. 3.—Segregation for chiasma number in the F_2 normal and desynaptic plants.

F_2 was transgressive when compared to that of the F_1 parents. The desynaptic F_2 plants not only showed a wider range in the number of chiasmata per cell but also a lower mean and mode than the F_1 and normal F_2 plants (fig. 4).

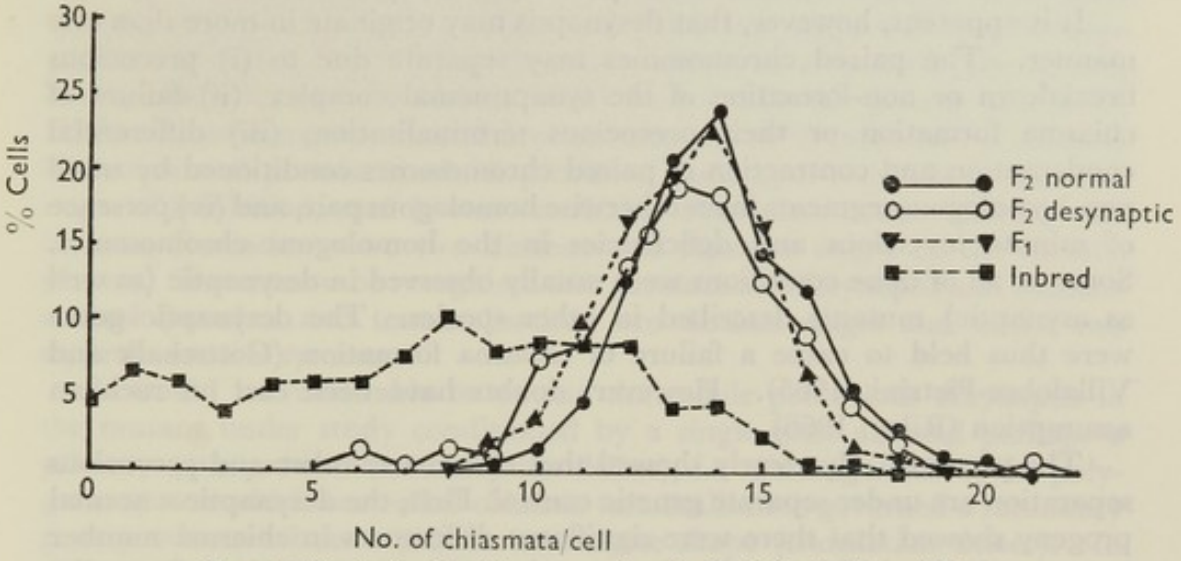


FIG. 4.—Variation in chiasma number in the inbred, F_1 and F_2.

The mean chromosome association and chiasma frequency of the selfed progeny closely approximated to that of the parental desynaptic clones grown under a variable environment (table 8). The desynaptic F_2 plants behaved like parental clones grown at 11° C. The F_1 and normal F_2 plants showed a similar chromosome behaviour (table 8).

TABLE 8

Mean number of bivalents and chiasmata in the parent, selfed progeny, F_1 and F_2

Plant type	Growth conditions	Mean no. of bivalents/cell		Mean no. of chiasmata/cell	
		N	*ds*	N	*ds*
1. Parental clones	variable	—	6·05	—	10·09
2. subclones	variable	—	4·84	—	6·58
3. subclones	11° C.	—	6·70	—	11·68
4. subclones	28° C.	—	5·39	—	7·41
5. Selfed progeny	variable	—	4·63	—	7·58
6. F_1 desynaptic × normal	variable	6·98	—	13·34	—
7. F_2 desynaptic × normal	variable	6·98	6·76	14·08	13·35

Phenotype = N normal, *ds* = desynaptic.
1 and 2 (from Ahloowalia, 1969*a*), 3 and 4 (from Ahloowalia, 1969*b*).

4. DISCUSSION

The study of the mutant on vegetative cloning, selfing, and crossing clearly established that the anomalous chromosome behaviour during the first meiotic division was genetically determined. Since chromosome pairing was normal at zygotene, and univalents were detectable from diplotene onwards, the mutant is *desynaptic*. If the surviving F_1 and F_2 plants represented a random sample, then it would appear that the desynaptic behaviour

was controlled by a single recessive gene, *ds*, as evidenced by nearly all normal F_1 and 3 : 1 segregation of normal to desynaptic plants in the F_2. Such a genetic control confirmed the previous speculation on the nature of desynapsis (Ahloowalia, 1969*a*) and agreed with the genetic control of similar mutants described in several other species (see Riley and Law, 1965).

It is apparent, however, that desynapsis may originate in more than one manner. The paired chromosomes may separate due to (i) precocious breakdown or non-formation of the synaptinemal complex, (ii) failure of chiasma formation or their precocious terminalisation, (iii) differential condensation and contraction of paired chromosomes conditioned by small non-homologous segments in an otherwise homologous pair, and (iv) presence of minute inversions and deficiencies in the homologous chromosomes. Some or all of these conditions were usually observed in desynaptic (as well as asynaptic) mutants described in other species. The desynaptic genes were thus held to cause a failure of chiasma formation (Gottschalk and Villalobos-Pietrini, 1965). However, doubts have been cast on such an assumption (Riley, 1966).

The present study clearly showed that chiasma number and precocious separation are under separate genetic control. First, the desynaptic × normal progeny showed that there were significant differences in chiasma number between plants which were alike in mean chromosome association. Secondly, when two such plants were crossed, the F_2 showed a transgressive segregation for chiasma number, implying a polygenic control, yet segregated for a major gene determining desynapsis. Thirdly, the F_2 showed that the desynaptic segregants could have either a relatively low or high chiasma number in spite of their similar bivalent number per cell. The most likely explanation of this kind of variation in chiasma frequency appeared to be an " independent " system of genetic control of chiasma frequency, polygenic in nature, superimposed upon a major gene determining chromosome desynapsis. Of course, the number of chiasmata, ultimately, depends on the bivalents, for a breakdown of all bivalents means absence of chiasmata. However, the semi-dependent variation of chiasmata number within cell populations with similar mean bivalent number suggests two different (perhaps closely linked) control systems. Since chiasma number was determined at metaphase I, when chiasmata have terminalised, the polygenic system, perhaps, governs chiasma terminalisation. The uncontrolled environment of the glasshouse might have further contributed to the variation in chiasma number, since a high temperature had been shown to increase desynapsis in this mutant (Ahloowalia, 1969*a*), and that the temperature fluctuations increased the rate of terminalisation of chiasmata.

The high rate of lethality, a reduced seed germination and occurrence of abnormal seedlings such as albinos and chlorotics in the inbred progeny and F_2 perhaps resulted from inbreeding since perennial ryegrass is normally cross-pollinated. Further, the wide variation of desynapsis in the selfed progeny might have been accentuated by inbreeding, since inbreeding in normal ryegrass has been shown to produce asynapsis and a reduction in mean chiasma number (Cooper and Thomas, 1961).

It is becoming increasingly clear that the various cytologically recognisable stages of meiosis are biochemically distinct and that phase specific changes take place in cell metabolism at the molecular level. A number of such changes have been shown to involve deoxyribonucleic acid and histones

of chromosomes in relation to the formation of the synaptinemal complex (Roth and Ito, 1967; Comings and Okada, 1970) and the progress of first meiotic prophase (Sheridan and Stern, 1967; Hotta, Ito and Stern, 1966; Ito, Hotta and Stern, 1967; Hotta, Parchman and Stern, 1968; and Stern and Hotta, 1969*a* and *b*). Similar and parallel studies on other biochemical components have indicated changes in DNA, RNA and proteins in developing anthers (Moss and Heslop-Harrison, 1967), in ribosomes (Mackenzie, Heslop-Harrison and Dickinson, 1967) and in enzyme pattern (Linskens, 1966). In some experiments (Stern and Hotta, 1969*b*) inhibitors of chiasma formation and protein synthesis produced desynapsis depending upon the stage of inhibition, and it was concluded that chromosome pairing and chiasma formation were two distinct and separate stages in the meiotic process. It must now be recognised that chromosome desynapsis and chiasma control (number and terminalisation) are distinct stages and under two separate control systems.

It would be reasonable to assume that while precocious desynapsis in the mutant under study conditioned by a single gene affected perhaps a primary gene product (transcription defect), *i.e.* messenger RNA, the polygenic system which controlled chiasma terminalisation governed a secondary gene product such as a protein or enzyme shape (translation defect). In rice, a considerably higher RNA content was present in desynaptic florets than in normal ones (Chao and Hu, 1961); Ansley (1954, 1958) has shown that asynaptic meiosis in the testes of *Scutigera forceps* and *Loxa flavicolis* had a changed ratio of DNA : histones.

In the absence of biochemical information on the mutant understudy, the hypothesis, however, is open to modifications.

5. Summary

1. In a desynaptic mutant of diploid ($2n = 14$) perennial ryegrass *Lolium perenne* L., homologous chromosomes paired normally to form bivalents up to diplotene, but separated before first metaphase.

2. All the plants of selfed progeny were desynaptic.

3. Except for two plants the F_1 from the cross between the desynaptic and normal was normal. These two plants, one diploid, the other hypertriploid ($2n = 3x+1 = 22$) were desynaptic.

4. The F_2 segregated for normals and desynaptics in a 3 : 1 ratio.

5. In both the F_1 and F_2, plants with relatively low and high chiasma number were present.

6. The precocious separation following pairing was determined by a single recessive gene, *ds*, in a homozygous condition while the number of chiasmata was controlled, in addition, by a polygenic system independent of the major desynaptic gene.

Acknowledgments.—Continued interest and encouragement by Dr T. Walsh, Director, Agriculture Institute, is highly appreciated.

6. References

AHLOOWALIA, B. S. 1967. Colchicine induced polyploids in ryegrass. *Euphytica, 14*, 170-172.

AHLOOWALIA, B. S. 1968. Technique for studying meiosis in ryegrass. In *Effects of Radiation on Meiotic Systems*, 159-161. Intern. Atomic Energy Agency, Vienna.

AHLOOWALIA, B. S. 1969*a*. Desynapsis in diploid and tetraploid clones of ryegrass. *Genetica, 40*, 379-392.

AHLOOWALIA, B. S. 1969*b*. Effect of temperature and barbiturates on a desynaptic mutant of ryegrass. *Mutation Res., 7*, 205-213.

AHLOOWALIA, B. S. 1970. Chromosome behaviour in a hypertriploid plant of ryegrass. *Theoret. Appl. Genetics, 40*, 138-142.

ANSLEY, H. R. 1954. A cytological and cytophotometric study of alternative pathways of meiosis in the house centipede (*Scutigera forceps* Rafinesque). *Chromosoma, 6*, 656-695.

ANSLEY, H. R. 1958. Histones of mitosis and meiosis in *Loxa flavicolis* (Hemipteran). *J. Biophys. Biochem. Cytol., 4*, 59-62.

BEADLE, G. W. 1930. Genetical and cytological studies of Mendelian asynapsis in *Zea mays*. *Mem. Cornell Univ. Agr. Expt. Sta., 129*, 1-23.

CHAO, C. Y., AND HU, W. L. 1961. The effect of temperature on a desynaptic gene in rice. *Bot. Bull. Acad. Sinica, 2*, 87-100.

COMINGS, D. E., AND OKADA, T. A. 1970. Mechanism of chromosome pairing during meiosis. *Nature, Lond., 227*, 451-456.

COOPER, J. P., AND THOMAS, P. T. 1961. Cytological unbalance resulting from intensive selection in *Lolium*. Rep., Welsh Pl. Breed. Sta. for 1961, 115-118.

ENNS, H., AND LARTER, E. N. 1960. Note on the inheritance of ds; a gene governing meiotic chromosome behavior in barley. *Can. J. Plant Sci., 40*, 570-571.

GOTTSCHALK, W., AND JAHN, A. 1964. Cytogenetische Untersuchungen an desynaptischen und männlich-sterilen Mutanten von *Pisum*. *Z. Vererbungsl., 95*, 150-166.

GOTTSCHALK, W., AND VILLALOBOS-PIETRINI, R. 1965. The influence of mutant genes on chiasmata formation in *Pisum sativum*. *Cytologia, 30*, 88-97.

HOTTA, Y., ITO, M., AND STERN, H. 1966. Synthesis of DNA during meiosis. *Proc. Natl. Acad. Sci. U.S., 56*, 1184-1191.

HOTTA, Y., PARCHMAN, L. G., AND STERN, H. 1968. Protein synthesis during meiosis. *Proc. Natl. Acad. Sci. U.S., 60*, 575-582.

ITO, M., HOTTA, Y., AND STERN, H. 1967. Studies of meiosis *in vitro*. II. Effect of inhibiting DNA synthesis during meiotic prophase on chromosome structure and behavior. *Develop. Biol., 6*, 54-77.

JAUHAR, P. P., AND SINGH, U. 1969. Desynapsis and the blockage of meiosis in *Pennisetum orientale* Rich. *Theoret. Appl. Genetics, 39*, 315-319.

LI, H. W., PAO, W. K., AND LI, C. H. 1945. Desynapsis in the common wheat. *Amer. J. Bot., 32*, 92-101.

LINSKENS, H. F. 1966. Die änderung des Protein-und Enzym-musters während der pollenmeiose und pollenentwicklung. Physiologische untersuchungen zur reifeteilung. *Planta, Berl., 69*, 79-91.

MACKENZIE, A., HESLOP-HARRISON, J., AND DICKINSON, H. G. 1967. Elimination of ribosomes during meiotic prophase. *Nature, Lond., 215*, 997-999.

MANN-LESLEY, M., AND FROST, H. B. 1927. Mendelian inheritance of chromosome shape in *Matthiola*. *Genetics, 12*, 449-460.

MARTINI, G., AND BOZZINI, A. 1966. Radiation induced asynaptic mutations in durum wheat. *Chromosoma, Berl., 20*, 251-266.

MILLER, O. L. 1963. Cytological studies in asynaptic maize. *Genetics, 48*, 1445-1466.

MOENS, P. 1969. Genetic and cytological effects of three desynaptic genes in the tomato. *Can. J. Genet. Cytol., 11*, 857-869.

MOSS, G. I., AND HESLOP-HARRISON, J. 1967. A cytochemical study of DNA, RNA and Protein in the developing maize anther. II. Observations. *Ann. Bot., 31*, 555-572.

PRAKKEN, R. 1943. Studies of asynapsis in rye. *Hereditas, 29*, 475-495.

PRITCHARD, A. J. 1965. Asynapsis in a tetraploid forage sorghum. *Australian J. Bot., 13*, 17-21.

RILEY, R., AND LAW, C. N. 1965. Genetic variation in chromosome pairing. *Adv. Genet., 13*, 57-114.

RILEY, R. 1966. Genetics and the regulation of meiotic chromosome behaviour. *Sci. Prog. Oxf., 54*, 193-207.

ROSS, J. G., SANDERS, M. E., AND FRANZKE, C. J. 1960. Asynapsis in *Sorghum*. *Hereditas, 46*, 570-580.

ROTH, T. F., AND ITO, M. 1967. DNA-Dependent formation of the synaptinemal complex at meiotic prophase. *J. Cell Biol., 35*, 247-255.

SHERIDAN, W. F., AND STERN, H. 1967. Histones of meiosis. *Exptl. Cell. Res., 45*, 323-335.

SOOST, R. K. 1951. Comparative cytology and genetics of asynaptic mutants in *Lycopersicon esculentum* Mill. *Genetics, 36,* 410-434.

STERN, H., AND HOTTA, Y. 1969*a*. DNA synthesis in relation to chromosome pairing and chiasma formation. *Genetics, 61,* (Supplement) 1, 27-39.

STERN, H., AND HOTTA, Y. 1969*b*. Biochemistry of meiosis. In *Handbook of Molecular Cytology,* 520-539. North Holland, London.

THOMAS, H., AND RAJHATHY, T. 1966. A gene for desynapsis and aneuploidy in tetraploid *Avena*. *Can. J. Genet. Cytol., 8,* 506-515.

WANG, S., YEH, P., LEE, S. S. Y., AND LI, H. W. 1965. Effect of low temperature on desynapsis in rice. *Bot. Bull. Acad. Sinica, 6,* 197-207.

LOCALISATION OF INTRASPECIFIC VARIATION IN MEIOTIC CHROMOSOME PAIRING

J. SYBENGA

Department of Genetics, Agricultural University, Wageningen, The Netherlands

Chromosomal differentiation is the main cause of the failure of meiotic pairing between homoeologous chromosomes in a hybrid between related species. Although the extent of preferential pairing may be increased by genetic control (*e.g.* the $5B^L$ system in wheat), the basis must reside in the chromosomes themselves. Rather than invoking cryptic structural rearrangements as the causal mechanism, there are reasons for explaining pairing differentials on the basis of mutational changes in the attraction system itself. If this is a correct point of view, comparable differences in attraction between homologous chromosomes should be detectable (on a more limited scale) between different genotypes of the same species. Such incipient differentiation is not expressed as failure of pairing in an intraspecific hybrid, but it may be detected in a situation where more than two chromosomes compete for pairing which is realised only between two at a time.

Combining one normal chromosome with one telocentric of one arm and two telocentrics of the other arm results in disomy for the first and trisomy for the latter (fig. 1). Such trisomics were constructed for the long and for the short arm of the satellite chromosome of rye, *Secale cereale*.

With complete pairing and not considering partner exchange, there are three basic associations. With chiasmata in all paired arms, two will lead, at MI, to a trivalent with a univalent telo, and one to a small bivalent between the two homologous telos of the trisomic arm, combined with a heteromorphic bivalent (the normal chromosome and the single telo of the disomic arm). When each of the three homologous arms has an equal probability of pairing with either of its two homologues, there will be twice as many trivalents with a univalent telo as there are bivalent pairs. When a significant deviation from this 2 : 1 ratio is observed, there must be heterogeneity in the attraction system. It may be a difference in qualitative homology or general efficiency of partner attraction.

Between primary attraction and observation at MI two processes take place that are important for the results:

1. Intimate pairing, perhaps centred around synaptonemal complex formation. Since the choice of partners may be assumed to be determined primarily by long-distance attraction (the zygomeres), intimate pairing is probably not important in this respect, but it may have an effect on chiasma formation.

2. Chiasma formation, which is essential for the recognition in MI of segments that have been brought together at earlier stages. It is clear from fig. 1, that with a low chiasma frequency in the trisomic arm, both pairing configurations will regularly be transformed into the same heteromorphic bivalent with two telocentric univalents. Distinction between the two pairing types is then not possible. Higher chiasma frequencies in specific combinations of two chromosomes than in other combinations will

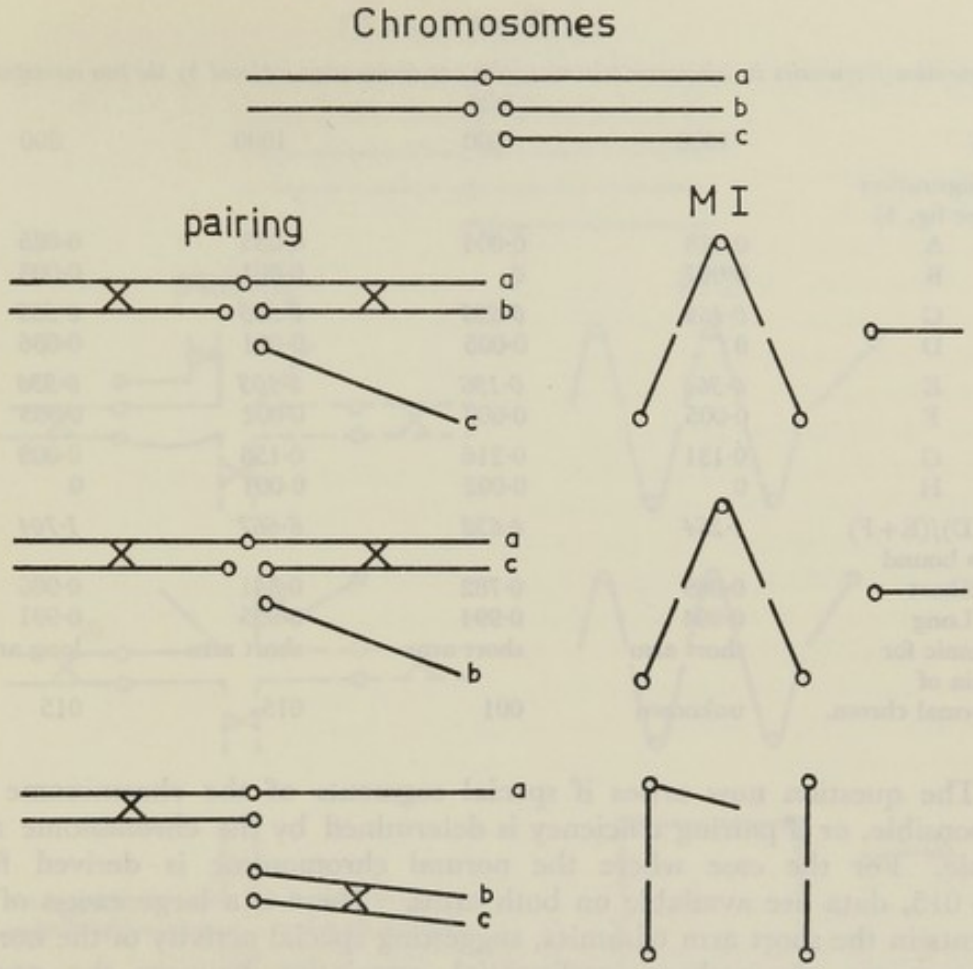


FIG. 1.—Chromosome constitution of the telocentric trisomics considered in the text, with the three pairing types without partner exchange and the corresponding meiotic first metaphase configurations.

simulate pairing preference. Since such chiasma-preference is *a priori* by no means less probable than pairing preference, material with high chiasma frequency in the trisomic arm is essential in an analysis of chromosome pairing.

With respect to chiasma formation, partner exchange may cause complications. As long as chiasmata are formed in at least two of the segments involved in partner exchange, it may be recognised. It can be shown that random failure of chiasmata in all except one of the segments involved results in trivalents with univalents and bivalent pairs in the same 2 : 1 ratio as expected with random pairing without partner exchange. Non-random failure of chiasmata, however, may again simulate non-random pairing. In the present material, total chiasma frequency is high, and partner exchange and proximal chiasmata appear to be relatively infrequent. It is therefore considered safe to apply deviations from the 2 : 1 ratio as a test for non-randomness of pairing.

In table 1, examples of considerable and variable deviation from randomness are given. More data are available, confirming the general trend. In the first example, the frequency of trivalents is low: the normal chromosome must be quite inefficient in finding a partner for its short arm. When this chromosome is derived from inbred lines 001 and 015 there are many trivalents: it is very efficient; 015 seems to be even better than 001.

TABLE 1

Configuration frequencies in telocentric trisomics with one chromosome replaced by the two corresponding telos

Cells	1000	600	1000	800
Configuration (See fig. 4)				
A	0·028	0·004	0·035	0·085
B	0·001	0	0·001	0·003
C	*0·469*	*0·638*	*0·699*	*0·565*
D	0	0·005	0·001	0·006
E	*0·366*	*0·136*	*0·103*	*0·330*
F	0·005	0·002	0·002	0·005
G	0·131	0·216	0·158	0·009
H	0	0·002	0·001	0
(C+D)/(E+F)	*1·264*	*4·638*	*6·667*	*1·704*
Arms bound				
Short	0·869	0·782	0·841	0·986
Long	0·994	0·994	0·995	0·991
Trisomic for	short arm	short arm	short arm	long arm
Origin of normal chrom.	unknown	001	015	015

The question now arises if special segments of the chromosome are responsible, or if pairing efficiency is determined by the chromosome as a whole. For the case where the normal chromosome is derived from line 015, data are available on both arms. There is a large excess of trivalents in the short arm trisomics, suggesting special activity of the normal chromosome, or perhaps preferential association between the normal chromosome and one of the telos. In the long arm trisomic, on the contrary, there is a shortage of trivalents: the pairing activity of the normal chromosome is low. Apparently, the two arms are quite independent in their primary attraction (zygomere) system.

For localisation within arms, interchanges were used. The preliminary results are not based on a very sophisticated system of analysis. A first impression was obtained from telocentric interchange trisomics (an interchange heterozygote with an additional telocentric chromosome, fig. 2). As the data of table 1 suggested that line 015 might be somewhat more efficient in attracting a partner for its short satellite arm than line 001, both being better than average, these two lines were crossed with a telocentric interchange trisomic, and the interchange trisomic progeny were isolated. Their normal chromosome was derived from the inbred lines, the telo and the interchange chromosome set from the interchange trisomic parent. Since this parent had a complex origin, it was heterozygous and differences in the progeny should not exclusively represent differences between 001 and 015. The figure shows the three pairing modes for the terminal segment and the corresponding MI configurations with chiasmata as indicated. With random pairing and without interstitial attachment of the telo, the telo will be associated with the normal chromosome in one-third of the cases, with the interchange chromosome in another third of the cases, while in the remaining third, it will be loose, as the normal and the interchange chromosome are associated.

The analysis of the association of the interchange segment or the corres-

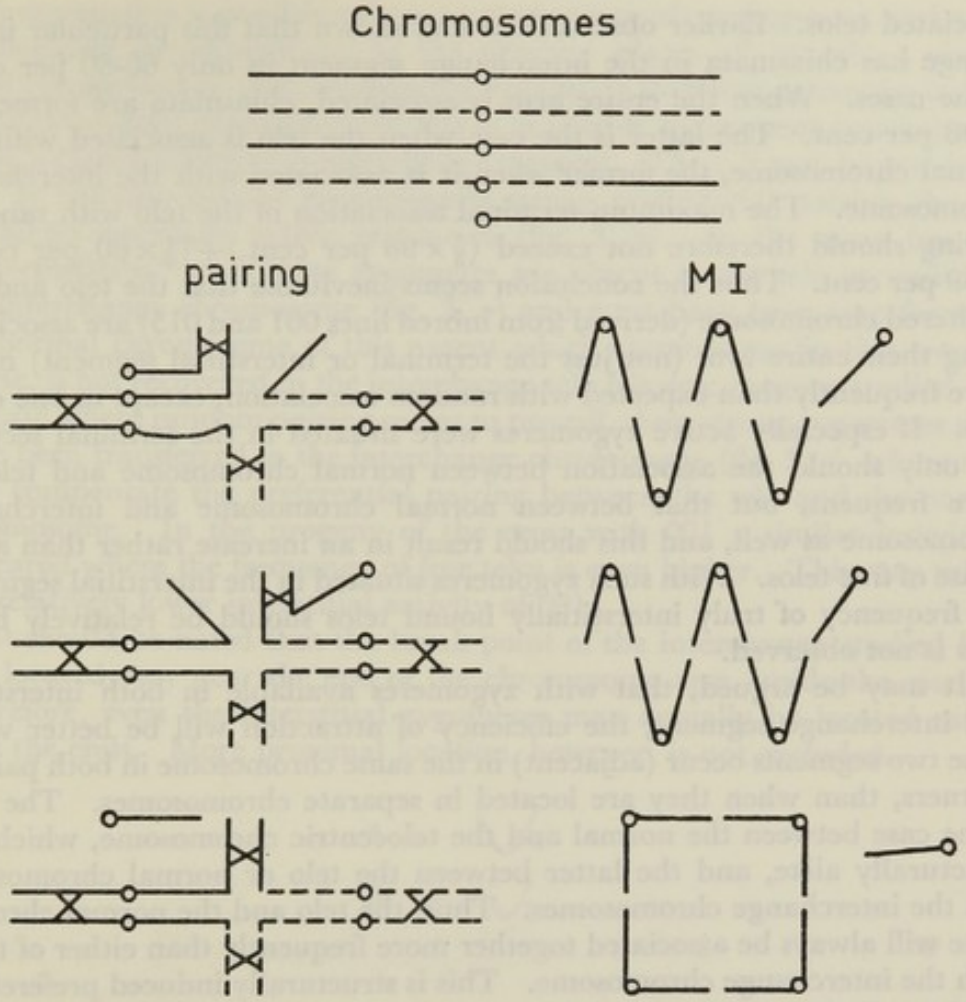


FIG. 2.—Chromosome constitution of the telocentric interchange trisomic with the three pairing modes without partner exchange and the corresponding meiotic first metaphase configurations.

ponding segment of the normal arms requires partitioning of the configurations with partner exchange and interstitial chiasmata, with the telo bound interstitially. For reasons not considered here, it may be concluded that by far the majority of these cases actually had the telos attached to the terminal segment only. Even when this fraction is not considered, it is clear that in most examples (table 2) there is a great excess of terminally

TABLE 2

Attachment of the telocentric chromosome in interchange telocentric trisomics in which the normal chromosome is derived from different inbred lines. Interchange 248

Cells	1000	500	500	500	500
Telo					
Terminal	0·827	0·758	0·800	0·946	0·520
Interstitial	0·043	0·074	0·110	0·012	0·058
Free	0·130	0·168	0·090	0·042	0·422
Bivalent arms bound	0·964	0·953	0·975	0·986	0·962
Normal chrom.	001	015	015	015	015
Maximum terminal association with random pairing		$\frac{1}{3}\times 0{\cdot}98+\frac{1}{3}\times 0{\cdot}8 = 0{\cdot}59$			
Average		$\frac{1}{3}\times 0{\cdot}95+\frac{1}{3}\times 0{\cdot}6 = 0{\cdot}52$			

associated telos. Earlier observations had shown that this particular interchange has chiasmata in the interchange segment in only 60-80 per cent. of the cases. When the entire arm is associated, chiasmata are formed in 85-98 per cent. The latter is the case when the telo is associated with the normal chromosome, the former when it is associated with the interchange chromosome. The maximum terminal association of the telo with random pairing should therefore not exceed $(\frac{1}{3} \times 98$ per cent.$) + (\frac{1}{3} \times 80$ per cent.$) = 59$ per cent. Thus the conclusion seems inevitable that the telo and the unaltered chromosome (derived from inbred lines 001 and 015) are associated along their entire arm (not just the terminal or interstitial segment) much more frequently than expected with random association, except in one clear case. If especially active zygomeres were situated in the terminal section, not only should the association between normal chromosome and telo be more frequent, but that between normal chromosome and interchange chromosome as well, and this should result in an increase rather than a decrease of free telos. With such zygomeres situated in the interstitial segment, the frequency of truly interstitially bound telos should be relatively high. This is not observed.

It may be argued, that with zygomeres available in both interstitial and interchange segment, the efficiency of attraction will be better when these two segments occur (adjacent) in the same chromosome in both pairing partners, than when they are located in separate chromosomes. The first is the case between the normal and the telocentric chromosome, which are structurally alike, and the latter between the telo or normal chromosome and the interchange chromosomes. Thus, the telo and the normal chromosome will always be associated together more frequently than either of them with the interchange chromosome. This is structurally induced preferential pairing. It does not operate when zygomeres are present in only one of the two segments, and it is most effective when zygomere activity is distributed equally over the two. In general, in rye, zygomere activity is concentrated in the ends. It is probable, because of the high frequency of the telo-normal association, that the extra activity of lines 001 and 015 is located in the interstitial segment, giving it an activity comparable to that of the ends (fig. 3, *a*). The same mechanism will reduce true exclusive interstitial attachment of the telo.

The difference between different plants of the same cross must be due to segregation of zygomeres from the trisomic parent, which was heterozygous.

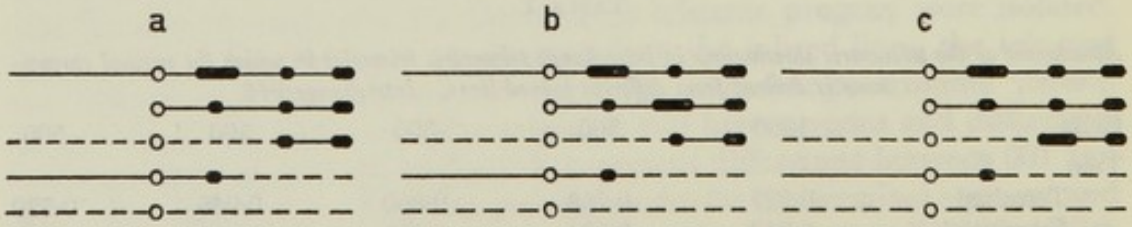


FIG. 3.—Diagram of suggested zygomere activities in the telocentric interchange trisomics with different frequencies in MI of terminally attached telos. Relative activities indicated by length of block; specificities not considered. (*a*) Active zygomere in interstitial segment of normal chromosome makes this comparable to interchange segment: preferential pairing between structurally like types (normal—telo). (*b*) Active zygomere in telo (segment corresponding to interchange segment) increases preferential pairing. (*c*) This zygomere transferred to interchange chromosome reduces preferential pairing.

Such segregation is possible only as a consequence of crossing-over, and not of chromosome recombination, as the progeny plants all contain a similar set of chromosomes. The segregating zygomeres therefore cannot be located in the interstitial segment, since this is not liable to frequent crossing-over. Their location must be the interchange segment. Then, it may be concluded that in the one case of exceptionally strong preferential pairing the telo contained especially active zygomeres (fig. 3, *b*). In the more frequent " intermediate " class these zygomeres are absent in the telo as well as in the interchange chromosome (fig. 3, *a*) and must have been transferred to the normal chromosome of this parent which chromosome in this cross, of course, is not recovered in the interchange telo trisomic progeny studied. In the case where random pairing seems to prevail, these strong zygomeres must have been transferred to the interchange chromosome (fig. 3, *c*), where they now compensate the preferential pairing between the telo and the normal chromosome. In the progeny of the cross with 001 a similar individual appeared where the frequency of free telos is even higher. This may reflect the relatively lower interstitial activity of 001.

It should be noted that the break point of the interchange studied here was located very near the end of the chromosome arm, inside the satellite. Therefore, even the interstitial zygomeres may actually be located rather near the ends. More proximal location, however, is not excluded.

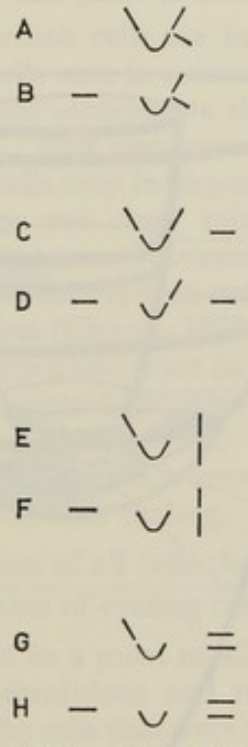


FIG. 4.—Configurations (see table 1).

Acknowledgments.—Thanks are due to Mr C. J. P. Seegeler who analysed one interchange telocentric trisomic and especially to Mr J. M. de Vries who analysed several and in addition the telocentric trisomics without interchange.

NON-DIVIDING CELLS IN MERISTEMS*

F. A. L. CLOWES
Botany School, Oxford University

1. Introduction

When we examine an apical meristem we commonly see that some 10 per cent. of its cells are in mitosis in actively growing roots and a smaller percentage in actively growing shoots. The cells in mitosis appear at first sight to be scattered throughout the meristem and they give the impression that all would come into mitosis if we could watch a living meristem for long enough. The fraction of cells that we see with their chromosomes displayed, then, would merely reflect the proportion of the mitotic cycle that is occupied by mitosis.

The impression we get from sections and squashes is false. The first indication that this was so came with the discovery of the quiescent centre in roots—a region surrounded by actively dividing cells where the average rate of mitosis is low (fig. 1). The presence of such a region can be deduced by merely analysing the pattern of cells, but we can demonstrate it more

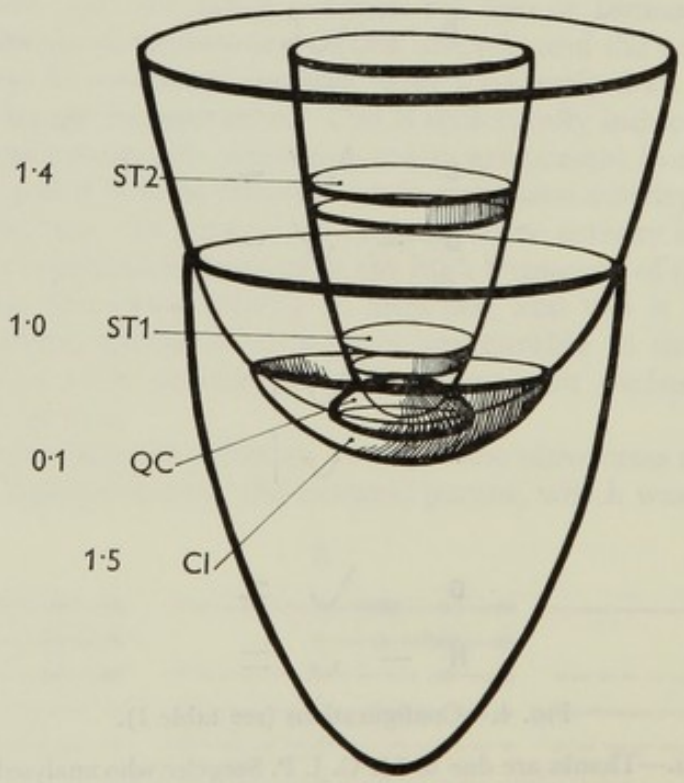


Fig. 1.—The root apex of *Zea mays* showing the four regions of the meristem for which the growth fraction has been calculated. CI, cap initials; QC, quiescent centre; ST1, stele just above the quiescent centre; ST2, stele 200 μm. above the quiescent centre. The rate of cell formation is given for each region expressed as cells formed per cell per day calculated from pulse-labelled roots at 23° C.

* Presented as a demonstration at the conference.

easily by giving the plant radioactively labelled nutrients (Clowes, 1954, 1956). The quiescent centre explains the absence of mitoses from the region that comes to light by marking the sites of dividing cells, but the absence of mitoses does not show the existence of a quiescent centre. It is probable that some shoot apices have a somewhat similar quiescent region at their summits, but, for technical reasons, this is difficult to prove and it is not universal.

Until recently we did not know whether the rates of mitosis that have been measured in the various regions of a meristem (Clowes, 1961, 1965) reflect the activity of all the constituent cells or whether they were made up of many mitotic cycles of differing durations, some of them infinitely long. This problem is particularly interesting in the quiescent centre because of the theories about its cause and function and their relation to cell kinetics in animals. The average rate of mitosis in the quiescent centre is often one-tenth of that in the surrounding regions. Is this so because all its cells pass through mitotic cycles of 200 or 300 hours duration or have some of its cells stopped dividing, leaving others with more normal rates of mitosis? We can now solve this problem and learn more about the meristematic regions by an analysis of pulse-labelled meristems fed with labelled thymidine.

2. Method

We need to find out which cells are in a mitotic cycle and which are not cycling. Clearly all cells seen in mitosis are cycling and we make the assumption that all cells that incorporate thymidine into DNA after pulse-labelling are also cycling. This assumption is not strictly valid because, even in a meristem, some cells may incorporate thymidine without proceeding to a mitosis and some can enter mitosis without entering a DNA-synthetic period (S). D'Amato and Avanzi (1965) have shown the first to be true of tetrahedral apical cells in fern roots and I have shown the second to be true after X-irradiation (Clowes, 1970*a*). However, the assumption is not likely to lead to any important error in normal angiosperm root apices. Cells that display neither mitotic chromosomes nor autoradiograph could be either cycling cells or non-cycling cells.

The quantity we want to measure is known as the Growth Fraction (GF) and is the fraction of all cells that cycling.

$$\text{So GF} = \frac{\text{fraction of all cells that are labelled (LC)}}{\text{fraction of cycling cells that are labelled}}.$$

The labelling is carried out in a pulse of 30 minutes and the meristems are then grown in label-free conditions and sampled at two-hourly intervals over two mitotic cycles. The cells that were in S during the pulse are labelled and they proceed through G_2 to mitosis and their daughters enter a second cycle. Cells not in S during the pulse also progress through cycles out of phase with the labelled cells. Cells that reach mitosis after the pulse will be labelled or unlabelled in the same proportion as labelled and unlabelled cells in any other phase of the mitotic cycle. Therefore the fraction of cycling cells that are labelled equals the fraction of mitoses that are labelled (LMM).

$$\therefore \text{GF} = \frac{\text{LC}}{\text{LMM}}. \qquad (1,2)$$

LC is more or less constant throughout the sampling period and is conveniently measured either immediately after the pulse or integrated over the first mitotic cycle. LMM varies from near zero to near one and is integrated over the first complete cycle (1) or (2) it can be estimated from comparing the areas under the curves for the fraction of all cells that are labelled mitoses (LMA) and the fraction of all cells that are mitoses (MI). An alternative way (3) of obtaining the same value for GF from different measurements is

$$GF = \frac{MI}{\text{labelled mitoses as a fraction of all labelled cells}}. \qquad (3)$$

The curve of pulse-labelled mitoses gives us estimates for the duration of the mitotic cycle (T) and of each of its phases G_1, S, G_2 and M. These may also be exploited to give a figure which is related to the growth fraction as measured by the three calculations outlined above thus:

$$GF = \frac{C}{A} = \frac{Ns}{A} \times \frac{C}{Ns}$$

where C is the no. of cycling cells
A is the no. of all cells
Ns is the no. of cells in S

$$\frac{C}{Ns} = \frac{T}{S} \text{ and } \frac{Ns}{A} = LC$$

$$\therefore GF = LC \times \frac{T}{S} \qquad (4)$$

and similarly

$$GF = MI \times \frac{T}{M} \qquad (5)$$

but the value for the duration of mitosis (M) used here must be independent of the mitotic index (MI).

These two equations (4) and (5) for the growth fraction provide figures only for those cycling cells that have the cycle time estimated from the separation of the peaks in the pulse-labelled mitosis curve. If there are cells with a longer mitotic cycle, they are excluded from the calculation and the values for GF will be lower than those obtained from the first three ways of calculating GF (1), (2) and (3). The fraction (6) of all cycling cells that have the mitotic cycle measured on the pulse-label curve may be obtained by comparing the ratio of the area under the LMA curve to that under the MI curve with the ratio of S to T. S, in these calculations, is the width of the first peak though the true value of S is this time minus the duration of the pulse. The development of these calculations is discussed in another article (Clowes, 1971).

3. Results

The pulse-labelled mitosis (LMM) curve is given in fig. 2 and the LMA curve in fig. 3 for the four regions shown in fig. 1 of the root meristem of *Zea* seedlings grown in water culture at 23° C. In table 1 are given (A) the true growth fraction calculated from three sets of observations, (B) the fraction of all cells with the cycle displayed in the LMM curves calculated from two sets of observations and (C) the fraction of all cycling cells that have the cycle displayed in the LMM curves.

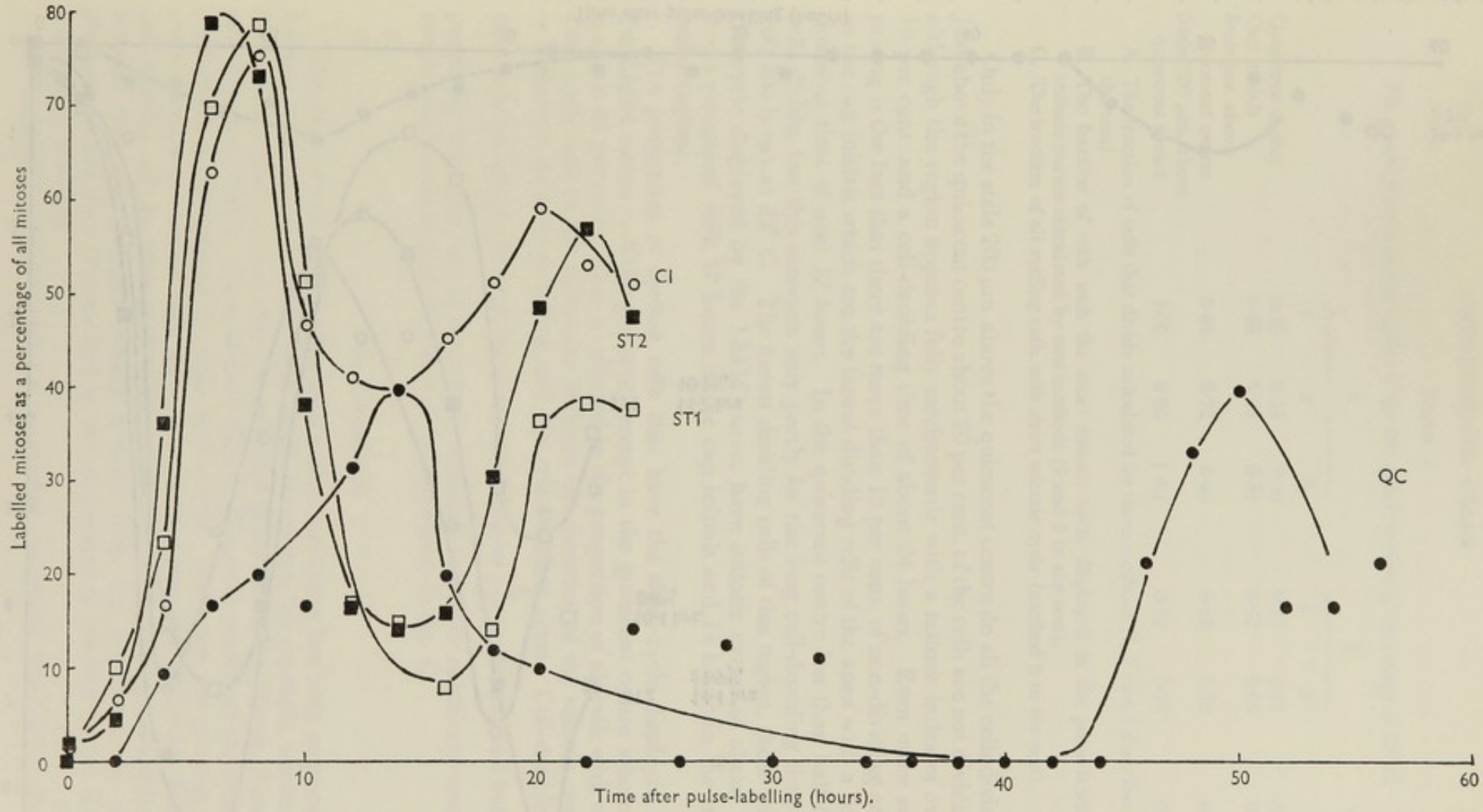


FIG. 2.—Pulse-labelled mitosis (LMM) curves for four regions of the root apical meristem of *Zea mays* at 23° C. grown in water after a 30-minute exposure to ^{3}H-thymidine (1 μCi/ml.). QC, quiescent centre; CI, cap initials; ST1, stele just above quiescent centre; ST2, stele 200 μm. above quiescent centre.

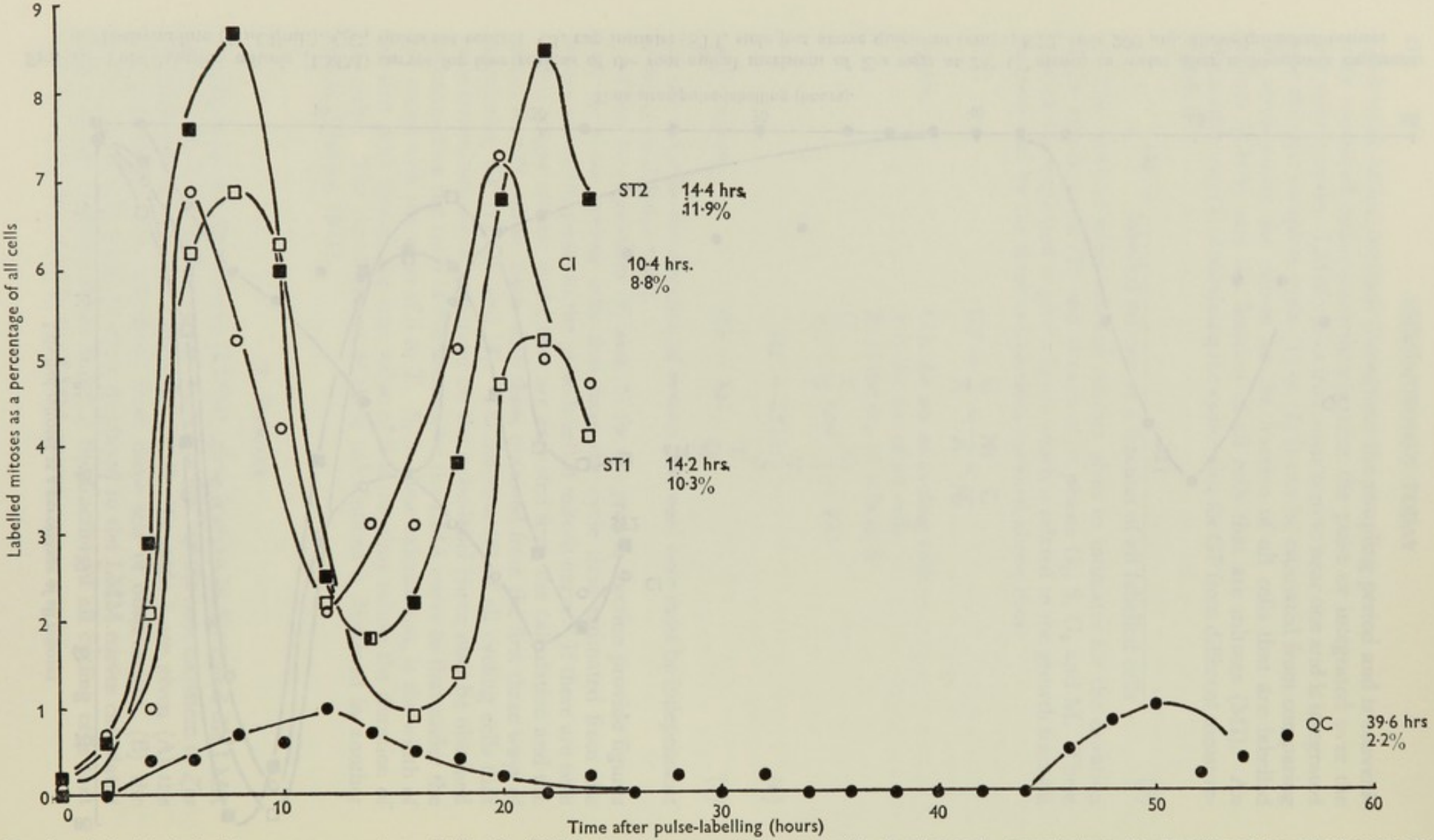


FIG. 3.—Pulse-labelled mitoses as a percentage of all cells (LMA) for the same regions as in fig. 2. Against each curve is given the duration of the mitotic cycle displayed in the LMM curves and the mitotic index as a mean percentage for the first complete cycle after labelling.

TABLE 1

The growth fraction in four regions of the root apical meristem of Zea mays *at 23° C.*

	A			B		C
	1	2	3	4	5	6
Quiescent centre	0·49	0·43	0·39	0·17	0·17	0·39
Cap initials	0·89	0·83	0·87	0·67	0·65	0·81
Stele just above quiescent centre	0·84	0·78	0·85	0·58	0·59	0·73
Stele 200 μm. above quiescent centre	1·00	0·96	1·01	0·82	0·78	0·79

A. The fraction of cells that divide calculated by three methods (1, 2 and 3 described in the text).

B. The fraction of cells with the short mitotic cycle displayed in the pulse-labelled mitosis curves calculated by two methods (4 and 5 in the text).

C. The fraction of all cycling cells with short mitotic cycle (method 6 in the text).

Only in the stele 200 μm. above the quiescent centre do all the cells divide. Just above the quiescent centre about 20 per cent. of the cells are not dividing although the region appears fully meristematic with a mitotic index of over 10 per cent. and a cell-doubling time of about 24 hours. Even more surprising is the fact that there are more than 10 per cent. of non-dividing cells in the cap initials which are the fastest dividing cells of the apex with a cell-doubling time of near 12 hours. In the quiescent centre less than half the cells divide, but this accounts only partly for the long cell-doubling time of 200-300 hours at 23° C. The fastest dividing cells of this region, those with the cycle displayed by the LMM curve, have mitotic cycles of about 40 hours compared with 10 hours in the cap initials and 14 hours in the two stelar regions.

The proportion of cycling cells that have the short-cycle displayed in the LMM curves is 70-80 per cent. except in the quiescent centre where it is about 40 per cent. This is reflected in the proportion of all cells with the short cycle and in the difference between the duration of the mitotic cycle as measured by pulse-labelling and the cell-doubling time. Cell-doubling time may be calculated from the pulse-label data as $\frac{S}{LC}$ or determined independently from the rate of metaphase accumulation after colchicine treatments and is used to find the rates of cell formation in fig. 1.

4. Discussion

The existence of non-dividing cells within meristems has been suspected before. Continuous labelling with DNA precursors, for example, leaves a few cells unlabelled even after several days (Clowes, 1961; Rasch, Rasch and Woodard, 1967). Some of these (600 in *Zea* roots) constitute the quiescent centre at the pole of the stele and cortex. They are conspicuous in autoradiographs because they form a compact group, but there are other non-labelled cells in other parts of the meristem. They are not conspicuous because they are scattered among labelled cells, but are shown by the plateaux of less than 100 reached in the percentage of labelled nuclei after continuous labelling.

The application of methods for measuring the growth fraction to plant

meristems has not revealed any meaningful figures before because whole root tips have always been used and these inevitably contain an unknown proportion of differentiated cells. Moreover most other workers have not measured the true growth fraction in apices, but the proportion of cells with the cycle displayed by pulse-labelling and this is lower than the growth fraction and depends heavily on the estimation of S. The duration of S is obtained from the width of the first peak in the LMM curve and the level at which this is measured is chosen somewhat arbitrarily.

As a result of the present measurements we could say that most meristematic regions contain cells that divide relatively fast, cells that divide more slowly and cells that do not divide at all. Probably there is no real distinction into three classes, but they can be considered like this because we can obtain parameters for them. The progeny of the cells of one class probably do not always stay in the same class. Non-dividing cells may also come into cycle and cycling cells may drop out of cycle. What controls this and the rates of mitoses?

Let us consider the problem of the quiescent centre in relation to the meristem first. In *Zea* it has about 270 cells that are cycling at any one time out of a total of 600. At 23° C. it produces a mere 60 cells a day compared with 10,000 a day produced by the 7000 cap initials on its distal margin and 170,000 a day produced by the 120,000 cells of the rest of the meristem. The lack of growth and division in the quiescent centre is not inherent in its cells because, under some circumstances, its average rate of mitosis can be speeded up to near that of the rest of a normal meristem. The region is not quiescent because it is starved of nutrients supplied by the root because we know that nutrients in the seed reach the cap initials on the far side of the quiescent centre. Similarly we know that nutrients supplied to the cap can reach the stele presumably after passing through the quiescent centre (Clowes, 1970*b*).

The shape of the region in apices of differing construction gives us a clue to the control mechanism. The quiescent centre revealed by autoradiography fits the geometry of the apex in such a way that it provides a more or less stable base on whose surface the dividing cells can grow without disrupting the files of cells. It is shaped like a biconvex lens in *Zea* (fig. 1), a hemisphere in *Sinapis*, a disc in *Pisum* so that the files of meristematic cells lie at right angles to its surface. Growth and division in the files of cells on the proximal surface lead to extension of the root; on the distal surface, to cap production compensated for by sloughing of the outer cells. Growth and division within the quiescent centre would disrupt the whole pattern of the root and therefore it does not occur except to compensate for cessation or slowing of growth in other regions of the meristem. We could think of pressure being applied to the quiescent centre from all directions restraining it from expanding. This pressure is relieved when part of the meristem slows its growth as after X-irradiation and other treatments that reduce the rate of mitosis or, in a few species, when the cap initials stop dividing of their own accord at intervals.

The same pressures could apply to individual cells within meristematic regions. The potential for cell production in all regions near the quiescent centre is greater than that actually achieved. It is restrained in a fraction of the cells by the need to grow conformably with other regions of the apex rather than by the attenuation of supplies of nutrients or by the control

exerted by hormones, though these may exert an overall control. The mechanism that stops cycling must be different within the meristem from that at the differentiating margins of the meristem because cells are slowed and arrested in G_1, in the first, but commonly go on synthesising DNA to the 4C level or higher in differentiation. The restraint on the non-dividing cells in the meristem could be primarily on cell growth and only secondarily on mitosis whereas the restraint at the end of meristematic activity and the onset of differentiation is on mitosis and not on growth. In animals, cells of many tissues appear to behave like those of the quiescent centre and surrounding meristematic regions in that they go out of cycle in G_1 and, when recalled into cycle, they do so by entering S. It may be that, in plants, pressure exerts the same kind of influence on the mitotic cycle as contact does in contact-inhibiting animal cells. Contact inhibition of cycling presumably cannot exist in plants with their immobile cells connected all the time by plasmodesmata, but our knowledge of communication between cells in plants is rudimentary as yet.

5. Summary

1. Pulse-labelling of the root meristem of *Zea* has been used to find the fraction of all cells that divide, the fraction that has the fast mitotic cycle displayed by the labelled mitosis curve and the fraction of all cycling cells with the fast cycle. Four regions of the meristem have been analysed separately.

2. More than half the cells of the quiescent centre are not in a mitotic cycle at all. Of those that do divide, the fastest have a cycle of 40 hours and the whole 600 cells contribute only 60 cells a day to the root.

3. The 6600 cap initials produce a whole root cap of 10,000 cells each day, but only 5600 contribute to this output and 4400 of these divide on average 2·3 times a day.

4. In the stele all the cells divide at 200 μm. above the quiescent centre, but only about 80 per cent. do so just above the quiescent centre. The whole meristem of 125,000 cells produces about 170,000 cells a day at 23° C.

5. The differences in cell proliferation in the different regions are controlled by the growth of the meristem as a whole rather than by differences in supplies to the cells.

6. References

CLOWES, F. A. L. 1954. The promeristem and the minimal constructional centre in grass root apices. *New Phytol.*, *53*, 108-116.

CLOWES, F. A. L. 1956. Nucleic acids in root apical meristems of *Zea*. *New Phytol.*, *55*, 29-34.

CLOWES, F. A. L. 1961. Duration of a mitotic cycle in a meristem. *J. Exp. Bot.*, *12*, 283-293.

CLOWES, F. A. L. 1965. The duration of the G_1 phase of the mitotic cycle and its relation to radiosensitivity. *New Phytol.*, *64*, 355-359.

CLOWES, F. A. L. 1970*a*. The immediate response of the quiescent centre to X-rays. *New Phytol.*, *69*, 1-18.

CLOWES, F. A. L. 1970*b*. Nutrition and the quiescent centre of root meristems. *Planta, Berl.*, *90*, 340-348.

CLOWES, F. A. L. 1971. The proportion of cells that divide in meristems. *Ann. Bot.* (in press).

D'AMATO, F., AND AVANZI, S. 1965. DNA content, DNA synthesis and mitosis in the root apical cell of *Marsilea strigosa*. *Caryologia*, *18*, 383-394.

RASCH, R. W., RASCH, E. M., AND WOODARD, J. W. 1967. Heterogeneity of nuclear populations in root meristems. *Cytologia*, *20*, 87-100.

THE ACTION OF 2-THIOCYTOSINE ON THE MITOSIS OF HUMAN LYMPHOCYTES IN CULTURE

J. TIMSON and D. J. PRICE

University Department of Medical Genetics, the Royal Infirmary, Manchester M13 9WL, United Kingdom

1. Introduction

Short-term cultures of human peripheral blood lymphocytes stimulated with phytohaemagglutinin (PHA) have been used to investigate the effects of a number of drugs on the rate of mitosis in human cells. In most cases the cultures have been terminated at 72 hours since it is known that at this time the maximum mitotic rate is obtained (MacKinney *et al.*, 1962). Actinomycin D and Puromycin (Neu *et al.*, 1966), Chloramphenicol (Pisciotta and DePrey, 1967), Testosterone (Timson, 1969*a*) and Paracetamol (Timson, 1968*a*) have all been shown to inhibit mitosis in PHA-stimulated human lymphocyte cultures.

This system has also been used to study the action of nucleic acid purines and pyrimidines and their precursors and analogues on human lymphocytes in mitosis. Orotic acid was found to reduce the mitotic rate at 10^{-2} and 10^{-3}M probably by competition for the available 5-phosphoribosyl-1-pyrophosphate (PRPP) in the cell leading to a reduced purine syntheis and hence DNA synthesis (Timson, 1969*b*). This effect of excess orotic acid may be the cause of the pathological changes found in orotic aciduria, a rare autosomal recessive condition in man first described by Huguley *et al.* (1959). 2-thiouracil reduced the mitotic rate of human lymphocytes *in vitro* at concentrations between 10^{-3} and 10^{-5}M (Timson, 1968*b*). Some of the side-effects of the therapeutic use of this compound in hyperthyroidism may be due to its anti-mitotic action. Thymine increased the mitotic rate in human lymphocyte cultures at concentrations between 10^{-3} and 10^{-5}M while adenine, guanine, cytosine and uracil did not show this effect (Timson and Price, 1970). McLeish (1953) showed that maleic hydrazide reduced the rate of mitosis in *Vicia faba* and it also causes a reduction in the mitotic rate at 10^{-2}M in human lymphocyte cultures (Timson, 1968*c*). Caffeine in this system was cytotoxic at 10^{-2}M, reduced the mitotic rate at 10^{-3}M and at 10^{-4}M stimulated mitosis in cultures from female donors and decreased it in cultures of male lymphocytes (Timson, 1970). 6-thioguanine is lethal to lymphocytes at 10^{-2} and 10^{-3}M and between 10^{-4} and 10^{-6}M is strongly anti-mitotic. This effect is reversed by adenine but not by guanine in equimolar concentrations. It is possible that 6-thioguanine acts mainly on the phosphoribosylpyrophosphate amidotransferase in this system (Price and Timson, 1970).

2-thiocytosine (2-TC) is an analogue of cytosine in which a sulphydryl group is substituted for the hydroxyl group in the number 2 position. Its potential as an anti-mitotic agent or antimetabolite does not appear to have been widely investigated. Mercer *et al.* (1953) reported 90-100 per cent. inhibition of formation of the RNA tobacco mosaic virus in tobacco leaf tissue at 10^{-4}M. This effect was reversible by uracil but not by cytosine or thymine.

2. Materials and methods

Venous blood was obtained from healthy adult volunteers of both sexes. The average age of the volunteers was 26·6 years (range 19 to 50). The blood was placed immediately into sterile bottles containing an anticoagulant (heparin in dextran) and after allowing the erythrocytes to settle the supernatant plasma and cells were withdrawn. 1·5 ml. of the cell/plasma suspension was made up to 9 ml. with TC199 for each culture. TC199 is a tissue culture medium containing no cytosine but 0·3 mg. of thymine, 0·3 mg. of uracil, 0·3 mg. of guanine hydrochloride and 5·1 mg. of adenine hydrochloride per litre (Morgan *et al.*, 1950). 2-thiocytosine (Sigma) in TC199 was added to give final concentrations of 10^{-2} to 10^{-6}M at 0, 24, and 48 hours in the 72-hour cultures and at 0 hours in the 96-hour cultures. Control cultures had TC199 without 2-TC added at the same time. Two drops of reconstituted PHA P (Difco) were added at the start of the culture period. All cultures were incubated at 37° C. At the end of the culture period 0·2 ml. of a solution of 1 mg. of demecolcine (" Colcemid ", Ciba) in 100 ml. of TC199 was added. After 2 hours at 37° C. the cells were spun down, resuspended in hypotonic saline for 15 minutes at 37° C., spun down and fixed in two changes of acetic acid-ethanol. They were then resuspended in 45 per cent. acetic acid, spread onto cold slides, airdried and stained with 10 per cent. Giemsa in *p*H 6·4 buffer. At least 1000 cells from each culture were examined and the number of mitoses recorded. These results were then expressed as a percentage of the control value. The average control value for the 72-hour cultures was 30·6 mitoses per 1000 cells and for the 96-hour cultures 18·7 mitoses per 1000 cells. All experiments were performed at least in triplicate on samples from different donors.

3. Results

Fig. 1 shows the effect of 72-hour exposure to 2-TC in 72-hour cultures. The Mitotic Index is here defined as the number of mitoses per 1000 cells and is expressed in the figure as a percentage of the control value. It can be seen that there is alternate inhibition and stimulation of the mitotic index at each change of concentration of 2-TC from 10^{-2} to 10^{-6}M. Table 1 gives the results obtained by varying the period of exposure to 2-TC to 24 and 48 hours in 72-hour cultures and of 96 hours exposure in 96-hour cultures. In table 2 the results obtained in 72-hour cultures in which PHA-stimulated lymphocytes were treated for 72 hours with equimolar concentrations of 2-TC and either cytosine or thymine or uracil are given. No abnormalities of structure of number of the chromosomes were observed in any of the control or experimental cultures.

4. Discussion

Fig. 1 shows an unusual type of response to changes in dose rates. More usually in this system inhibition of mitosis at the higher concentrations is followed by a gradual rise to control values as the dose rate is reduced. Alternatively where stimulation of the mitotic rate is observed after inhibition at higher concentrations this is followed by a fall to control values, giving when expressed as a graph, an approximation to a normal curve.

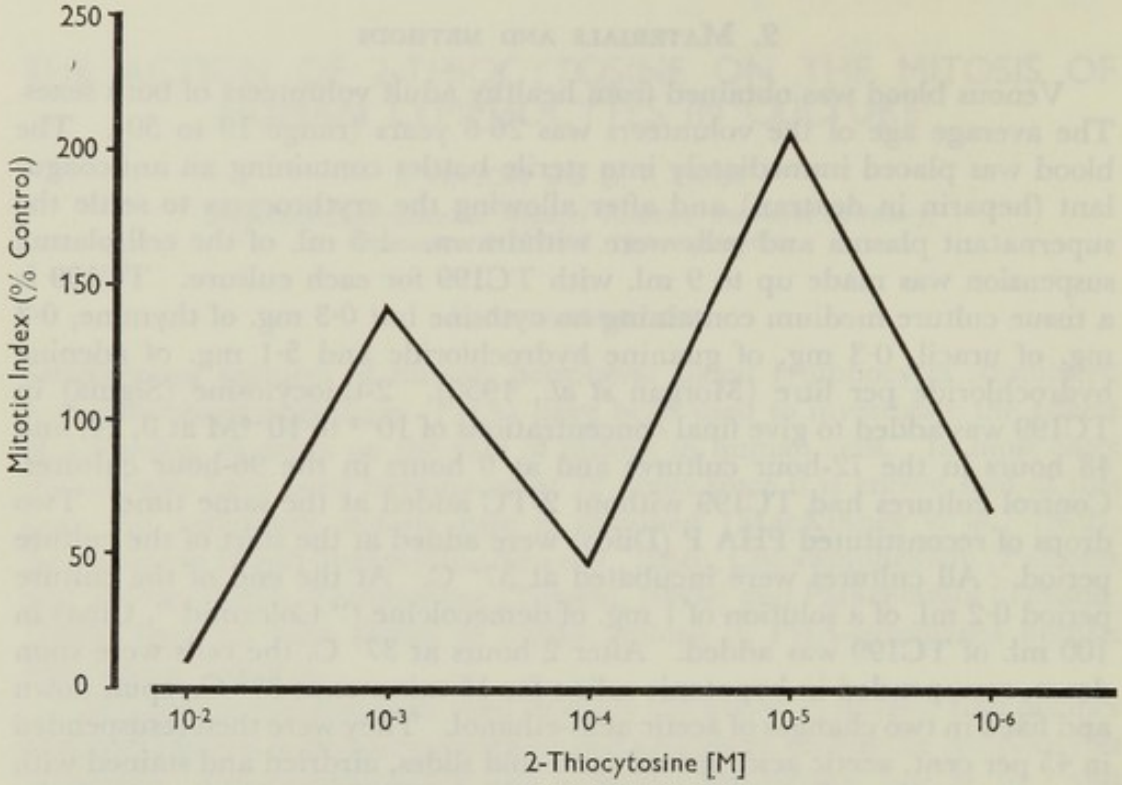


FIG. 1.—Effect of 2-thiocytosine on the mitotic rate of human lymphocytes in culture (72-hour exposure).

TABLE 1

Effect of exposure to 2-thiocytosine for different periods of time on the mitotic rate of human lymphocytes in culture

Molar concentration of 2-thiocytosine	Period of exposure (hours)			
	24 % control	48 % control	72 % control	96 % control
10^{-2}	27·4	35·4	10·2	85·6
10^{-3}	78·7	94·4	139·1	119·3
10^{-4}	63·3	75·5	45·3	42·8
10^{-5}	97·4	108·4	209·4	101·6
10^{-6}	53·1	61·5	67·2	87·2
0 (control)	100·0	100·0	100·0	100·0

2-thiocytosine over the range of concentrations studied, however, causes an oscillation of response measured as mitotic index after exposure of the lymphocytes to 2-TC for 72 hours. Examination of table 1 shows that while this oscillation is greatest after 72 hours exposure to 2-TC it is still present after 48 hours of growth without 2-TC followed by 24 hours exposure to 2-TC although to a much lesser extent. It is also still clearly present after 48 and 96 hours exposure. Since maximum DNA production and mitotic rate is reached in this system at 72 hours (MacKinney *et al.*, 1962) this suggests that the effect on mitosis of 2-TC is by incorporation of 2-TC into DNA or RNA leading to misreading followed by failure of DNA replication and/or defective protein production. Table 2 shows that cytosine is able to remove most of the oscillation probably by direct competition. Thymine considerably reduced the amplitude of the oscillation but the approximate normal curve response expected following thymine treatment (Timson and

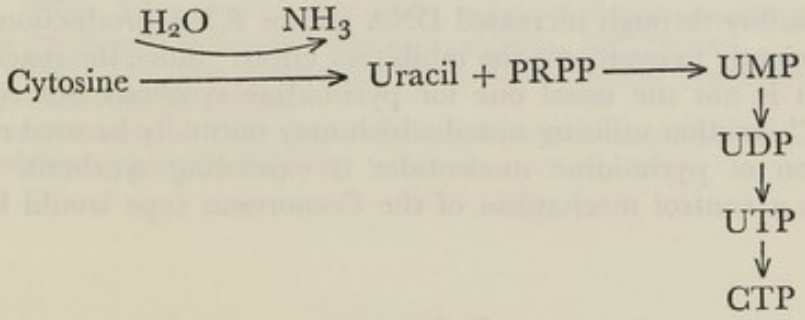
TABLE 2

Effect of cytosine, thymine and uracil at equimolar concentrations on 2-thiocytosine treated human lymphocytes in culture (72-hour exposure)

Molar concentration of each pyrimidine	2-Thiocytosine plus		
	Cytosine % control	Thymine % control	Uracil % control
10^{-2}	98·3	8·0	42·6
10^{-3}	86·2	110·6	122·7
10^{-4}	98·3	105·3	93·2
10^{-5}	101·7	117·0	102·6
10^{-6}	77·6	97·3	84·3
0 (control)	100·0	100·0	100·0

Price, 1970) with a peak of about 500 per cent. of control at 10^{-4}M was not found. Thymine and 2-TC are, therefore, mutually antagonistic except at 10^{-2}M where both are antimitotic and when combined at this concentration the effect appears to be additive. With uracil the oscillation still occurs but with reduced amplitude suggesting that uracil is relatively ineffective in reversing the action of 2-TC in this system. These results are the reverse of those of Mercer *et al.* (1953) where the inhibition of tobacco mosaic virus production by 2-TC was reversible only with uracil and not with cytosine or thymine. This suggests that the effect of 2-TC in human lymphocytes is on DNA rather than RNA.

Cytosine is not known to be directly incorporated into the nucleic acid production pathways. Cytidine nucleotide is produced by amination of UTP. Cytosine is, however, converted into uracil in the degradation pathway for pyrimidine nucleotides. Uracil can react with PRPP to yield UMP which can be converted to UDP and UTP and hence to CTP. The following pathway is therefore possible:



It is therefore theoretically possible for 2-TC to follow the same pathway to yield 2-thioCTP leading to the production of abnormal DNA and/or RNA. This might well lead to inhibition of mitosis. This would explain the effect of equimolar cytosine if the first stage of the above pathway preferentially utilised cytosine. It does not, however, satisfactorily explain the relative lack of effect of uracil since it would be expected that this would suppress the conversion of 2-TC to 2-thioCTP. Utilisation of the above pathway would involve the formation of 2-thiouracil which has been shown to inhibit mitosis in this system between 10^{-3} and 10^{-5}M (Timson, 1968*b*). It is therefore possible that 2-TC is incorporated via an unknown pathway. Thus while 2-TC appears to affect the mitotic rate of human lymphocytes in culture via the nucleic acid pathway this alone is not sufficient to explain

the oscillation found and the above pathway may well not be the only one utilised.

CDP and CTP are involved as co-enzymes in the synthesis of lecithins and CTP is involved in the conversion of sialic acid to CMP-N-acetyl-neuraminic acid in the glycoprotein production pathway. It is possible that if 2-thioCTP were found in the cell these pathways might be affected but it seems unlikely that such effects would be expressed by oscillation of the mitotic index with dose rate.

Comorosan (1970) has described a new mechanism for the biochemical control of cellular reactions. In this a dose-dependent stimulation of enzyme activity alternates with control levels following extremely low-level ultraviolet irradiation of the substrate. It is suggested that the energy necessary for this mechanism is of the order available from cellular biochemical energy generating systems. Thus in an $A \rightleftharpoons B$ type reaction if the corresponding oscillations differ in phase (*i.e.* are not overlapping) then a particular energy level would stimulate $A \rightarrow B$ while leaving $B \rightarrow A$ unchanged. Comorosan (1970) has shown this to be the case with the pyruvate $\rightleftharpoons$ lactate reaction *in vitro*.

The following hypothesis is therefore provisionally suggested to explain the oscillation in response of PHA-stimulated human lymphocytes to 2-TC *in vitro* as measured by the rate of mitosis.

(i) The basic action of 2-TC is to inhibit mitosis by the production of 2-thioCTP and/or 2-thiouracil following the pathway outlined above or by incorporation into some, at present, unknown pathway. This is supported by the suppression of most of the inhibitory effect by cytosine and thymine, and by the gradual rise towards the control value of the mitotic indices of the inhibitory dose rates (fig. 1).

(ii) The energy produced during the metabolism of 2-TC in the cell may be able to act as a substrate activator in the manner described by Comorosan (1970) so that at certain concentrations a reaction leading to increased mitosis possibly through increased DNA and/or RNA production is stimulated sufficiently to overcome the inhibitory effect. Since the reaction pathway given is not the usual one for pyrimidine synthesis but relies on a " salvage " reaction utilising uracil which may normally be used only when degradation of pyrimidine nucleotides is exceeding synthesis. In such conditions a control mechanism of the Comorosan type would be advantageous.

5. Summary

1. The action of 2-thiocytosine (2-TC) on the rate of mitosis of human lymphocytes *in vitro* was investigated. After 72 hours exposure to 2-TC an oscillation of response was observed such that at 10^{-2}, 10^{-4} and 10^{-6}M inhibition of mitosis occurred while at 10^{-3} and 10^{-5}M stimulation was found.

2. After 24, 48 and 96 hours exposure to 2-TC the same oscillation of response was detected although to a lesser extent.

3. When cytosine was also added in equimolar concentrations the oscillation disappeared; equimolar thymine had a similar effect and the expected stimulation due to thymine was also largely removed. Uracil failed to remove the oscillation although it was reduced.

4. A provisional explanation of this phenomenon is given in terms of a basic inhibitory effect of 2-TC on mitosis together with a stimulation caused by low-level activation of a substrate of the kind observed by Comorosan (1970).

Acknowledgments.—We wish to thank the Smith Kline and French Foundation for financial support, the Department of Medical Illustration, Manchester Royal Infirmary, for help with fig. 1, and Mrs Susan Brooks for typing the manuscript.

6. References

COMOROSAN, S. 1970. New mechanism for the control of cellular reactions: the biochemical flip-flop. *Nature, 227*, 64-65.

HUGULEY, C. M., BAIN, J. A., RIVERS, S. L., AND SCOGGINS, R. B. 1959. Refractory megaloblastic anaemia associated with excretion of orotic acid. *Blood, 14*, 615-634.

MACKINNEY, A. A., STOHLMAN, F., AND BRECHER, G. 1962. The kinetics of cell proliferation in cultures of human peripheral blood. *Blood, 19*, 349-358.

MCLEISH, J. 1953. The action of maleic hydrazide in *Vicia*. *Heredity, 6* (Suppl.), 123-146.

MERCER, F. L., LINDHORST, T. E., AND COMMONER, B. 1953. Inhibition of tobacco mosaic virus biosynthesis by 2-thiopyrimidines. *Science, 117*, 558-559.

MORGAN, J. F., MORTON, H. J., AND PARKER, R. C. 1950. Nutrition of animal cells in tissue culture. 1. Initial studies on a synthetic medium. *Proc. Soc. exp. Biol. Med., 73*, 1-8.

NEU, R. L., LEAO, J. C., AND GARDNER, L. I. 1966. Effects of actinomycin D and puromycin on chromosomes of cultured human lymphocytes. *Nature, 211*, 881.

PISCIOTTA, A. V., AND DEPREY, C. 1967. Inhibition of mitosis by chloramphenicol in phytohaemagglutinin stimulated lymphocytes. *Blood, 30*, 457-464.

PRICE, D. J., AND TIMSON, J. 1971. 6-thioguanine: antimitotic effect on human lymphocytes *in vitro* prevented by adenine. *Brit. J. Cancer* (in press).

TIMSON, J. 1968*a*. Paracetamol and mitosis. *Lancet, ii*, 1084.

TIMSON, J. 1968*b*. The effect of 2-thiouracil on the division of phytohaemagglutinin-stimulated human lymphocytes *in vitro*. *Biochem. Pharmacol., 17*, 325-326.

TIMSON, J. 1968*c*. The effect of maleic hydrazide on the mitosis of phytohaemagglutinin-stimulated human lymphocytes cultured *in vitro*. *Caryologia, 21*, 157-159.

TIMSON, J. 1969*a*. Effect of testosterone on the division of phytohaemagglutinin stimulated human lymphocytes *in vitro*. *Wilhelm Roux'Archiv., 162*, 95-96.

TIMSON, J. 1969*b*. Effect of orotic acid on the mitosis of phytohaemagglutinin stimulated human lymphocytes in culture. *Wilhelm Roux'Archiv., 164*, 199-200.

TIMSON, J. 1970. The effect of caffeine on the mitosis of human lymphocytes in culture. *Brit. J. Pharmacol., 38*, 731-734.

TIMSON, J., AND PRICE, D. J. 1970. Use of thymine to increase the yield of mitoses in lymphocyte cultures. *Lancet, i*, 1397-1398.

ENDOSPERM FAILURE AND INCOMPATIBILITY IN CROSSES BETWEEN *TRITICUM* AND *SECALE*

J. P. MOSS

Department of Agricultural Botany, University of Reading

1. Introduction

Different species of wheat and, in some cases, different cultivars, vary in their crossability with rye (Backhouse, 1916; Riley and Chapman, 1967). Some crosses produce viable seed, others (especially the tetraploid wheats) produce a large but inviable shrivelled seed, and others produce only a very small inviable seed. Backhouse (1916) and Lein (1943) showed that crossability was controlled by two genes, and Riley and Chapman (1967) located these on chromosomes 5B and 5A.

In many of the species that produce a shrivelled seed the embryo is viable if excised and placed on a suitable medium, and this indicates that one barrier to natural hybridisation occurs after fertilisation. Zeven and Heemert (1970) have recently shown there is no difference in pollen tube growth between cultivars of *Triticum aestivum*, which do not produce a viable seed when pollinated with *Secale segetale*, and cv. " Chinese Spring ", which does produce a viable seed. They conclude that the barrier is not in the stigma, style or ovary wall, though they have not seen pollen tubes entering the micropyle.

The present study was initiated in an attempt to ascertain the cause of the failure of the endosperm, and whether this failure was the only barrier to crossability.

2. Materials and Methods

All species used in the present study are listed in table 4 (p. 128). Plants for the study of chromosome behaviour in the endosperm were grown under controlled conditions in the glasshouse, emasculated, bagged and hand pollinated. The spikes were fixed in 1 : 3 acetic alcohol and stored in 80 per cent. alcohol until required. The seeds were removed from the florets and placed in 60 per cent. and 40 per cent. alcohol for 15 minutes each to increase the water content and to soften them. The ovaries were hydrolysed for 10 minutes in 1 N HCL at 60° C., and stained in leuco basic fuchsin for one to two hours. The ovaries were then dissected in aceto-carmine under a binocular microscope at 25 to 50 times magnification. The debris was removed, and a coverslip gently placed on top. All stages from meiosis in the embryosac mother cell to mature endosperm have been observed by this means. The slides were scored before making permanent wherever possible.

Ovaries for sectioning were fixed in formalin acetic alcohol overnight (1 part formalin, 1 part glacial acetic acid and 18 parts 70 per cent alcohol by volume), transferred through a series of alcohols and alcohol chloroform mixtures into chloroform, and embedded in 54° wax under reduced pressure for 30 to 40 minutes. Sections were cut at 5 or 10μ and stained in Feulgen reagent and fast green.

3. Results

(i) *The embryosac in selfed* Triticum *species*

The embryosac is pear shaped, and contains two synergids, the egg nucleus, two polar nuclei and a variable number of antipodal cells (plate I, fig. 1, table 1). The egg cell and polar nuclei react weakly to Feulgen stain, though the antipodal nuclei are darkly stained.

Table 1

Number of antipodal cells in embryosacs of species of Triticum *and* Secale

	Minimum	Maximum	Mean	No. of embryosacs
A. *Triticum*				
Diploids				
T. aegilopoides	13	29	19·5	13
T. monococcum	5	26	15·0	70
All diploids	5	29	15·7	83
Tetraploids				
T. carthlicum	4	26	17·6	15
T. dicoccoides	6	30	14·8	12
T. dicoccum	12	28	16·6	10
T. orientale	10	26	15·7	21
T. timopheevi	7	25	16·0	73
All tetraploids	4	30	16·1	131
Hexaploids				
T. aestivum	3	21	12·0	88
B. *S. cereale*				
Diploid	5	20	8·4	13
Tetraploid	9	17	12·4	5

(ii) *The antipodal cells*

In the early stages of embryosac formation the antipodals divide, to increase from the three originally produced to the numbers observed (table 1). At first the antipodals are the same size as the other cells, and as the embryosac matures endomitosis occurs to produce the giant antipodal cells observed at the time of fertilisation, when the antipodals are in a state resembling interphase, with large, well stained nuclei (plate I, fig. 1). They are many times larger than the diploid nucellar cells, and are not all of the same size. After fertilisation, the antipodals follow a constant pattern of degeneration, first becoming stranded (plate I, fig. 2), then having a very dense, amorphous appearance, and they are not seen in embryos more than six days after pollination.

When the strands appear in the nucleus, they can either be uniformly distributed, or there can be a number of darker areas, the endochromocentres (Odenbach, 1965; Lange, 1969) within the nucleus. In a few cases these resolve themselves into structures resembling giant chromosomes (plate II, fig. 6), though without the detail that has been seen by Sharma (1964) in *Hyacinthus*, Enzenberg (1961) in *Phlomis*, or Beaudry (1951) and Jones and Omidiji (1970) in Gramineae. The number of chromocentres and the number of bodies seen approximate to, but never exceed, the haploid number of the species. As the endosperm develops, the nucleus and cytoplasm of the antipodals are no longer distinguishable, and the antipodal cell is seen as a darkly staining amorphous mass, which finally breaks up into globules and disperses (plate II, fig. 7).

(iii) *The development of embryo and endosperm*

The embryo divides about once every 24 hours, to produce a 32 or 64 cell embryo by the fifth day after pollination. The endosperm divides more rapidly, the first divisions are synchronous and no cell walls are formed (plate I, fig. 2). This synchrony ceases after about four days, cell walls are produced, and the thousand cell endosperm begins to produce starch.

(iv) *Types of abnormal nuclei in the endosperm*

The types of chromosome abnormalities which occur in the endosperm of species of *Triticum*, *Secale* and their hybrids are as follows:

(*a*) Chromosome breakage and reunion.
(*b*) Bridges, with and without fragments.
(*c*) Dumb-bell and hyperpolyploid nuclei.

In the first few days of endosperm development, no cell walls are formed, and the nuclei are dispersed in a common cytoplasm (plate I, fig. 2). The bridges formed by breakage and reunion can persist into the following interphase to give rise to the dumb-bell nuclei and, by restitution, to the hyperpolyploid nuclei. Many of these hyperpolyploid nuclei are then unable to divide normally, forming instead tripolar spindles, which in turn give rise to nuclei of varying sizes, or unseparated daughter nuclei at anaphase (dumb-bell nuclei), or whip tail nuclei, which have separated, but the chromatin has been drawn out into a long " tail ". Any micronuclei produced are able to continue dividing during the early synchronous divisions, in phase with the other nuclei and apparently assisted by them in the common cytoplasm, but when cell walls have been formed, most micronuclei do not divide.

(v) *Frequency of nuclei with chromosome aberrations*

The frequency of the different types of abnormalities differs in the endosperms of wheat, rye, and their hybrids, and in endosperm at different stages of development (table 2). There are less than 1 per cent. of aberrant

TABLE 2

Frequency of nuclei with chromosome abnormalities in T. aestivum *cv. Chinese Spring*, S. ancestrale *and their hybrid, expressed as percentage of total aberrant nuclei*

	T. aestivum	*S. ancestrale*	*T. aestivum* × *S. ancestrale*	
Age of endosperm (Days after pollination)	4	4	4	5
Bridges, fragments and micronuclei	60·0	100·0	32·1	21·6
Spindle faults, hyperpolyploid nuclei and amorphous chromatin masses	40·0	0·0	67·9	78·4
Total aberrant nuclei	10	6	207	283
Total nuclei scored (approx.)	2000	1000	5000	7000

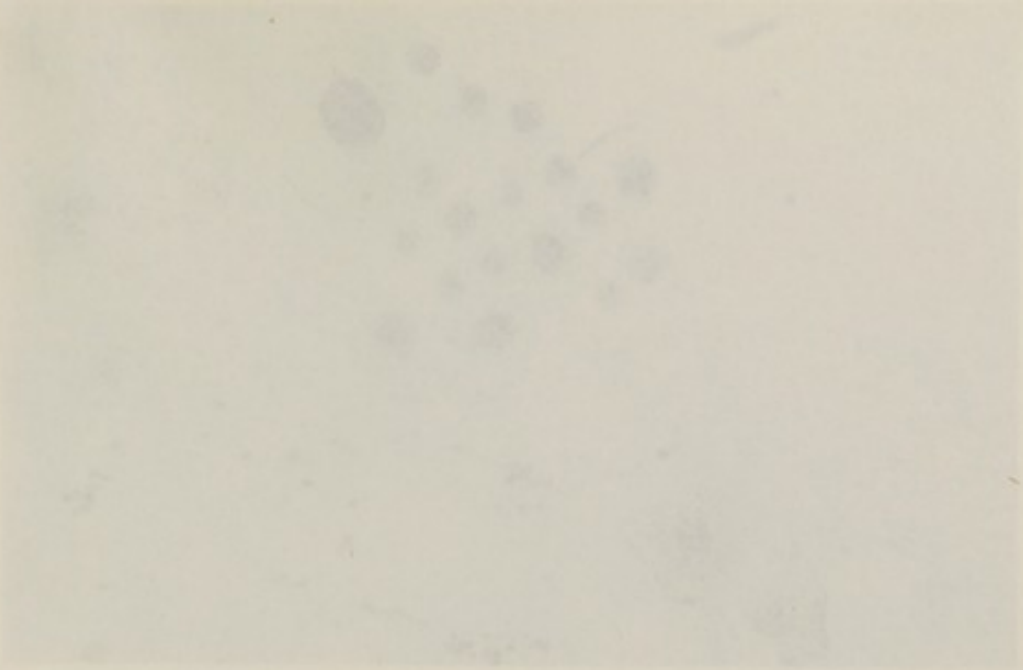


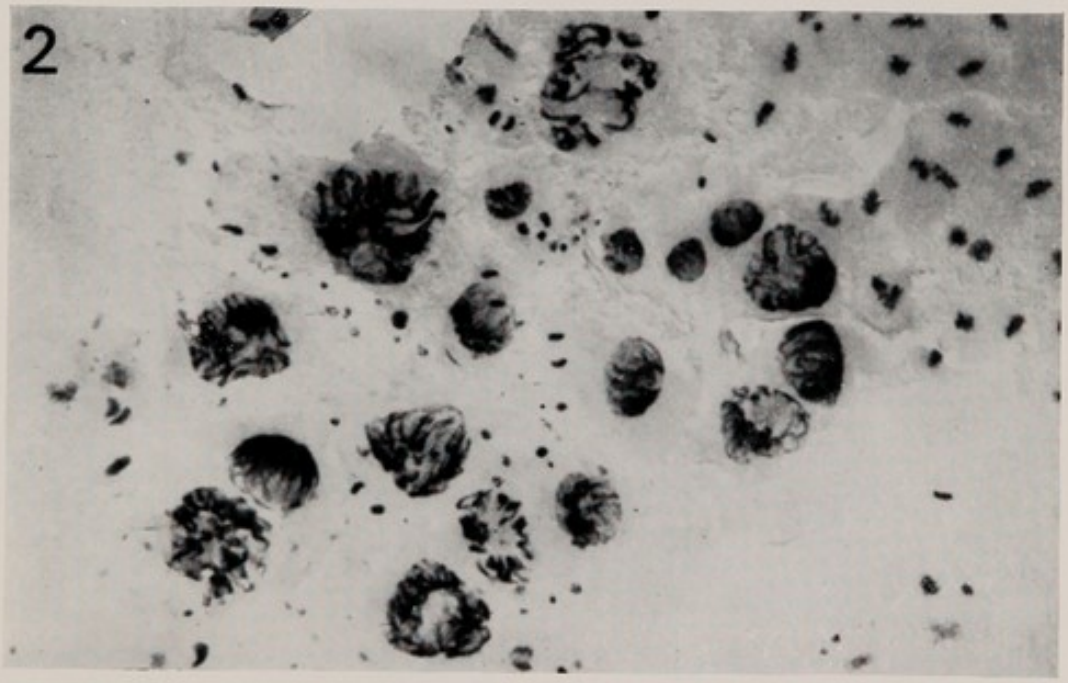
Plate I

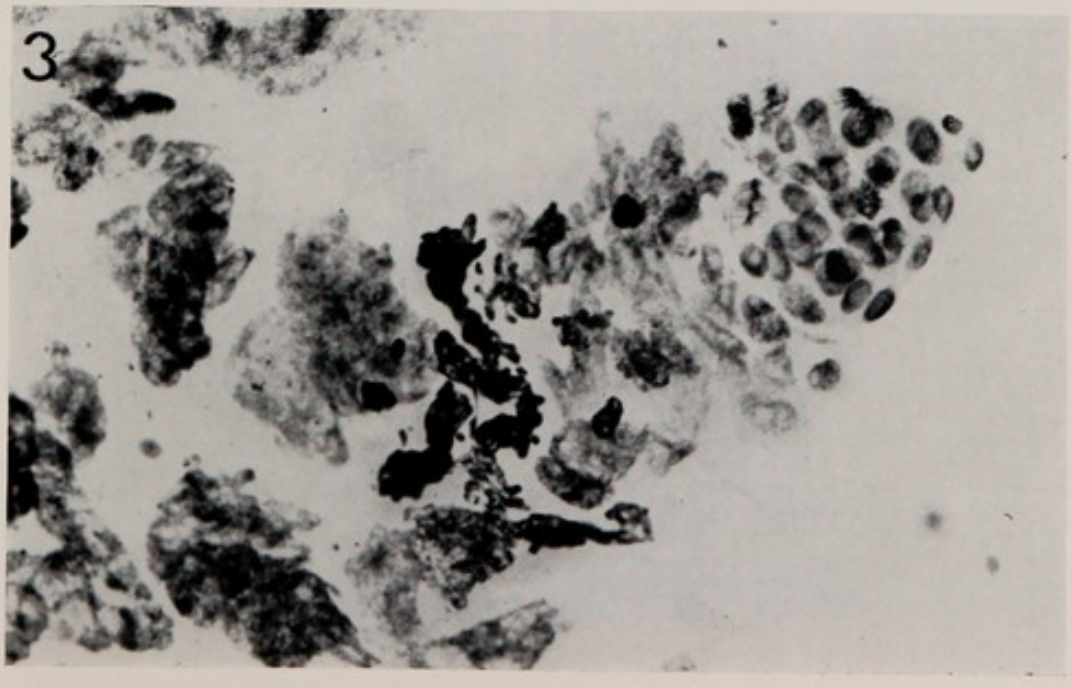
FIG. 1.—Embryosac of *T. carthlicum* comprising two synergids, egg nucleus, two polar nuclei (fused) and 18 antipodal cells (×80).

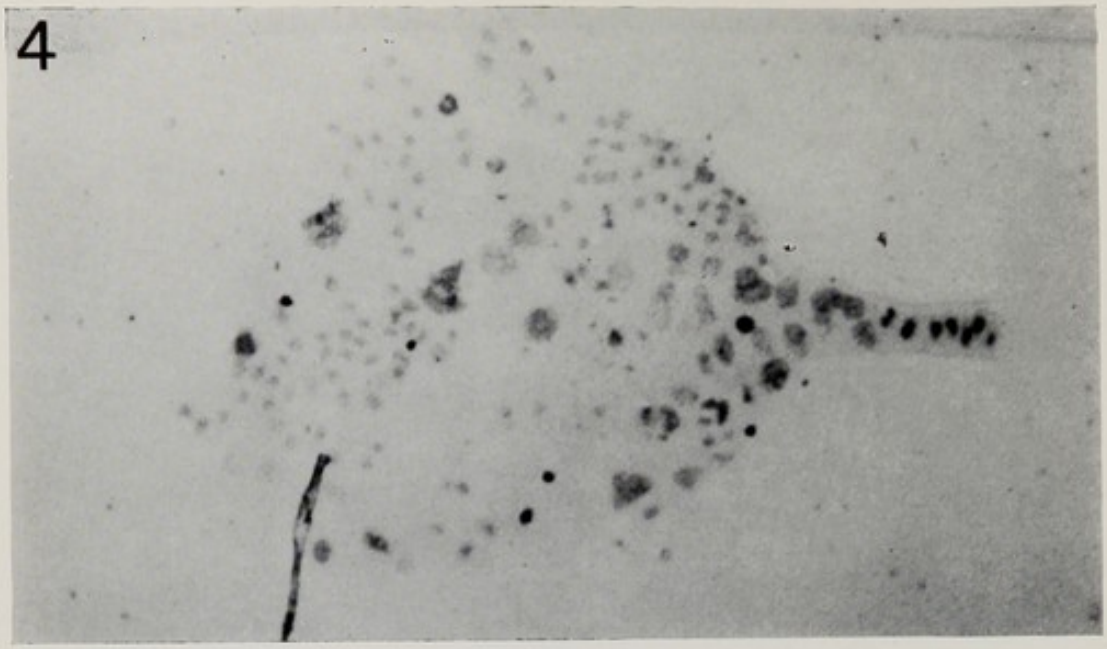
FIG. 2.—The antipodal cells of *T. aestivum*. The stranded nature of some nuclei can be seen. The nuclei at top right are endosperm nuclei (×100).

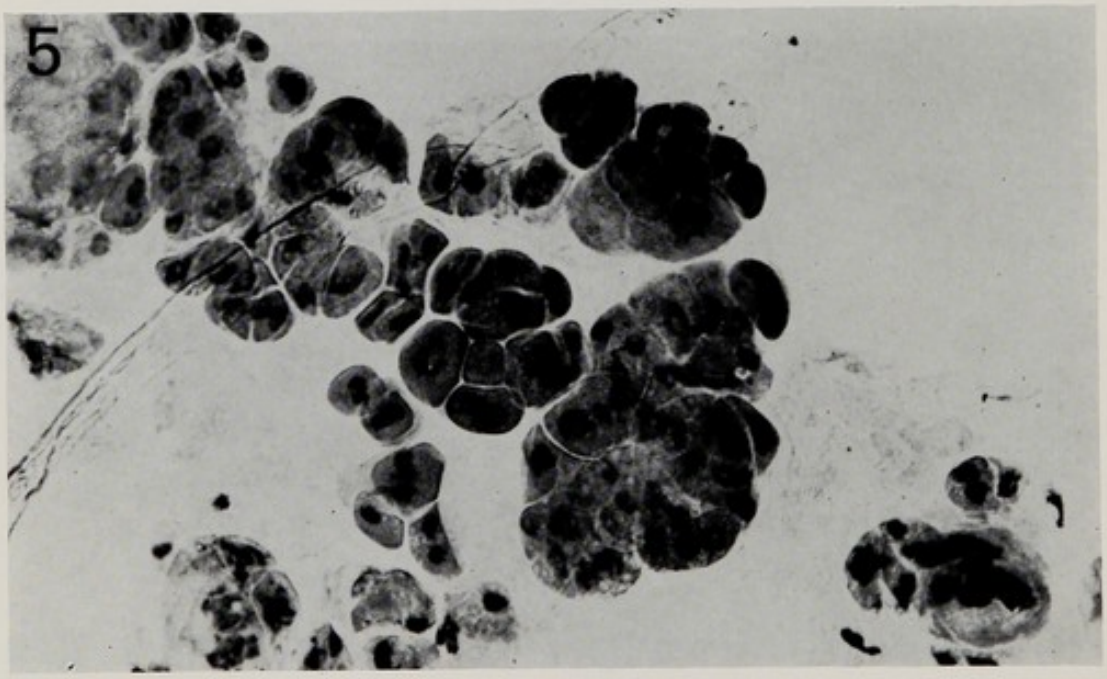
FIG. 3.—Degenerating endosperm in *T. aestivum* × *S. ancestrale*. The normal embryo is at the top right, the region adjacent to it consists of hyperpolyploid and degenerating nuclei (×200).

1


2


3


4


5

6

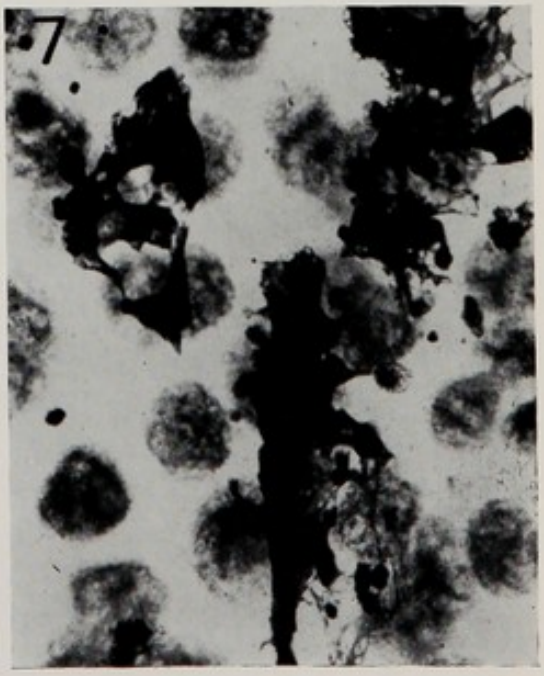

7

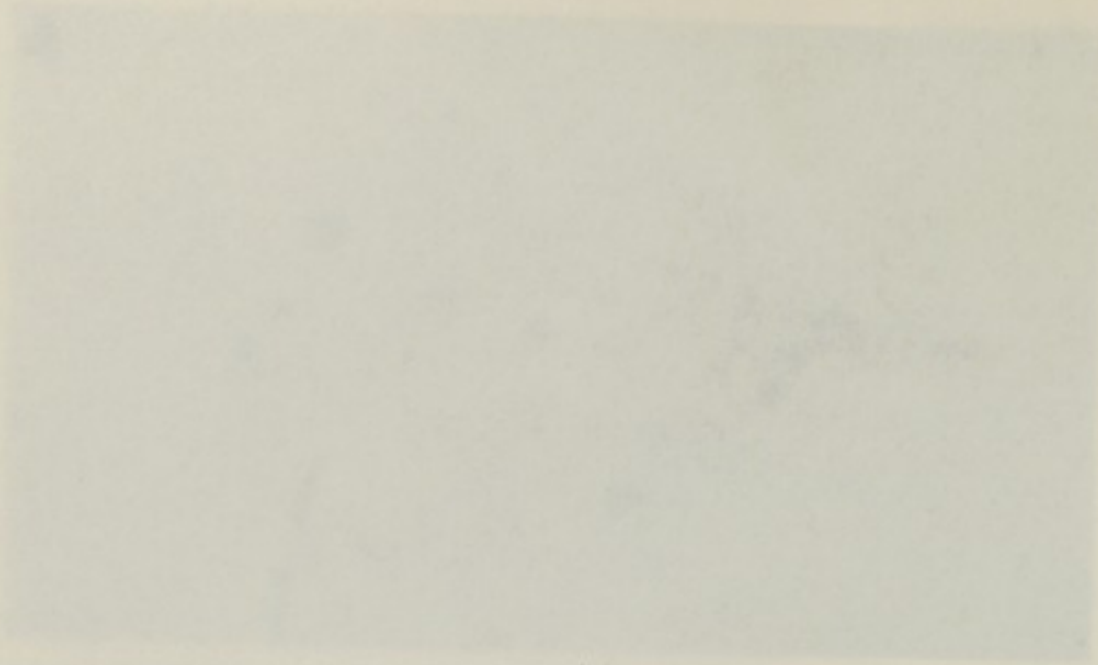
Plate II

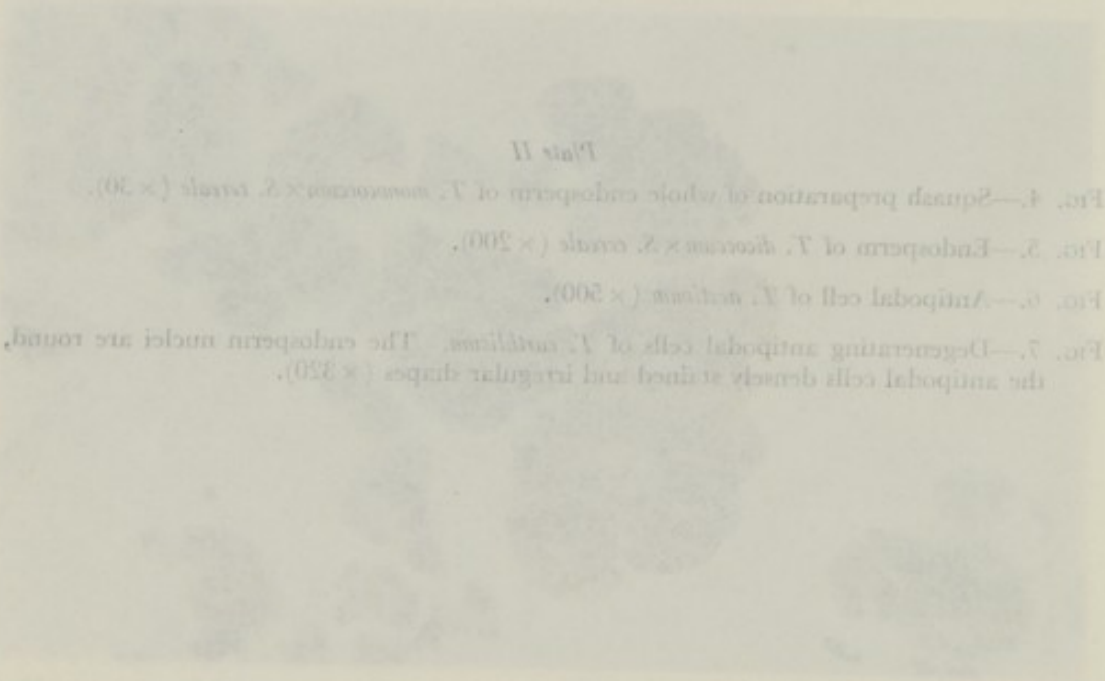
FIG. 4.—Squash preparation of whole endosperm of *T. monococcum* × *S. cereale* (× 30).

FIG. 5.—Endosperm of *T. dicoccum* × *S. cereale* (× 200).

FIG. 6.—Antipodal cell of *T. aestivum* (× 500).

FIG. 7.—Degenerating antipodal cells of *T. carthlicum*. The endosperm nuclei are round, the antipodal cells densely stained and irregular shapes (× 320).





nuclei in the selfed endosperm, and most of these are simple bridges, fragments and micronuclei.

The frequency of aberrant nuclei at four days is eight times greater in the hybrid than in either parent, but the proportion of aberrant nuclei in the divisions immediately following breakage is lower in the hybrid. Most of the abnormalities are those involving the spindle, either as tri-polar spindles, dumb-bell or hyperpolyploid nuclei, or a complete degeneration into amorphous chromatin masses.

The five day hybrid endosperm shows a different position to any of the above. The cell number has doubled, synchronisation is breaking down and the rate of division is slower. The frequency of the different types of aberration has changed, there being fewer in the divisions immediately following breakage, and a higher proportion of spindle faults.

(vi) *Position of aberrant nuclei*

It is possible, with the technique used, to remove all unwanted material and leave the embryo and endosperm intact on the slide. I was thus able to score the positions in the endosperm of the different types of aberration (table 3). There is a marked increase in the number of spindle faults and

TABLE 3

Distribution of abnormal nuclei in hybrid endosperms four days after pollination, in relation to embryo

Region	Proximal	Median	Distal
Bridges, fragments and micronuclei	18	26	23
Spindle faults and hyperpolyploid nuclei	28	6	3
Complete degeneration	90	10	26

hyperpolyploid and degenerating nuclei in the region near the embryo. In most hybrids the cells next to the embryo are hyperpolyploid, and in many cases there is a zone of degenerating nuclei in this region (plate I, fig. 3). These findings have been confirmed in sections.

(vii) *The behaviour of the embryosac in certain* Triticum × Secale *crosses*

(a) *Hexaploids*

T. aestivum cv. Chinese Spring × *S. cereale*

The frequency of aberrations is higher than in either parent, but over 90 per cent. of cells do not contain any visible aberrations, and about 75 per cent. of florets pollinated produce viable hybrid seed (table 4).

Five other cultivars have been studied. About 1 per cent. of pollinated florets produce seeds, though these are very shrunken and completely inviable, and the embryo dies before the seed ripens. The endosperm develops slowly, becomes cellular at an early stage (2 days after pollination) and ceases developing about 6 days after pollination. There is no evidence of fertilisation having occurred in those florets which do not produce any seed.

(b) *Tetraploids*

All hybrid seed produced by the tetraploids is shrivelled at maturity, though in many cases the embryo remains viable up to 21 days after pollination, and hybrids can be produced by embryo culture. The seed set varies from nil to 40 per cent. (table 4).

TABLE 4

Seed set per 100 ovules in intergeneric crosses (with number of pollinations in brackets)

	Male parent		
Female parent	*S. ancestrale*	*S. cereale* (2x)	*S. cereale* (4x)
2x			
T. monococcum		0·0 (104)	0·0 (26)
T. aegilopoides		0·0 (42)	0·0 (38)
4x			
T. carthlicum		10·5 (38)	4·9 (82)
T. timopheevi	38·5 (52)	23·8 (80)	12·9 (132)
T. polonicum	6·3 (16)	2·4 (126)	8·0 (100)
T. durum	14·3 (14)	8·4 (26)	15·4 (130)
T. orientale	0·0 (36)	0·0 (36)	0·0 (24)
T. pyramidale	12·1 (58)		0·0 (100)
T. dicoccum	40·7 (96)	13·3 (90)	11·0 (100)
T. dicoccoides	39·4 (38)		35·0 (80)
6x			
T. aestivum			
Jufy I and Svenno		1·5 (204)	
Chinese Spring	74·8 (127)	74·0 (74)	53·6 (112)

Although the development of the embryo appears normal, the older endosperm consists entirely of aberrant nuclei, mostly hyperpolyploid nuclei which form groups of cells of different sizes (plate II, fig. 5). The caryopsis elongates, but as no bulk of endosperm is produced, it forms a shrivelled seed that will not germinate.

Measurements were made of all ovaries prior to dissection. Some ovaries which had elongated were found to contain undivided egg cell and polar nuclei, though they were the same length as fertilised ovaries. Other unfertilised ovaries had not elongated. This ovary stimulation was most marked in *T. timopheevi* × *S. cereale*, a cross in which the ovary elongates rapidly in the first few days after pollination (Moss, 1965).

(*c*) *Diploids*

The endosperm of the hybrids with the diploids contains few normal cells. At 5 days it consists of a number of hyperpolyploid nuclei (plate II, fig. 4) which form groups of cells of different sizes as in the tetraploids. These do not divide further or produce starch, and the developing seed collapses entirely. The embryo appears normal for the first few days, but then ceases to divide, and dies. At maturity one cannot differentiate between fertilised and unfertilised florets.

(viii) *The use of tetraploid* Secale cereale *as pollen parent*

The crossability with rye depends more on the species of wheat than on whether the rye is diploid or tetraploid (table 4). In every case where

there are comparable figures, there is no evidence of the tetraploid producing an increase or decrease in seed set, or of producing a more or less viable mature hybrid seed than the diploid.

(ix) *The use of* Secale ancestrale, S. segetale *and* S. vavilovii *as pollen parents*

These three species are all closely related to *Secale cereale*, and have been used in 16 intergeneric crosses with seven different *Triticum* species. There is no difference between *S. ancestrale* and *S. cereale* in endosperm behaviour, though seed set is greater with *S. ancestrale* and less with the other two species (Moss, 1965).

4. Discussion

(i) The antipodals

Although in some cases the antipodals contain structures resembling metaphase chromosomes, I have never seen mitotic divisions after fertilisation, and the antipodals soon break down and disappear. The increase in size, without any cells in anaphase or telophase, may be a result of endomitosis by abbreviation of the cell cycle (Nagl, 1970).

There is no correlation in my results between the position of the antipodals and the aberrant nuclei, as reported by Cooper and Brink (1944), Boyes and Thompson (1937) and Kihara and Nishiyama (1932) in *Hordeum* × *Secale* crosses and intergeneric crosses in wheat and oats respectively.

Their exact function remains uncertain, but they are degenerating and being absorbed during the first few days of the growth of the seed. This is a time of very rapid growth (Moss, 1965), when the nutritional demand must be great, and there is no evidence that their function is anything other than purely nutritive, and their increase in number and size would be to provide a reserve of nutrients. This is supported by the behaviour of the endosperm in *Avena* crosses, where regeneration of the endosperm occurs near the antipodal cells (Kihara and Nishiyama, 1932), though Beaudry (1951) found that the endosperm began to degenerate in this region in *Elymus* × *Agropyron* crosses.

(ii) Ovary stimulation

The term ovary stimulation has been used in intergeneric hybridisation to indicate the enlargement of the ovary without the production of a viable or culturable embryo. The results presented here show that enlargement can occur without either the egg cell or the polar nuclei dividing, though they may have been fertilised. This stimulation, mainly in *T. timopheevi* × *S. cereale*, is most likely caused by the fertilisation, without further development, of the egg cell and polar nuclei, and not by the growth of the pollen tubes, as Zeven and Heemert (1970) observed pollen tubes which had grown as far as the micropyle in 25 incompatible hexaploid wheat-rye crosses, but I have observed no stimulation without subsequent development in five similar crosses.

(iii) Types and frequency of aberrations in endosperm

The types of chromosome breakage and the presence of hyperpolyploid cells were the same as have been reported in other studies, but the frequency

of breakage in the selfed species was greater than expected. Brock (1954) did not find any breakage in selfed *Lilium*, nor Dyer (1963) in selfed *Trillium* or *Paris*.

Both Brock and Dyer conclude that chromosome breakage leads to the failure of the endosperm, and a similar situation exists in *Triticum* × *Secale* crosses. In the young endosperm there is a larger proportion of nuclei in the divisions immediately following breakage than in the older endosperm, when hyperpolyploid nuclei are more common. This indicates that in these crosses (as in *Lilium* × *Lilium* and *Paris* × *Trillium*) chromosome breakage, causing bridges and the loss of fragments, is the primary cause of endosperm failure. The resulting nuclei may degenerate, or attempt to divide and produce hyperpolyploid nuclei by restitution, or continue dividing in a bridge breakage fusion cycle (McClintock, 1941). Some of the hyperpolyploid nuclei are unbalanced, having lost chromosomes or parts of chromosomes, or because they are the products of tripolar spindles, and may degenerate for this reason. Others do not divide again, and give rise to an endosperm with few large cells (plate II, fig. 4). These cells near the embryo become hyperpolyploid and cease dividing or degenerate.

The cause of endosperm failure is undoubtedly chromosome breakage, bridges and the formation of hyperpolyploid restitution nuclei which lead to split spindles and unbalanced nuclei. The cause of chromosome breakage is less certain. In the wheat-rye crosses reported here the frequency of chromosome breakage decreases as the rate of division decreases, and it is possible that there are errors in replication at high rates of division, though breakage is rare in the selfed species. The presence of a zone of irregularities near the embryo could indicate a reaction between the endosperm and the embryo. Other hypotheses have been put forward. Watkins (1932), Thompson and Cameron (1928) and Rutishauser (1956) all thought the cause was a change in the normal ratio of chromosome numbers between the tissues concerned. However, differences within the diploids, tetraploids and hexaploids indicate that this is not the cause of endosperm failure. Also there are differences in the degree of breakdown in interspecific crosses in *Triticum*, where the ratios of chromosome numbers are the same (Boyes and Thompson, 1937). This indicates that the genotype itself is important, and the necessity for a careful study of a range of cultivars within certain species.

(iv) The causes of incompatibility in *Triticum* × *Secale* crosses

Lein (1943) showed that two genes, Kr_1 and Kr_2, were responsible for incompatibility between wheat and rye. The majority of *T. aestivum* cultivars have the genotype Kr_1Kr_1, Kr_2Kr_2, but " Chinese Spring " is homozygous recessive and crosses readily with rye. If these genes control chromosome behaviour in the endosperm, we would expect a large number of hybrid seed in incompatible crosses to begin development and then collapse. This only occurs in 1·5 per cent. of florets pollinated with rye, and the other 98·5 per cent. show no signs of fertilisation or endosperm development. Thus endosperm failure is not the only cause of incompatibility. It is more important as an incompatibility mechanism in the tetraploids than in the hexaploids, as in the former up to 50 per cent. of seeds can begin development. The substitution line " Chinese Spring " " Hope 5A "

genotype kr_1kr_1, Kr_2Kr_2, sets 26·2 per cent. seed (Riley and Chapman, 1967) and it is possible that this genotype is similar to that of the tetraploids, though other genes may be involved.

Thus, two incompatibility mechanisms are operative in *Triticum* × *Secale* crosses. One, the *Kr* system, operates between the time of penetration of the pollen tube into the micropyle and the first division of the fertilised egg cell and polar bodies. This is the more important of the two in the hexaploids. The second operates when the embryo and endosperm have begun to develop, causing endosperm failure and the production of an inviable seed. This is the more important in the tetraploids, which may not have both dominant *Kr* genes. " Chinese Spring " is compatible with rye because neither barrier is completely effective, as fertilisation occurs and there are sufficient normal cells in the endosperm to produce 75 per cent. of viable seeds.

5. Summary

The collapse of developing seed in *Triticum* × *Secale* crosses is caused by endosperm failure which results from chromosome breakage and subsequent aberrations.

2. Chromosomal and nuclear aberrations are not uniformly distributed in the endosperm but are more frequent near the embryo.

3. There is no evidence for the antipodal cells having any other role than a nutritive one.

4. " Ovary stimulation " is not caused by the growth of pollen tubes, but can occur in ovaries where there is no development of embryo or endosperm.

5. The proportion of ovules fertilised is larger in the tetraploids than in the hexaploids.

6. Two incompatibility mechanisms are operative in *Triticum* × *Secale* crosses, the *Kr* system before or at the time of fertilisation, and endosperm failure during seed development.

Acknowledgments.—This study was started in the Botany School, Oxford and I would like to thank Professor C. D. Darlington for all his help and encouragement. I would also like to thank Mrs J. Gallon (Christine Cox) for technical assistance. Financial support from the Ministry of Agriculture and the Science Research Council is gratefully acknowledged.

6. References

BACKHOUSE, W. O. 1916. A note on the inheritance of crossability. *J. Genet.*, *6*, 91-94.

BEAUDRY, J. R. 1951. Seed development following the mating *Elymus virginicus* × *Agropyron repens* L Beauv. *Genetics*, *36*, 109-133.

BOYES, J. W., AND THOMPSON, W. P. 1937. The development of the endosperm and embryo in reciprocal interspecific crosses in cereals. *J. Genet.*, *34*, 203-227.

BROCK, R. D. 1954. Spontaneous chromosome breakage in *Lilium* endosperm. *Ann. Bot.*, *18*, 7-14.

COOPER, D. C., AND BRINK, R. A. 1944. Collapse of seeds following the mating of *Hordeum jubatum* × *Secale cereale*. *Genetics*, *29*, 370-390.

DYER, A. F. 1963. Endosperm development after controlled pollination within and between species of *Trillium* and *Paris*. *Chromosoma*, 14, 549-567.

ENZENBERG, U. 1961. Beitrage zur Karyologie des Endosperms. *Österr. Botan. Zeitsch.*, *108*, 245-285.

JONES, J. K., AND OMIDIJI, M. O. 1970. Chromosomes in antipodal cells of *Aegilops* species and of *Hordeum vulgare* (Abstract). *Chromosomes Today*, *3*, 303.

KIHARA, H., AND NISHIYAMA, I. 1932. The genetics and cytology of certain cereals. II. Different compatibility in reciprocal crosses of *Avena*. *Jap. J. Bot.*, *6*, 245-305.

LANGE, W. 1969. Cytogenetisch en embryologisch onderzoek aan kruisingen tussen *Hordeum vulgare* en *H. bulbosum*. Ph.D. Thesis, Wageningen.

LEIN, A. 1943. Die Genetische Grundlage der Kreuzbarkeit zwiscen Weizen und Roggen. *Z. indukt. Abstamm.-u Vererb Lehre*, *81*, 28-61.

MCCLINTOCK, B. 1941. The stability of broken ends of chromosomes in *Zea mays*. *Genetics*, *26*, 234-282.

MOSS, J. P. 1965. D.Phil. Thesis, Oxford University.

NAGL, W. 1970. Inhibition of polytene chromosome formation in *Phaseolus*, by polyploid mitoses. *Carylogia*, *35*, 252-258.

ODENBACH, W. 1965. Histologische und cytologische Untersuchungen der Entwicklungsvorgänge nach der Bestäubung von Gerste mit Roggen. *Z. PflZücht*, *53*, 1-52.

RILEY, R., AND CHAPMAN, V. 1967. The inheritance in wheat of crossability with rye. *Genet. Res.*, *9*, 259-267.

RUTISHAUSER, A. 1956. Chromosome distribution and spontaneous chromosome breakage in *Trillium grandiflorum*. *Heredity*, *10*, 367-407.

SHARMA, V. B. 1964. D.Phil. Thesis, Oxford University.

THOMPSON, W. P., AND CAMERON, D. R. 1928. Chromosome numbers in functioning germ cells of species hybrids in wheat. *Genetics*, *13*, 456-469.

WATKINS, A. E. 1932. Hybrid sterility and incompatibility. *J. Genet.*, *25*, 125-162.

ZEVEN, A. C., AND C. VAN HEEMERT. 1970. Germination of pollen of weed rye (*Secale segetale*) on wheat (*Triticum aestivum* L.) stigmas and the growth of the pollen tubes. *Euphytica*, *19*, 175-179.

CHROMOSOMALLY MARKED POPULATIONS OF CELLS IN HUMAN BONE MARROW

SYLVIA D. LAWLER

Department of Clinical Research, Royal Marsden Hospital/Institute of Cancer Research, Fulham Road, London, S.W.3

1. Introduction

Chromosomally marked populations of human bone marrow cells may be found in untreated patients with chronic or acute myeloproliferative disorders. Alternatively, they can be induced by, for example, β irradiation with radioactive phosphorus, which is the treatment favoured in some centres for the management of cases of polycythaemia vera—a chronic myeloproliferative disorder.

Unfortunately the technique for preparing chromosome spreads results in a loss of certain cytological characteristics of a cell. When looking at a metaphase spread of a bone marrow cell it is not possible to know whether the cell is an erythrocytic or granulocytic precursor in division. Red cell precursors have been identified by the demonstration of iron in metaphase spreads by the use of iron stains (Clein and Flemans, 1966) or radioactive iron (Rastrick *et al.*, 1968). It is legitimate to assume that the polyploid cells seen in bone marrow metaphases are platelet precursors in mitosis, because such cells are known to be polyploid from cytological observations.

In a normal individual the formed elements in the peripheral blood having a myeloid origin are differentiated cells that are incapable of further proliferation. Thus the granulocytic white cells of normal peripheral blood cannot be stimulated to go into mitosis. In both chronic and acute myeloproliferative disorders less differentiated cells that are capable of dividing in *in vitro* cultures may spill over from the bone marrow into the peripheral circulation. In these circumstances it is possible to obtain dividing cells of bone marrow origin by culturing peripheral blood without the addition of mitotic stimulants.

The chromosomal constitution of normal individuals is usually determined by stimulating the lymphocytes of peripheral blood to divide by activating them with mitotic agents. Normal lymphocytes do not enter mitosis unless stimulated. When examining a patient with a disease of the bone marrow it is usual to culture the lymphocytes in order to make sure that an abnormality found in bone marrow cells is acquired and not constitutional.

2. Chromosomes and clinical state in polycythaemia vera

Chromosomally marked populations of bone marrow cells can be exploited in various ways. First, they may be studied in relation to the disease as evinced by the fluctuation in the levels of the formed elements in the peripheral blood. It is not uncommon (about 25 per cent. of cases) for patients with chronic myeloproliferative disorders to have some degree of aneuploidy of the bone marrow cells at the time of diagnosis. This aneuploidy may take the form of extra C group chromosomes in a variable proportion of cells either in males or females; or of an almost entirely Y− population in constitutionally normal males. The significance of the missing Y chromosomes must be viewed in the context that *minor* populations of bone marrow cells lacking a Y chromosome may sometimes be found in

elderly, haematologically normal males. (O'Riordan *et al.*, 1970; Secker-Walker, 1971). Amongst the patients with polycythaemia vera studied at the Royal Marsden Hospital is a former jockey who has fathered sons, whose bone marrow at the time that polycythaemia vera was diagnosed was entirely populated by 45,X,Y− cells. As shown in table 1 after treatment the level of haemoglobin fell, and also the white blood cell count. When the level of haemoglobin fell the proportion of cells lacking a Y chromosome

TABLE 1

Polycythaemia vera, case 8, male
Serial studies

	Hb. (g/100 ml.)	%45,X,Y− cells
Feb. 1965	23·2	100
July 1965	14·8	69
Jan. 1966	16·7	72
Aug. 1967	17·5	100
May 1968	13·2	66
May 1970	15·9	97

was reduced. This evidence suggests that the cells marked by lack of a Y chromosome belong to a population that is less sensitive to regulatory controls than the 46,XY population.

Another patient had a clone of cells marked by a chromosomal abnormality, a partial deletion of the long arm of a C group chromosome, that was presumably induced by irradiation (Kay *et al.*, 1966).

The percentage of cells marked by the Cq− chromosome varied over the four years this patient has been followed up and the frequency correlated with the blood levels (Lawler *et al.*, 1970). Here again it would appear that the clonal population was less sensitive to regulatory controls, predominance of karyotypically normal cells being associated with a fall in blood levels towards normal.

Competition between different chromosomally marked populations could be demonstrated in another patient with polycythaemia vera shown in table 2. Initially, before treatment commenced, the patient had a minor population of aneuploid cells. Following treatment this population at first decreased. Three years later the patient was found to have a mixture of three types of cells, normal cells, diploid cells with a deletion in one of the F group chromosomes (an abnormality that occurs in about 15 per cent. of patients with polycythaemia vera (Millard *et al.*, 1968), and a hyperdiploid population of variable karyotype. One year later the proportion of hyperdiploid cells was substantially the same, but the ratio of diploid cells with F?− to normal cells had altered. In the latest sample examined one karyotypic rearrangement of the hyperdiploid cells had come to predominate. Cytologically the bone marrow was hypoplastic, with an excess of undifferentiated blast cells. So the success of the clone of cells with the formula 49,XX,C+2,Marl+, illustrated in plate I, fig. 1, is associated clinically with the development of a leukaemic state.

3. Chronic Myeloid Leukaemia and the Xg Blood Group System

Unlike polycythaemia vera in which the most commonly associated chromosome anomaly, the F?− has been found in only a minority of cases,

Plate I

1
2
3
B
4
5
X
6
12
Mar1
CASE 3, P.R.V. BM. DIR.
49, XX, C+2, Mar 1+
D
13
14
15
E
16
17
18
F
19
20
G
21
22

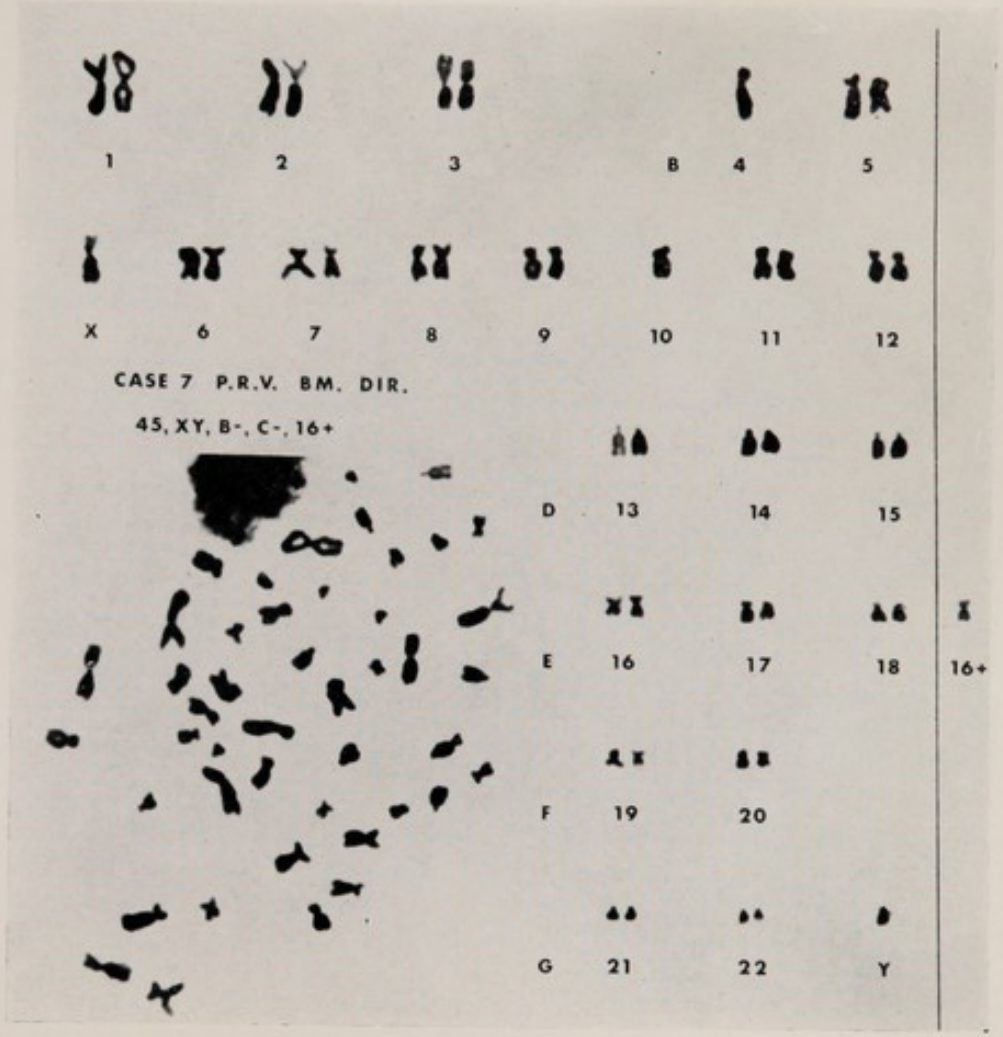

1
2
3
B
4
5
X
6
7
8
9
10
11
12
CASE 7 P.R.V. BM. DIR.
45, XY, B-, C-, 16+
D
13
14
15
E
16
17
18
16+
F
19
20
G
21
22
Y

TABLE 2

Polycythaemia vera, case 3, female
Bone marrow chromosome analysis

Date	<46	46 Normal	46 F?−	47 C+	48 C+2	48 B+C+	48 B+2	48 C+2, E+,D−	48 C+,Marl+	48 C+2,Marl+ D,E,ForG−	49 C+2,Marl+	Total cells
Aug. 1965	3	30		1	5							39
Nov. 1965	11	29		1	1							42
Mar. 1968	5 (3F?−)	13 + 1*	33		7	9	1	1	1		1	72
July 1969	1	10	11			1			1		3	27
May 1970			1							6	12	19

*Pseudodiploid cell, 46,XX,3+,B+,G−2.

chronic myeliod leukaemia is associated with the presence of a specific chromosome abnormality, the Philadelphia chromosome, Gq−. In classical cases almost all the bone marrow metaphases carry this chromosome, thus it is present in precursors of all the formed elements of the blood. The cytogenetic evidence that the red-cell precursors in this disease are members of a single clone is supported by genetical evidence. Fiaklow and his colleagues (1967) found three families with chronic myeloid leukaemia whose fibroblasts showed that they were heterozygous AB at the G-6-PD locus, but their red cells and granulocytes showed only type A. The G-6-PD locus, carried on the X chromosome, has been shown in normal females to undergo the random fixed inactivation postulated by Lyon. It is not known whether the process of inactivation includes all the loci on the X chromosome in man and this doubt applies particularly to the blood group Xg. The single clonal origin of bone marrow cells in chronic myeloid leukaemia has been exploited in the investigation of the behaviour of Xg locus. If the same X chromosome is expressed in all the red cells in these patients then only half Xg^a/Xg heterozygous females with chronic myeloid leukaemia would be expected to show the Xg^a antigen on their red cells. In a sample of patients this would result in a shift in the frequency of the Xg(a+) phenotype towards the frequency expected in males. No such disturbance in the distribution of the Xg groups was found among 48 females all of whom were known to have the Ph′ chromosome (Lawler and Sanger, 1970).

Fialkow and his colleagues (1970) in Seattle and Mexico have reached similar conclusions. Two of their patients typed as Xg(a−) and family studies demonstrated that they could be Xg/Xg homozygotes.

Provided that the Ph′ positive cells in chronic myeloid leukaemia are of monoclonal origin and the Xg^a antigen is manufactured in the erythrocytic series, these studies indicate that the Xg blood group locus is not inactivated when present in a normal X chromosome.

4. Clones and Gene Localisation

The presence of chromosomally identifiable clones in the bone marrow provides an approach to the study of gene localisation. There are many genetic markers that can be tested for by sampling peripheral blood. It is therefore important to make such tests on patients who have, or who are likely to acquire, chromosomally abnormal bone marrow cells. When a patient with polycythaemia vera was first examined in 1965 the majority of the bone marrow cells had a normal karyotype (Kay *et al.*, 1966). At that time his Rhesus type was CDe/cDE. His bone marrow in May 1970 contained a major line of cells with the formula 45,XY,B−,C−,16+, illustrated in plate I, fig. 2. The blood (no transfusion had been given) was tested again and a mixed population of red cells was found in the Rhesus system. The mixture consisted of the original CDe/cDE cells together with cells that were apparently CDe/CDe, this population totally lacking c and E antigens. The two populations were separable, the other blood groups were the same in both populations and agreed with the original testing. It is of course tempting to assume that the apparently hemizygous red cell population, CDe/- - -, represents a monosomic genetic contribution associated with chromosomal loss (Callender *et al.*, 1971).

Other rare examples of populations of red cells of mixed Rhesus types have been described, six of them in haematologically normal individuals (Race and Sanger, 1968), and two in patients with myeloproliferative disorders (Levan *et al.*, 1964; Mannoni *et al.*, 1970). Unfortunately only one other case has had the chromosomes of the bone marrow cells examined. This was a treated patient with polycythaemia vera described by Levan *et al.* whose myeloid cells had 48 chromosomes, but the authors stated that structural rearrangement resulting in the loss of chromosomal material could also have taken place.

Further evidence that these Rhesus mixtures are associated with the loss of a particular chromosome can only be obtained by bone marrow chromosomal analysis in these rare cases.

5. Summary

A chromosomally marked population of bone marrow cells can be exploited in various ways:

1. As a marker for a population resistant to homeostatic controls.
2. As a source of a monoclonal population for phenotypic studies.
3. As a means of studying gene localisation.

6. References

CALLENDER, SHEILA T., KAY, H. E. M., LAWLER, SYLVIA D., MILLARD, ROSEMARY E., RACE, R. R., AND SANGER, RUTH. 1971. *Brit. J. Med.*, *i*, 131-133.

CLEIN, G. P., AND FLEMANS, R. J. 1966. Involvement of the erythroid series in blastic crisis of chronic myeloid leukaemia. Further evidence for the presence of Philadelphia chromosome in erythroblasts. *Brit. J. Haemat.*, *12*, 754-758.

FIALKOW, P. J., GARTLER, S. M., AND YOSHIDA, AKIRA. 1967. Clonal origin of chronic myelocytic leukaemia in man. *Proc. natn. acad. sci.*, *58*, 1468-1471.

FIALKOW, P. J., LISKER, R., GIBLETT, ELOISE R., AMD ZAVALA, C. 1970. Xg Locus: Failure to detect inactivation in females with chronic myelocytic leukaemia. *Nature, Lond.*, *226*, 367-368.

KAY, H. E. M., LAWLER, SYLVIA D., AND MILLARD, ROSEMARY E. 1966. The chromosomes in polycythaemia vera. *Brit. J. Haemat.*, *12*, 507-527.

LAWLER, SYLVIA D., AND SANGER, RUTH. 1970. Xg. Blood-groups and clonal-origin theory of chronic myeloid leukaemia. *Lancet*, 584-585.

LAWLER, SYLVIA D., MILLARD, ROSEMARY E., AND KAY, H. E. M. 1970. Further cytological investigations in polycythaemia vera. *Europ. J. Cancer*, *6*, 223-233.

LEVAN, A., NICHOLS, W. W., HALL, B., LOW, B., NILSSON, S-B., AND NORDEN, A. 1964. Mixture of Rh positive and Rh negative erythrocytes and chromosomal abnormalities in a case of polycythaemia. *Hereditas, Lund*, *52*, 89-105.

MANNONI, P. P., BRACQ, CHRISTINE, YVART, J., AND SALMON, CH. 1970. Anomalie de fonctionnement du locus Rh au cours d'une myelofibrose. *Nouv. Rev. Franc. Hemat.*, *3*, 381-388.

MILLARD, ROSEMARY E., LAWLER, SYLVIA D., KAY, H. E. M., AND CAMERON, C. B. 1968. *Brit. J. Haemat.*, *14*, 363-374.

O'RIORDAN, MAUREEN L., BERRY, ELIZABETH, W., AND TOUGH, ISHBEL, M. 1970. Chromosome studies on bone marrow from a male control population. *Brit. J. Haemat.*, *14*, 83-90.

RACE, R. R., AND SANGER, RUTH. 1968. *Blood Groups in Man.* Fifth edition. Blackwell Scientific Publications, Oxford and Edinburgh.

RASTRICK, J. M., FITZGERALD, P. H., AND GUNZ, F. W. 1968. Direct evidence for presence of Ph′ chromosome in erythroid cells. *Brit. J. Med. 1*, 96-98.

SECKER-WALKER, LORNA. 1971. The chromosomes of bone marrow cells of haematologically normal men and women. *Brit. J. Haemat.* (in press).

INSTABILITY OF THE PARACENTRIC REGION AND SELECTIVE REDUPLICATION OF CHROMOSOME 2 IN MAN

M. FRACCARO, J. LINDSTEN, L. TIEPOLO and N. RICCI

EURATOM Unit for Human Radiation and Cytogenetics, and Institute of General Biology of the Medical Faculty, University of Pavia; Department of Clinical Genetics, Karolinska Hospital, Stockholm; Department of Internal Medicine, University of Ferrara

1. Introduction

Spontaneous preferential discontinuity of specific chromosomal regions is either revealed by *ad hoc* studies in healthy subjects (*e.g.* Lubs and Samuelson, 1967) or detected in the occasional individual whose chromosomes are examined because of phenotypic abnormalities. During 1964 we studied and filed a case with a distinct and variable instability of the region close to the centromere in the long arm of one chromosome No. 2. Our interest in this case was revived by a personal communication from Dr Lejeune and a note by Lejeune *et al.* (1968) reporting two related cases with a similar chromosomal peculiarity. Subsequently, we discovered that we had overlooked a similar observation made by Conen and Erkma (1966) in an untreated subject with leukaemia and Down's syndrome. While re-studying the first one, we found two further cases, referred to for chromosome analysis by Dr C. Andreoli, Vercelli, and Dr P. Zetterqvist, Stockholm. Ferrante *et al.* (1968), Reeves and Lawler (1970) and Bühler *et al.* (1970) reported a similar abnormality in a mentally retarded girl, in a healthy female and in a male child with hypospadia, respectively. Further, we know from personal communications that several colleagues have also observed the same peculiarity of one chromosome No. 2 (among others, J. H. Hamerton, J. de Grouchy, M. Ferguson Smith, M. Cohen and B. Noel).

2. Material and methods

Personal and family data of the three subjects are set out in table 1.

Table 1

Clinical and family data of the three subjects

Sex and age	Reason for chromosome examination	Phenotype	Family data
A. G. female 4.5.1964	Internal malformations	Died at 8 months. Severe hydrocephalus. Atresia of oesophagus. Malformed heart with single ventricle, agenesis of mitral valves, open foramen ovale, persistent ductus arteriosus. Right double renal pelvis and ureters.	Third of four sibs. One sister and two brothers. Mother 35 and father 36 at her birth.
Z. R. male 9.5.1965	Mental retardation	Severe mental deficiency. Unspecified alterations of the eye fundus. Onychogryphosis.	First of two boys. Two previous abortions at 2nd month. Mother 25 and father 28 at his birth.
B. F. female 3.7.1951	Secondary amenorrhea	No malformations or defects.	Only child. Mother 25 at her birth.

Cultures. Blood cultures were made by a standard method, using a concentration of $1 \times 10^6 - 1{\cdot}5 \times 10^6$ white cells/ml. of culture medium. Fibroblast cultures from a skin biopsy of A. G. were kindly made by Dr H. P. Klinger, New York.

Autoradiography. The following experiments were performed using blood cultures processed at 72 h and tritiated thymidine (^{3}H-TdR) with a specific activity of 6·2 C/mM.

(1) *Continuous labelling* with ^{3}H-TdR for the last 6 h with a dose of 0·5 μc/ml. of culture medium (experiments 4, 5, 7 in table 2).
(2) *Excess of ^{3}H-TdR* at a dose of 2 μc/ml. of culture medium for the last 6 h of culture (experiment 6 in table 2).
(3) *Presence of TdR* at a dose of 10 μg/ml. of culture medium for the last 6 h of culture (experiment 7 in table 2).
(4) *Second division* after labelling was studied after a pulse of 20 min. with a dose of 1 μc/ml. TdR at 48 h of culture (experiment 8 in table 2).
(5) *Initial phase of DNA synthesis.* Cultures were labelled at 48 h with 1 μc/ml. of ^{3}H-TdR and after 8 h washed and chased with a 100× dose of TdR. (experiment 9 in table 2).
(6) *Continuous labelling of two cycles of replication* was studied by adding 0·3 μc/ml. for the last 48 h of culture (experiment 10 in table 2).

Kodak AR 10 stripping film was used in all experiments. Grain counts were made at the microscope with photographic control.

Electronmicroscopy. Single, intact cells in metaphase from preparations which had been previously fixed in 3 : 1 methyl alcohol : acetic acid were removed from the slides and transferred to the grids following the instructions of Barnicot and Huxley (1961). These cells were contrasted with uranile acetate according to Watson (1958) and photographed with a Siemens Elmiskop—I.

3. Results

General

In the three subjects with the abnormal No. 2 chromosome all cells had an otherwise normal karyotype. The patterns of sex chromatin and of chromosome DNA replication were also normal. The parents of subjects B. F. and A. G. had normal karyotypes in 25 and 30 blood metaphases, respectively. Only a few cells could be analysed in the parents of Z. R. and these were normal.

Skin fibroblast cultures

For subject B. F. a first skin culture failed to grow while a second had limited growth and only 5 cells could be analysed, all normal. Fifty cells from a skin culture from subject A. G. were analysed. Of these 36 had a normal karyotype, 11 had less than 46 chromosomes with random loss of various chromosomes, 2 were 47,C+ and one cell had one chromatid break on each of the arms of chromosome No. 1. It was not possible to obtain a skin culture from Z. R.

TABLE 2

Synopsis of the results in the three subjects. For details of experiments see text

Subject and culture (date)	Type of culture and scoring	Normal cells	Instability of one No. 2	Duplication of long (short) arm of one No. 2	Loss of long (short) arm of one No. 2	Additional No. 2	Total analysed cells	Total abnormal No. 2 (per cent.)
A. G.								
1 (6.7.1964)	72 hours	28	16	—	(2)		46	18 (39)
2 (27.11.64)	72 hours	166	58	2 (1)	—	—	227	61 (27)
Z. R.								
1 (15.9.67)	72 hours	98	58	8 (1)	8	1	174	76 (44)
B. F.								
1 (21.6.68)	72 hours	149	76	2	—	—	226	77 (34)
2 (4.7.68)	72 hours	31	19	—	—	—	50	19 (38)
3 (4.7.68)	48 hours	76	24	—	—	—	100	24 (24)
4 (4.7.68)	72 hours. ^{3}H-Tdr for 6 hours	61	18	—	—	—	79	18 (23)
4a (—)	among labelled cells of 5	15	4	—	—	—	19	4 (21)
5 (11.10.68)	72 hours. ^{3}H-TdR for 6 hours	73	21	—	—	—	94	21 (22)
5a (—)	among labelled cells of 6	25	10	—	—	—	35	10 (29)
6 (11.10.68)	72 hours. ^{3}H-TdR in excess for 6 hours	93	7	—	—	—	100	7 (7)
6a (—)	among labelled cells of 7	35	3	1	—	—	39	4 (10)
7 (4.4.70)	72 hours. TdR for 6 hours	76	9	2	—	—	87	11 (13)
8 (4.4.70)	72 hours. 20 min. ^{3}H-TdR at 48 hours	123	7	—	—	—	98	8 (8)
9 (4.4.70)	72 hours. ^{3}H-TdR at 48 hours for 8 hours	90	8	—	—	—	98	8 (8)
10 (4.4.70)	72 hours. ^{3}H-TdR for last 48 hours	92	5	—	—	—	97	5 (5)

Blood cultures

Table 2 summarises the results from the blood cultures of the three subjects. In this table are entered separately only some of the variations observed in the abnormal No. 2, of which a short description follows, based on the patterns reproduced in plate I. In the majority of the abnormal cells one of the two chromosomes No. 2 had a distinct gap in the long arm very close to the centromere. This gap was variable in length as exemplified in plate I, a to i and, with the exception of one cell (plate I, c), was present on only one of the two chromosomes No. 2. In most of these cells a characteristic, single or bipartite, lightly staining structure could be seen attached to the centromeric region of the short arm. In several cells it was possible to see tiny, faintly stained single or double filaments connecting the two separated sections of the abnormal chromosome. In eight cells of case Z. R. the entire long arm was missing (plate I, j) and in two cells of case A. G. the short arm was missing. In one cell of case Z. R. there were three normal chromosomes No. 2. One cell of case B. F. had a single outstretched chromatid of the long arm (plate I, v).

Duplication of the long arm and, less frequently, of the short arm was found in two different patterns. In the first (plate I, p-u), there was close contiguity between the short (respectively long) arm and the two long (respectively short) arms. Only this category is entered in table 1 under the heading " duplication of long (short) arm ". In the second category, there were two chromosomes No. 2 either both normal (plate I, k and m) or one with the gap (plate I, l and v) and in addition an extra chromosome of the size of either the long (plate I, l, m, v) or the short arm (plate I, k). The extra separated long arm had in most instances the appearance of an acentric fragment (e.g. plate I, l, m, v). Conversely, the extra, separated short arm had the appearance of a chromosome of the D group (e.g. plate I, k). The reduplicated arms were in all cells of the same size and degree of contraction as the short arm of the other chromosome No. 2, with the exception of the cell showed in plate II, in which the four chromatids of the two long arms were outstretched to an unusual length.

DNA replication

In the cultures labelled for the last 6 hours the abnormal No. 2 was apparently synchronous in replication with its normal homologue. This impression was tested quantitatively. A sample of 30 labelled metaphases contained 10 cells with the abnormal chromosome and these cells had on both No. 2 chromosomes a total of 813 grains of which 384 were on the normal and 429 on the abnormal No. 2. The normal metaphases had on both chromosomes No. 2 a total of 1617 grains. The ratios of the total number of grains on chromosomes No. 2 to the total grains on chromosomes 1 and the six chromosomes of the D group (13-15) were 813/2397 (0·339) in the 10 abnormal cells and 1617/4651 (0·348) in the 20 normal cells. Thus, the impression of synchrony was confirmed quantitatively. A close inspection of the abnormal chromosomes revealed that the zone of discontinuity never had superimposed radioautographic grains (plate II).

The experiments designed to study the second division after labelling, the initial phase of the S period and the effect of continuous labelling

during two division cycles did not give relevant information on the replication of the abnormal No. 2. One cell with reduplication of the long arm from the experiment with excess ^{3}H-TdR had the remarkable pattern reproduced in plate III. This cell came in contact with ^{3}H-TdR towards the end of the S period as indicated by the presence of the late replicating X chromosome. The four chromatids of the duplicated long arm were covered with grains and at least as late replicating as the X chromosome. Removal of the grains revealed that the duplicated arms were despiralised and outstretched. The frequency of cells with the abnormal No. 2 in the cultures labelled with ^{3}H-TdR for the last 6 hours was lower than in unlabelled cultures processed in the same way, as seen by comparing cultures 1 and 2 with 4 and with 4 and 5 pooled. In a culture exposed to a fourfold excess dose of ^{3}H-TdR (No. 6 in table 2) the overall proportion of cells with the abnormal No. 2 was only 7 per cent., which is significantly lower than the one in the parallel experiment with a normal dose of ^{3}H-TdR (No. 5 in table 2). These findings called for a check on the effect of ^{3}H-TdR on the incidence of general chromosome breakage (table 3). The proportion of cells with breaks, and the mean number of breaks per cell are not significantly different in the cultures labelled for 6 hours with the standard and the excess dose of ^{3}H-TdR while the incidence of abnormal No. 2 is lower in the excess cultures. Thus, an excess dose of ^{3}H-TdR results in a lower incidence of abnormal chromosomes No. 2.

At this time it was impossible to obtain blood from the patient for further experiments. After one and a half years, blood was available for experiments 7 to 10 of table 2. In all these experiments the prevalence of abnormal cells was consistently low but lower in the experiments with ^{3}H-TdR as compared with those with TdR. Again the incidence of the abnormal No. 2 was independent of that of overall chromosome breakage (table 3).

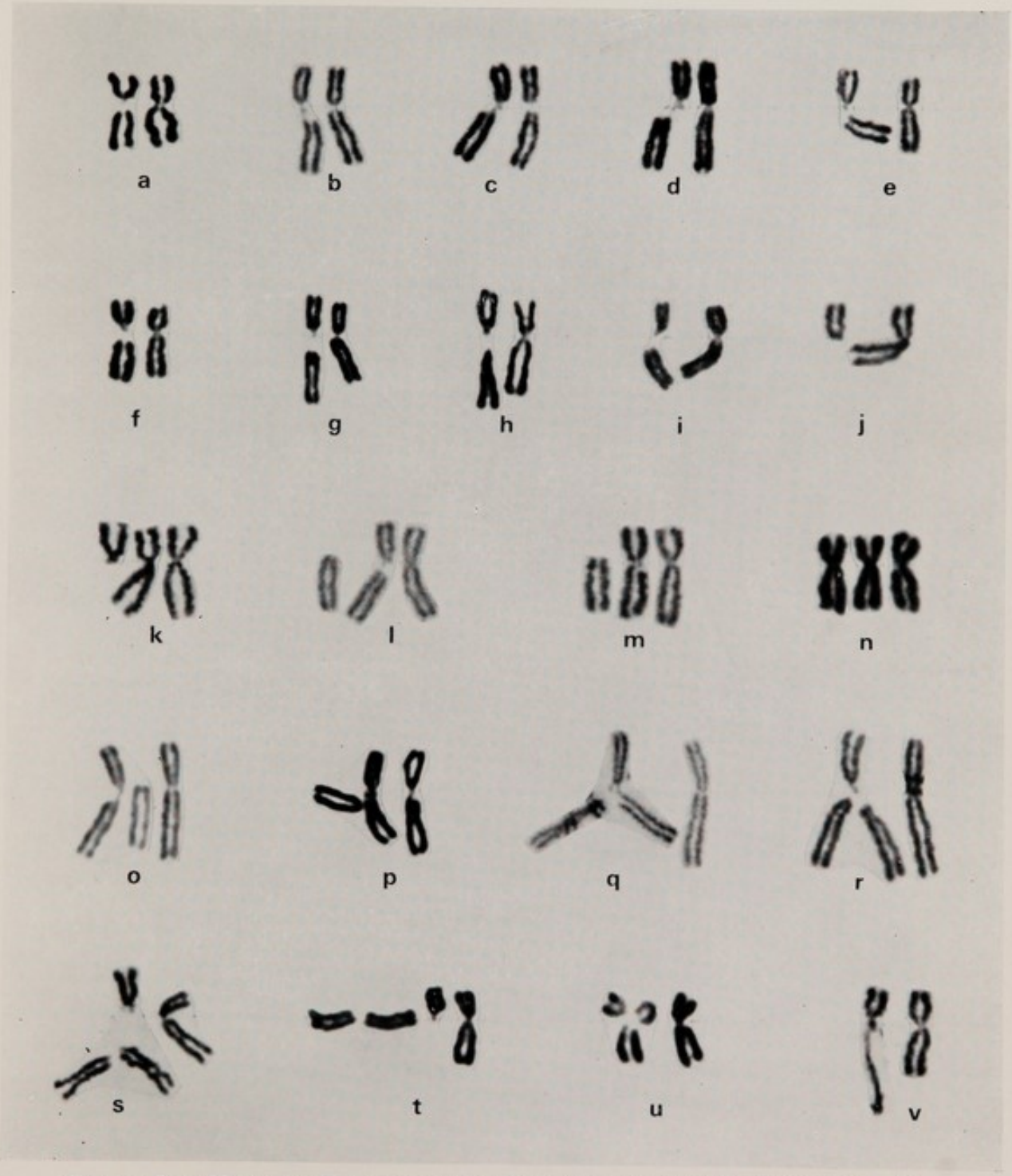
Electron microscopy

Electron photomicrographs of selected abnormal chromosomes showed in correspondence with discontinuity, a much lower electron density than contiguous and/or homologous zones but no disruption of continuity along the chromatid(s). With the unspecific staining procedure employed in these preliminary experiments we could not decide whether the zones of lower electron density consisted of DNA, proteins or both.

4. Discussion

The discontinuity in a procentric region of the long arm was variable in size and visible on either one or both chromatids. Electron microscopy revealed that in the gap region there was no complete disruption of chromosome material. This region, however, was obviously fragile as indicated by the far displacement within the cell or loss of the portion of the long arm distal to the gap. Autoradiography revealed that the abnormal chromosome No. 2 was synchronous in replication with its homologue in both arms. Autoradiographic grains were never located on the discontinuous zone and this indicates that either the discontinuity itself originates after the S period or that in the gap region there is no DNA or not enough DNA to be revealed by autoradiography. An excess dose of tritiated thymidine

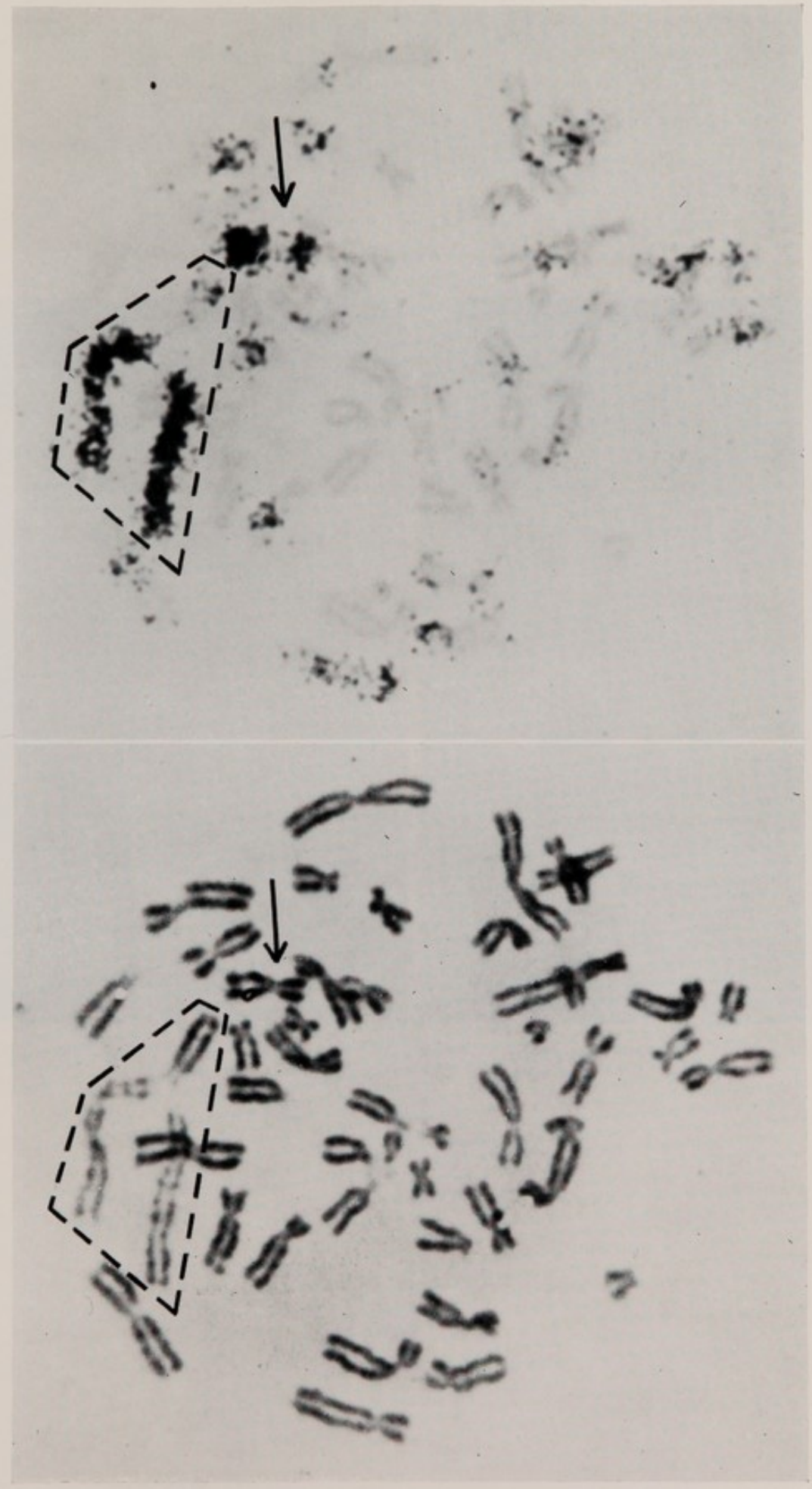
Plate I



Cut-outs from 22 blood cells to demonstrate the varying aspects of the abnormality. The normal chromosome(s) are always placed at the right side.

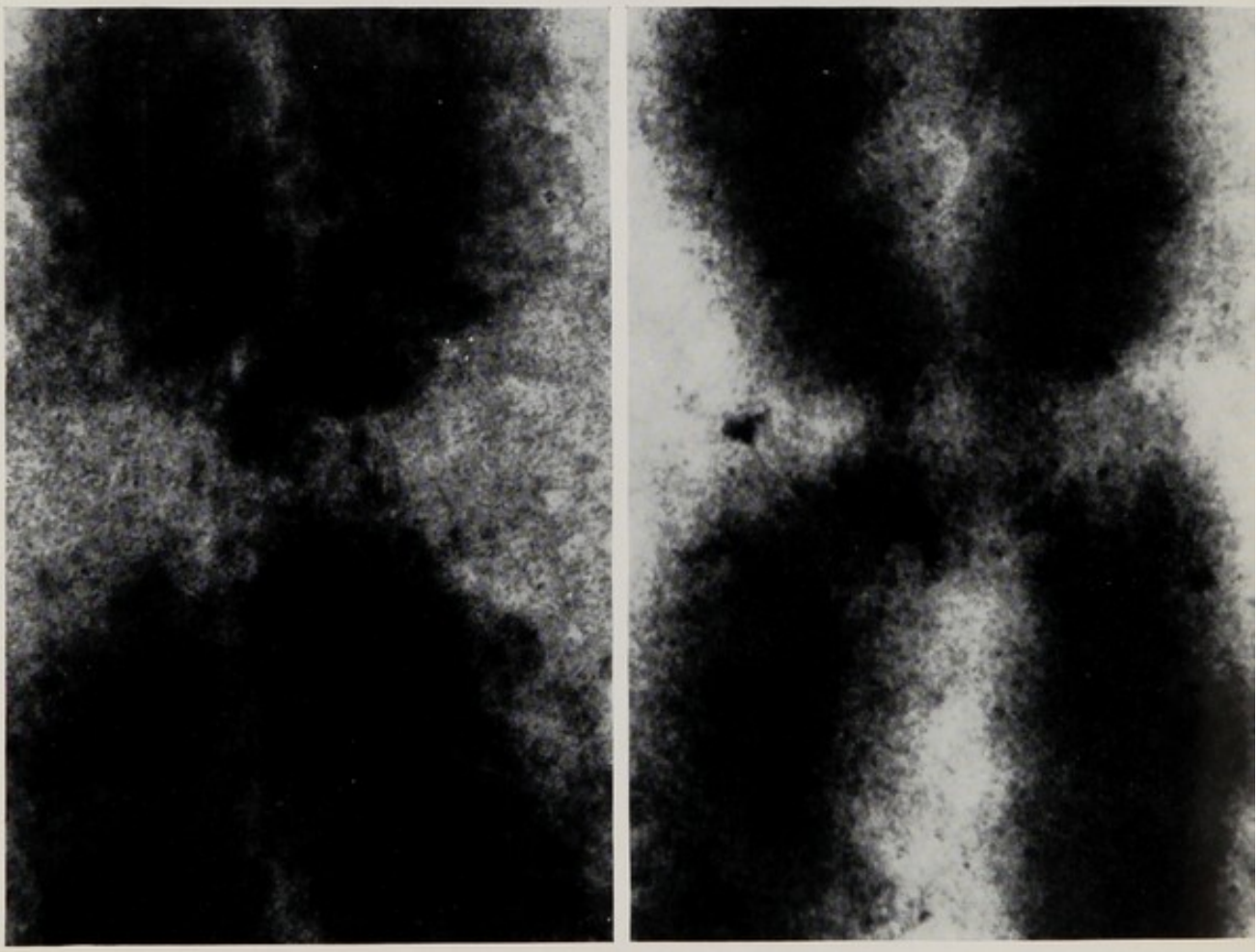
Plate II

Cut-outs from 6 cells labelled for the last 6 hours of culture (*above*) and from the same cells without radioautographic grains. The cells are arranged in decreasing order of grain intensity.



Cell with selective reduplication of the long arms of one No. 2 (boxed) in which the long arms are outstretched and heavily labelled. The arrow points to the late-replicating X chromosome.

Plate IV



Electromicrographs of a chromosome No. 2 with a discontinuity in the paracentric region of the long arm (*left*) and its normal homologue (*right*).

TABLE 3

Incidence of chromosome breakage other than in chromosome No. 2 in various cultures of subject B. F.

Culture number as in table 2	Breakage other than No. 2					Proportion of abnormal No. 2 as in table 2 with exclusion of arm duplication	
	number of cells scored	number of cells with breaks	% cells with breaks	total number of breaks	mean number of breaks per cell		
1	100	10	10	10	0·1	75/226	33%
4a	16	14	88	45	2·8	4/19	21%
5a	30	24	80	47	1·6	10/35	29%
6a	39	29	74	63	1·6	3/39	8%
8	130	7	5	7	0·05	7/130	5%
9	98	32	33	114	1·2	8/98	8%
10	97	34	35	88	0·9	5/97	5%

given for the last 6 hours of culture did not increase the overall frequency of chromosome breakage in *all* chromosomes but produced a significant decrease of the incidence of the abnormal No. 2. This indicates that the excess dose rapidly eliminates cells with the abnormal chromosome, or exerts some kind of repair effect or influences the rate of DNA replication in cells with the abnormality so that fewer of them are sampled at the end of the culture. Of special interest is the occurrence of reduplication of the entire section of the long arm distal to the discontinuity and more rarely also of the short arms. We have little doubt, in agreement with Lejeune *et al.* (1968), that this is a true reduplication of the chromosome long (respectively short) arms. The existence of several cells in which the reduplicated arms are displaced within the cell (plate I, k, l, m, o) indicates that two of the four chromatids of the reduplicated arms might be lost during cell division. Therefore, the phenomenon of selective reduplication is probably much more frequent than revealed by the actual observations. The gap might be combined with loss or alteration of element(s) controlling chromosome replication, which we know to be located on the chromosome itself (Cattanach and Isaacson (1967), in the mouse; Halfer *et al.* (1969), in *Drosophila melanogaster*). This point is impressively supported by the observation of the cell in plate III in which the reduplicated long arms are very late replicating in complete asynchrony with the short arm and the homologous normal No. 2.

However, explanations alternative to that of selective endoreduplication can be offered. One is that of sister chromatid fusion although this interpretation assumes the capacity of autonomous DNA duplication of an acentric segment and is made unlikely by the morphological appearance of continuity of both arms displayed by several of the reduplicated chromosomes. Perhaps more attractive is the hypothesis of chromatid mis-segregation at anaphase which results in the migration to one pole of a chromosome composed of one short (long) and two long (short) arms. This hypothesis implies an unequal division of the centromere rather than selective disruption of the long arms of the No. 2. The very existence of reduplication also of the *short* arm indicates that the process of reduplication is *not* exclusively dependent on the discontinuity which in all cells was observed only in the long arm. To explain the coexistence of long and short arm reduplication we have to assume either the existence of a functional defect also on the short arm or again a misfunction of the centromere which in most cases results in a failure of the long arm to segregate at anaphase but occasionally the same happens to the short arm. It should be noted that in the case reported by Conen and Erkman (1966) the discontinuity was observed in the short arm of No. 2.

Reduplication of individual chromosome arms was noted by Lejeune *et al.* (1966) in one cell of each of five individuals with an otherwise normal karyotype and by Sinkus (1969) in blood cultures of a cancer patient treated with mitomycin C, but never before with such a consistency as in the case of chromosome No. 2.

Preferential discontinuity has been found also in other chromosomes. Six cases involving a chromosome of the C group have been reported recently (see for review Reeves and Lawler, 1970; and also Kunze-Mühl *et al.*, 1970) and we have also observed one such case which will be reported elsewhere. The same phenomenon was observed in chromosome No. 3 (Brøgger, 1967) and in chromosome No. 16 (Day *et al.*, 1967). While

Lubs and Samuelson (1967) found that these two chromosomes have the highest frequency of spontaneous breakage in normal human subjects, they did not find in the same series preferential breakage of chromosome No. 2.

The abnormal No. 2 was not observed in the parents of two of our cases, while in the third the family was not thoroughly investigated. The parents of the case described by Ferrante *et al.* (1968) had normal karyotypes. Familial aggregation of the anomaly was found by Lejeune *et al.* (1968) and in two families mentioned by Shaw (1968). The normal daughter of the index case of Reeves and Lawler (1970) had no abnormality of chromosome No. 2 but the frequency of breakage in the other chromosomes was increased over the control values.

The cause of the abnormality described in this paper remains obscure. Variations in its prevalence within and between subjects may be due to sampling accidents, and/or varying culture conditions but they may reflect individual and temporal differentials in the reaction to internal or external agents. Whatever the cause, we are inclined to think that the abnormality has no relation with the phenotype. Our three cases were ascertained independently and had different phenotypes and it should be noted that the case of Reeves and Lawler (1970) was ascertained in an aged but healthy woman who was one of a panel of normal controls.

5. Summary

1. Three independently ascertained subjects with different phenotypes had in their cultured lymphocytes a variable but consistent proportion of cells with a definite gap in the paracentric region of the long arm of one chromosome No. 2.

2. In a few cells in all subjects there was selective reduplication of the entire long arm and more rarely of the short arm.

3. Electromicroscopy revealed that the discontinuity was not complete and within its limits, autoradiography revealed that there was no radioactive DNA in the gap.

4. The abnormality may be associated with an impairment of the replication control, resulting in the occasional reduplication of the long arm.

5. The abnormality is not directly related to the phenotypes of its carriers and its prevalence is probably affected by external and internal conditions.

6. References

BARNICOT, N. A., AND HUXLEY, H. E. 1961. The electron microscopy of unsectioned human chromosomes. *Ann. Hum. Genet., London, 25,* 253-258.

BRØGGER, A. 1967. Abnormally frequent, spontaneous breakage of the human chromosome number 3. *Rep. Fifth Scand. Congr. Cell Res., Åbo, Finland, June 1-3, 1967.*

BÜHLER, E. M., LUCHSINGER, U., BÜHLER, U. K., MEHES, K., AND STALDER, G. R. 1970. Non-condensation of one segment of a chromosome No. 2 in a male with an otherwise normal karyotype (and severe hypospadias). *Humangenetik, 9,* 97-104.

CATTANACH, B. M., AND ISAACSON, J. H. 1967. Controlling elements in the mouse X chromosome. *Genetics, 57,* 331-346.

CONEN, P. E., AND ERKMAN, B. 1966. Combined mongolism and leukemia. *Am. J. of Diseases of Children, 112,* 429-443.

DAY, E. J., MARSHALL, R., MACDONALD, P. A. C., AND DAVIDSON, W. M. 1967. Deleted chromosome 18 with paternal mosaicism. *Lancet, ii,* 1307.

FERRANTE, E., BRUNI, L., AND GRIMALDI, S. 1968. Rottura paracentromerica del braccio lungo di un cromosoma 2 in una bambina di 9 mesi affetta da cistationinuria, ritardo psicomotorio e cataratta. *Rivista di Clinica Pediatrica, 81*, 612-619.

HALFER, C., TIEPOLO, L., BARIGOZZI, C., AND FRACCARO, M. 1969. Timing of DNA replication of translocated Y chromosome sections in somatic cells of *Drosophila melanogaster*. *Chromosoma, 27*, 395-408.

KUNZE-MÜHL, E., FISCHER, P., AND GOLOB, E. 1970. Spezifische Aberration eines Chromosoms der C-Gruppe bei mehreren Mitgliedern einer Sippe. *Humangenetik, 9*, 91-96.

LEJEUNE, J., BERGER, R., AND RETHORÉ, M.-O. 1966. Sur l'endoréduplication sélective de certains segments du génome. *C.R. Acad. Sc. Paris, 263*, 1880-1882.

LEJEUNE, J., DUTRILLAUX, B., LAFOURCADE, J., BERGER, R., ABONYI, D., AND RETHORÉ, M.-O. 1968. Endoréduplication sélective du bras long du chromosome 2 chez une femme et sa fille. *C.R. Acad. Sc. Paris, 266*, 24-26.

LUBS, H. A., AND SAMUELSON, J. 1967. Chromosome abnormalities in lymphocytes from normal human subjects. *Cytogenetics, 6*, 402-411.

REEVES, B. R., AND LAWLER, S. D. 1970. Preferential breakage of sensitive regions of human chromosomes. *Humangenetik, 8*, 295-301.

SHAW, M. W. 1968. Autosomal abnormalities. In Lozzio, C. B. and Chernoff, A. I. (eds.) *Symposium on Human Cytogenetics*, 5-11. Birth Defects Evaluation Center, Knoxville, Tennessee.

SINKUS, A. H. 1969. Asynchronous endoreduplication of chromosomes as a possible source of aneuploidy and pseudodiploidy. *Genetika, Moscow, 5*, 144-150 (in Russian).

WATSON, M. L. 1958. Staining of tissue sections for electron microscopy with heavy metals. *J. Biochem. Biophys. Cytol., 4*, 475.

ON THE ORIGIN AND NATURE OF ACHROMATIC LESIONS

JYOTI P. CHAUDHURI

University of Ulm, Department of Clinical Physiology and Cytogenetics Section, D79 ULM, Western Germany

1. Introduction

Chromosomes in their number and form show such a constancy and their abnormalities are generally so distinct that they provide very sensitive and dependable parameters in measuring various biological responses. But unfortunately there are some types of chromosome abnormalities which are neither distinctly identifiable nor clearly understood. Achromatic lesions, commonly called gaps, are one of the poorly understood chromosome abnormalities. They occur very frequently—spontaneously as well as after certain treatment like irradiations, exposures to radiomimetic chemicals or viral infections. Generally they are of the chromatid type although isochromatid gaps are not very uncommon. Sometimes they are hardly distinguishable from a chromatid break. Even the conventional criteria for distinguishing them is rather vague (Fig. 1). Whether a chromatid fragment

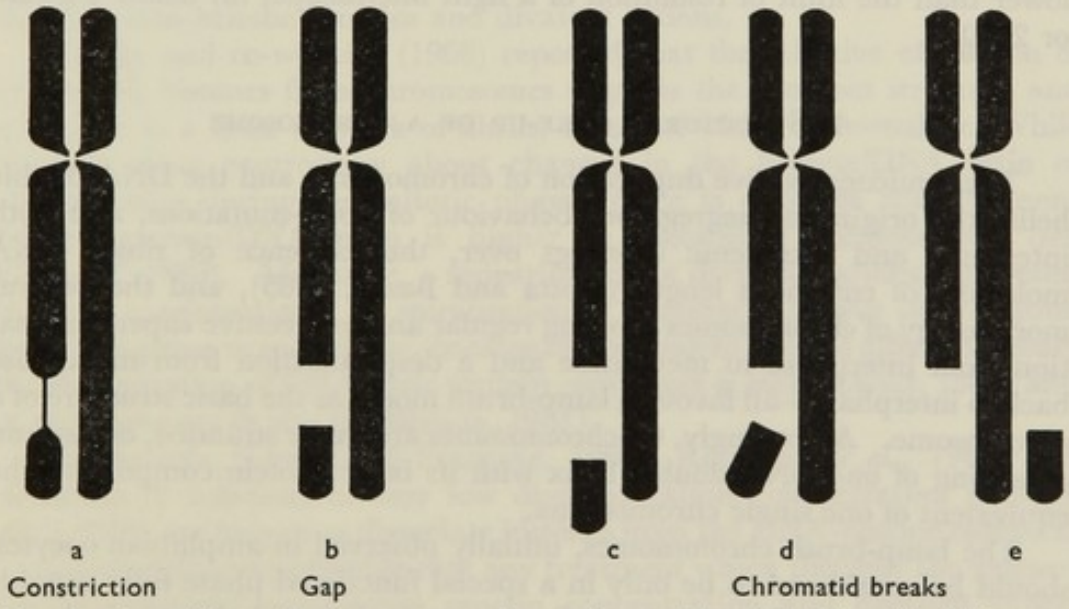


Fig. 1.—According to the current convention a chromatid break is distinguished from a gap by the topological or axial displacement of the distal acentric part.

to the rest of the chromatid or shows a 180° torsion in the microscopic lies co-axial preparation may well be a matter of chance. It is conceivable therefore that the configurations in fig. 1 b and e may both represent true chromatid breaks.

In a recent screening of 37 thorotrast patients in an experiment which enabled us to estimate chromosome abnormalities in two subsequent cell divisions (Chaudhuri and Knörr-Gärtner, 1970) no consistency was found in the frequency of gaps. Their occurrence was also found to be very irregular

in new-born rats exposed to various doses of ^{3}H-thymidine *in utero* (Chaudhuri *et al.*, in press). An examination of chromosomes in the NASA astronauts has also revealed the predominant occurrence of gaps and chromatid breaks which were proportional to the duration of flight rather than the exposures to irradiation (Gooch and Berry, 1969).

Determination of chromatid breaks as such is of considerable interest because they serve to mark the phase of the cell cycle exposed to the insult.

2. Gaps are a reality

Achromatic lesions or gaps are those parts of the chromosome or chromatid which are not stainable and therefore look like breaks although they do not represent any material discontinuity. The best proof of the real existence of such an abnormality has come from the observation that during anaphase separation the part of a chromatid lying beyond the gap trails faithfully behind the centromeric part of the chromosome. Some material continuity across a gap is thus beyond doubt.

3. Bridge across the gap

The " achromatic " or non-stainable material providing continuity across the gap is also not visible through a high resolution phase-contrast objective. The connection across a gap is then of an order of dimension lower than the limit of resolution of a light microscope, *i.e.* below 0·2 μm. or 2000 Å.

4. Structural make-up of a chromosome

The semiconservative duplication of chromosomes and the DNA double helix, the origin and segregatory behaviour of point-mutations, and both intergenic and intragenic crossings over, the existence of single DNA molecules of enormous length (Hotta and Bassel, 1965), and the general morphology of chromosomes showing regular and progressive superspiralisation from interphase to metaphase and a despiralisation from metaphase back to interphase—all favour a lamp-brush model as the basic structure of a chromosome. Accordingly, G_1 chromosomes are single stranded, one strand consisting of one DNA double helix with its basic protein component, the equivalent of one single chromonema.

The lamp-brush chromosomes, initially observed in amphibian oocytes, should be considered to be only in a special functional phase fully capable of giving rise to chromosomes of conventional morphology. It is not unexpected that the chromosomes of organisms showing extreme gametic dimorphism should adopt a lamp-brush form during oogenesis. The lamp-brush nature of the oogonial chromosomes in higher animals like rats, monkeys and humans (see Baker, 1970) is consistent with this view.

Electron microscope studies of chromosomes in section often reveal a bundle of coiled fibres of 100-200 Å, the order of size of a chromatid of lamp-brush chromosomes. Recent electron microscopic investigations of interphase and metaphase chromosomes using air-water interphase spread, critical point drying and wholemount techniques generally reveal basic chromatin fibres of about 200-300 Å, which represent the second order of

spiralisation of a DNA-histone complex (Lampert, 1969). The nature of the first order of spiralisation in the structural organisation of a chromosome has been explained in detail by Pardon and Richards (see also p. 38) in their X-ray diffraction studies on the molecular structure of nucleo-histones.

Normal metaphase chromosomes, usually seen as consisting of a pair of solid shaft chromatids, when photographed using light of wavelength 530-550 nm. reveal a spiralised form for each chromatid. A hypotonic treatment of 0·550 mol KCl, $NaNO_3$ and Na-acetate (4 : 2 : 1) also reveals the spiralised structure of chromatids (Ohnuki, 1965). These chromatid spiralisations seen under a light microscope have been interpreted by DuPraw (1966) on the basis of electron microscopic studies to represent the 4th and final order of spiralisation. Compact folding of these spirals would give rise to apparently cylindrical chromatids.

In this light, a gap would represent an extremely despiralised portion of a chromosome, the dimension of the despiralised fibres being less than 2000 Å and, perhaps, even approaching the fineness of 100-250 Å.

5. Origin of gaps

If gaps can now be regarded as extremely despiralised zones or as localised failures of compact folding in metaphase chromosomes, their origin should be associated with those moieties which are responsible for such spiralisations or foldings. Besides DNA, at least three other regular components of the chromosome may be involved in the spiralisation cycle of chromosomes—histones, non-histone proteins and divalent cations.

Mirsky and co-workers (1968) reported that the selective extraction of lysine-rich histones from chromosomes unpacks the compact structure and gives rise to a loose network of fibrils, an effect which is reversible. While there is some controversy about changes in the histone/DNA ratio of chromosomes during the mitotic phases, there is evidence that the non-histone protein/DNA ratio is higher in metaphase chromosomes (see Hancock, 1969). Moreover, a redistribution of divalent cations may result in the " condensation " or spiralisation of chromatin during its transition from interphase to metaphase, because these divalent cations affect markedly the configuration of DNA-basic protein complexes *in vitro* (Kabat, 1967) and of chromosomes *in vivo* (Steffenson, 1961).

Irradiations (Lloyd and Peacocke, 1968; Robinson *et al.*, 1966) and exposures to solutions of very low divalent cation concentration (Mirsky *et al.*, 1968) are known to dissociate histones from the DNA-histone complex.

It is therefore conceivable that any treatment which disturbs the nucleo-histone complex, non-histone protein concentration and divalent cation distribution, may cause localised failures in spiralisation of chromatin fibres and may give rise to an achromatic lesion. As it does not represent a structural discontinuity it may undergo easy recovery in the next cycle of spiralisation. The observation of a specific chromosome abnormality of achromatic lesion type in a normal young woman showing quick regression (Gooch and Fischer, 1969) also favours such reversibility. The morphological similarity between achromatic lesions, centromeric regions and primary and secondary constrictions supports the view that achromatic lesions are extremely despiralised parts of chromosomes capable of re-spiralisation (fig. 2).

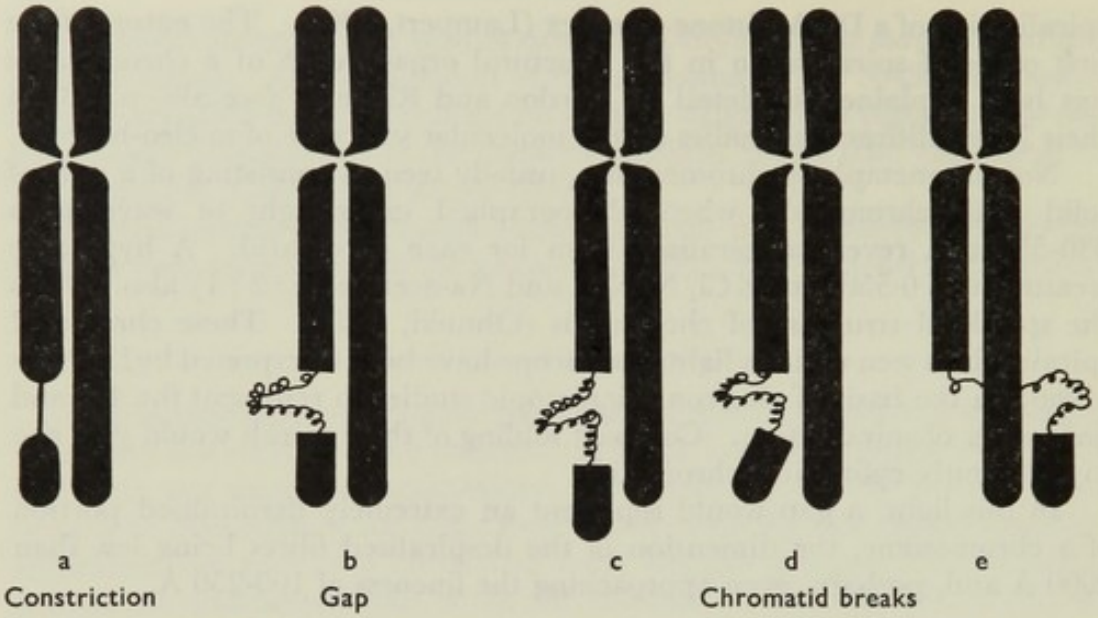


FIG. 2.—An extremely despiralised part of a chromatid may result in a gap. Such extreme despiralisation may also allow displacement of the distal part assuming the configuration of a typical chromatid break.

6. SUMMARY

1. If a G_1 chromosome is single stranded and if the apparently cylindrical chromatids of metaphase chromosomes are compactly folded spirals of the fourth order of single continuous nucleoprotein chains, achromatic lesions can be explained on the basis of despiralisation or non-spiralisation.

2. At least three constituents of a chromosome are responsible for its spiralisation and each of them responds to more than one kind of treatment. Consequently, a host of different factors including the preparative techniques may influence the yield of gaps. This may explain the variability in their frequency.

REFERENCES

BAKER, T. G. Comparative aspects of the effects of radiation during oogenesis, *in* Mammalian Radiation Genetics Meeting, Munich, 7-9th July, 1970 (Sponsored by IAEA, Vienna and Deutsche Strahlenforschung Gesellschaft, Munich).

CHAUDHURI, J. P., AND KNÖRR-GÄRTNER, H. 1970. Effect of chronic irradiation from incorporated thoriumdioxide on human chromosomes. *International Congress of Radiation Research*, (Abs.), Evian, p. 42.

CHAUDHURI, J. P., HAAS, R. J., SCHREML, W., KNÖRR-GÄRTNER, H., AND FLIEDNER, T. M. 1971. Cytogenetic and teratologic studies on the rats continuously infused with various doses of tritiated thymidine during pregnancy. *Teratology* (in press).

DUPRAW, E. J. 1966. Evidence for a " folded-fibre " organization in human chromosomes. *Nature*, *209*, 577-581.

GOOCH, P. C., AND BERRY, C. A. 1969. Chromosome analysis of Gemini astronauts. *Aerospace Medicine*, *40*, 610-614.

GOOCH, P. C., AND FISCHER, C. L. 1969. High frequency of a specific chromosome abnormality in leukocytes of a normal female. *Cytogenetics*, *8*, 1-8.

HANCOCK, R. 1969. Conservation of histones in chromatin during growth and mitosis in vitro. *J. Mol. Biol.*, *40*, 457-466.

HOTTA, Y., AND BASSEL, A. 1965. Molecular size and circularity of DNA in cells of mammals and higher plants. *Proc. Natl. Acad. Sci., Wash.*, *53*, 356-362.

KABAT, D. 1967. Fibrous complexes of deoxyribonucleic acid with certain globular proteins. Role of divalent metal ions in the organization of nucleoprotein structures. *Biochemistry*, 6, 3443-3458.

LAMPERT, F. 1969. Feinstruktur und Trockengewicht menschlicher Chromosomen. *Naturwissenschaften, 56*, 629-633.

LLOYD, P. H., AND PEACOCK, A. R. 1968. The dissociation of histone from deoxyribonucleohistone by gamma radiation. *Biochem. J., 109*, 341-345.

MIRSKY, A. E., DURDICK, C. J., DAVIDSON, E. H., AND LITTAU, V. C. 1968. The role of lysine-rich histone in the maintenance of chromatin structure in metaphase chromosomes. *Proc. Natl. Acad. Sci. Wash., 61*, 592-597.

OHNUKI, Y. 1965. Demonstration of the spiral structure of human chromosomes. *Nature, 208*, 916-917.

ROBINSON, M. G., WEISS, J. J., AND WHEELER, C. M. 1966. Irradiation of deoxyribonucleohistone solutions. II. Labilization of the DNA-histone linkage. *Biochem. Biophys. Acta., 124*, 181-186.

STEFFENSON, D. M. 1961. Chromosome structure with special reference to the role of metal ions. *Int. Rev. Cytol., 12*, 163-197.

STÜTZER, H. R. 1970. Eine Technik zur lichtmikroskopischen Darstellung der Struktur menschlicher Chromosomen. *Eitz-Mitt. für Wiss. u. Techn., 5*, 57-60.

THE PARTICIPATION OF CHROMOSOME ARMS IN RADIATION-INDUCED CHROMOSOME TYPE EXCHANGE

JOHN R. K. SAVAGE
M.R.C. Radiobiology Unit, Harwell, Berkshire

1. Introduction

The initial lesions induced within the nucleus by the radiation qualities and doses generally used in radiation cytology are almost certainly at random, but the observed structural exchanges which arise from these lesions do not always reflect this randomness.

Randomness can be investigated at at least three levels

(*a*) between nuclei within a population,

(*b*) between chromosome arms within nuclei,

(*c*) within a given arm.

Level (*a*) has been investigated most thoroughly for both *chromosome* and *chromatid* type exchanges, and since the subject has recently been reviewed elsewhere (Savage, 1970) it will not be treated further here.

Levels (*b*) and (*c*) have been the subject of study in a few favourable organisms where it is possible to make clear distinctions between chromosomes and chromosome arms in the karyotype. However, such investigations have involved almost exclusively *chromatid* type exchanges, since both asymmetrical and symmetrical forms can be scored, and the " point " of exchange along a given arm determined with some precision (*e.g.* Reiger and Michaelis, 1959; Evans and Bigger, 1961; Fox, 1966).

In general it is found that participation of arms in exchange (level *b*) is not random, shorter arms taking part more frequently than expected on the basis of length measured at metaphase.

There is also a tendency for non randomness within an arm (level *c*), preferential exchange occurring in those regions which show " cold starvation " and late labelling with tritiated thymidine and generally referred to as heterochromatin.

Chromosome type exchanges have neither of the advantages mentioned above. Only asymmetrical exchanges can be scored unambiguously, and the " point " of exchange can only be inferred indirectly from fragment measurements, or distances between centromeres. The problem is further complicated in organisms with low chromosome numbers by the fact that as the radiation dose rises, an increasing proportion of the observed exchanges have been modified by secondary exchange events, and are not necessarily related to any associated acentric fragment (see Savage and Papworth, 1969 for full discussion).

It follows therefore that in such organisms, analysis of exchanges with related fragments can only be performed at low radiation doses, when the probability of double events is negligible.

This paper reports the results of an experiment fulfilling these conditions.

2. Materials and methods

Chromosome exchanges induced in the developing microspores of *Campelia zanonia* L.HBK were used for analysis. The radiobiology of this species has been reported elsewhere (Savage and Pritchard, 1969).

Doses of 100 and 150 rads of X-rays (250 kv, 14 mA, H.V.L. 1·2 mm. Cu) were given to inflorescences in 100 per cent. oxygen, which together with unirradiated controls were then returned to a constant environmental growth chamber with 14-hour day, 22° C. and R.H. 70 per cent. until fixation 4 days later. Permanent acetic orcein preparations were made of cells in metaphase.

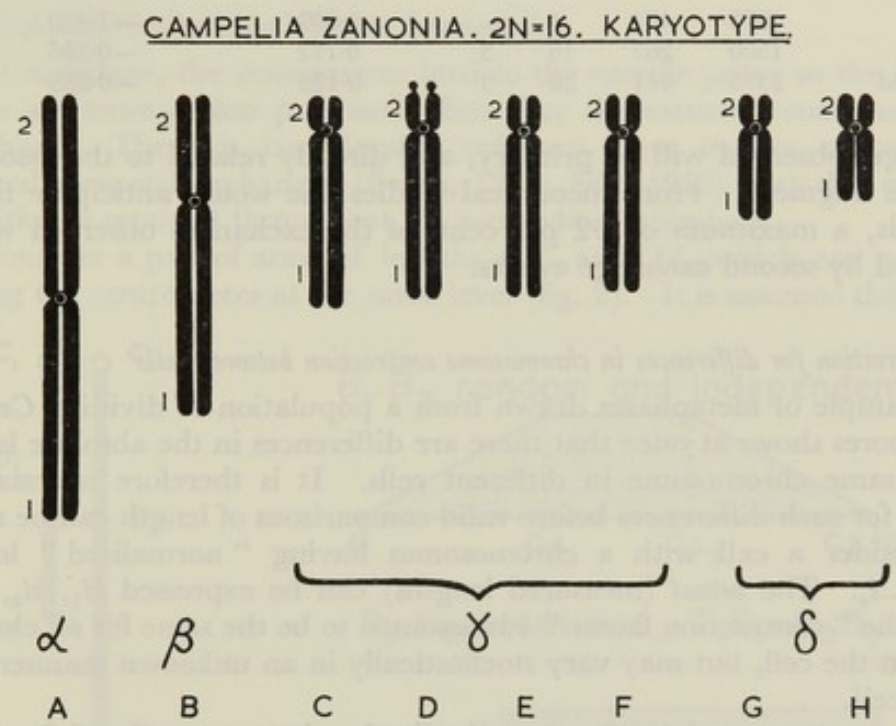


FIG. 1.—The haploid karyotype of *Campelia zanonia*.

The 8 haploid chromosomes (fig. 1) comprise 4 distinct groups α-δ, further distinction into long and short arms being possible for groups β-δ. Thus, all simple asymmetrical exchanges can be classified into 28 categories, 4 centric rings and 24 dicentrics (Savage and Papworth, 1969).

Measurements of acentric fragments and arm lengths were made at constant magnification using a stepped eye-piece graticule.

3. Theoretical considerations

3.1 *Effect of distortion*

As mentioned above, it is essential when classifying or measuring chromosome exchanges and their fragments, to ensure that one is dealing with a primary, unmodified aberration. It is impossible to observe a given arm taking part in more than one *chromosome* exchange, although they must do so. Hence, as the radiation dose increases, an increasing proportion of exchanges fail to be recorded. The consequence of this is an underdispersed distribution of exchanges between nuclei (Savage and Papworth, 1969). A simple test therefore for the importance of double events is to check the exchange distribution for deviations from a Poisson.

Table 1 gives the results of a test for the 100 and 150 rad data used here, based on the dispersion index (Savage, 1970).

It can be concluded that distortion is negligible, and that nearly all

TABLE 1

Distribution of exchange aberrations between cells

X-ray dose (rads)	Frequency of cells with exchanges				Mean exchanges per cell	Test of goodness of fit to a Poisson	
	0	1	2	3		Unit normal deviate	P
100	1875	174	4		0·089	−1·420	0·158
150	1500	267	16	3	0·172	−0·286	0·778
Combined	3375	441	20	3	0·128	−0·395	0·693

exchanges observed will be primary, and directly related to the associated acentric fragment. From theoretical studies one would anticipate that at 150 rads, a maximum of 1·2 per cent. of the exchanges observed will be modified by second exchange events.

3.2 *Correction for differences in chromosome contraction between cells*

A sample of metaphases drawn from a population of dividing *Campelia* microspores shows at once that there are differences in the absolute lengths of the same chromosome in different cells. It is therefore necessary to correct for such differences before valid comparisons of length can be made.

Consider a cell with n chromosomes having " normalised " lengths $l_1, l_2, \ldots l_n$. The *actual* (measured lengths) can be expressed $kl_1, kl_2, \ldots kl_n$ where the " contraction factor " k is assumed to be the same for all chromosomes in the cell, but may vary stochastically in an unknown manner from cell to cell.

We are interested in the size distribution between cells of fragments from a particular exchange A. It is clear that we must consider " normalised " lengths because the measured lengths will involve k whose distribution between cells is unknown.

Normalisation can be achieved by regarding a particular chromosome arm in the cell, say S as a standard; let the " normalised " length of S be s.

In a particular cell suppose an A fragment of length l_c is observed, and the measured length of S in the same cell is s_c. The contraction factor k associated with this cell is, by definition, s_c/s, whence it follows that the "normalised" length corresponding to l_c is $l_c \div s_c/s = \dfrac{l_c s}{s_c}$.

For this particular cell, the *ratio* of the normalised fragment length to the normalised standard length is $\dfrac{l_c s}{s_c} \div s = l_c/s_c$, *i.e.* the same as the ratio of the measured lengths.

Now in considering the *distribution* of normalised fragment lengths it is quite immaterial what units the lengths are measured in. In fact we can regard s as the unit of length (even though it is unknown) in which case l_c/s_c becomes the normalised fragment length in s units.

In some cells, direct measurement of s_c may not be possible. In this case, another arm is used as standard, say T the normalised length of which is t. If, in a given cell, the measured length of T is t_c, the observed length of S would have been $s_c = t_c \times s/t$. We can obtain an estimate of s/t by measuring the ratio s_c/t_c in a number of cells (this ratio, by definition being

independent of k) and taking the mean. From this value, s_c can be calculated.

In the subsequent results, fragment lengths have been computed by these methods, and are expressed in s units.

The standard arm chosen was β_2 and where this was not available, α_1, α_2, β_1 or δ_1 in that order of preference.

3.3 *Expected distribution of acentric fragment size*

At anaphase, the centromeres lead to the spindle poles, so the chromosomes are more or less polarised when they commence decondensation in telophase. There is considerable evidence from studies of radiation-induced *chromatid* exchanges (Evans, 1961; Fox, 1966) that this polarised condition is retained throughout the succeeding interphase.

Consider a pair of arms of lengths, C_1L_1 and C_2L_2 which are polarised, having the centromeres at the same level (fig. 2). It is assumed that lesions

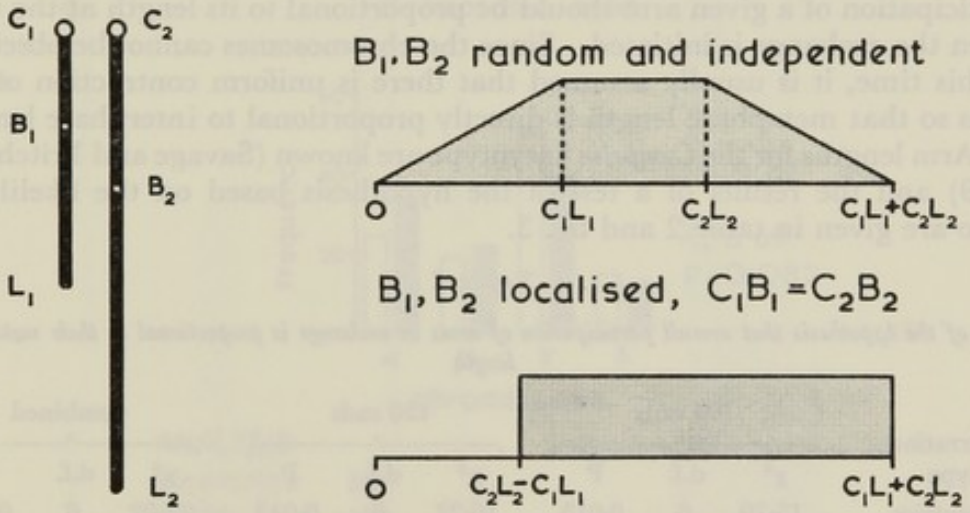


FIG. 2.—Expected forms of the frequency distributions of acentric fragment sizes following asymmetrical exchange between B_1 and B_2.

B_1, B_2 which may give rise to exchange can occur with equal probability anywhere along an arm. Asymmetrical exchanges will produce an acentric fragment of length $B_1L_1 + B_2L_2$.

Two limiting conditions can be envisaged regarding the positions of B_1,B_2 for them to be involved in exchange:

(1) B_1, B_2 are located randomly and independently in C_1L_1, C_2L_2 respectively.

(2) The participating lesions must be highly localised, *i.e.* the distances C_1B_1, C_2B_2 are identical, the common distance varying randomly between 0 and C_1L_1.

From what is known of the proximity requirements of lesions for exchange, and observations on the positions of *chromatid* exchanges, condition 2 would seem to be more probable. However, the ideal arrangement of fig. 2 is unlikely to be achieved in reality since the centromeres (except in the case of opposite arms of the same chromosome) will not necessarily be at the same level, and the two arms are unlikely to lie parallel along their entire lengths but may only fulfil the proximity requirement at certain points.

The forms of the frequency distributions of fragment size for the two limiting conditions have been derived (D. G. Papworth, unpublished) and

are shown in fig. 2. The most obvious difference lies in the existence of a minimum fragment size for condition 2 when $C_1L_1 < C_2L_2$.

4. Results

At the 100 rad dose 2053 metaphases were examined, and 182 chromosome exchanges observed. Of these, 170 (118 dicentrics and 52 centric rings) could be assigned without difficulty to one of the 28 exchange categories, and the associated fragment measured.

At 150 rads, 1786 cells yielded 308 exchanges, 260 of which (203 dicentrics and 57 centric rings) were classified and the fragments measured.

The rejected exchanges comprise those from cells having two or more (Savage and Papworth, 1969) together with any of uncertain classification.

4.1 *Overall participation of arms in exchange*

If exchange is really random, then the simplest hypothesis is that participation of a given arm should be proportional to its length at the time when the exchange is initiated. Since the chromosomes cannot be observed at this time, it is usually assumed that there is uniform contraction of the arms so that metaphase length is directly proportional to interphase length.

Arm lengths for the *Campelia* karyotype are known (Savage and Pritchard, 1969) and the results of a test of the hypothesis based on the likelihood ratio are given in table 2 and fig. 3.

Table 2

Test of the hypothesis that overall participation of arms in exchange is proportional to their metaphase length

Aberration type	100 rads			150 rads			combined		
	χ^2	d.f.	P	χ^2	d.f.	P	χ^2	d.f.	P
Dicentrics	15·79	6	0·015	16·22	6	0·013	22·02	6	0·0012
Rings	2·83	3	0·42	4·09	3	0·25	6·69	3	0·083

It is necessary to analyse centric rings separately from dicentrics because of probability differences which exist for these two classes of aberration (Savage and Papworth, 1969). There is no significant evidence to suggest that the relative frequencies with which the arms participate in exchange are different at the two doses (dicentrics, $\chi^2_6 = 10{\cdot}77$; $P = 0{\cdot}096$: rings, $\chi^2_3 = 0{\cdot}248$; $P = 0{\cdot}97$) so the data may legitimately be combined.

The results suggest that for dicentrics, participation in exchange is not proportional to arm length, but for centric rings the hypothesis cannot be rejected, though the agreement with expectation is only marginal.

4.2 *Participation of arms in individual exchanges*

A likelihood ratio test may also be used to compare observed and expected frequencies for the 24 categories of dicentrics.

In this case, there was significant evidence that the relative frequencies with which different types of dicentric occur are not the same at the two radiation doses ($\chi^2_{21} = 39{\cdot}21$; $P = 0{\cdot}009$) so that the data cannot be combined.

Because of low numbers in several categories, and the necessity to pool some of them, the analysis is not so reliable.

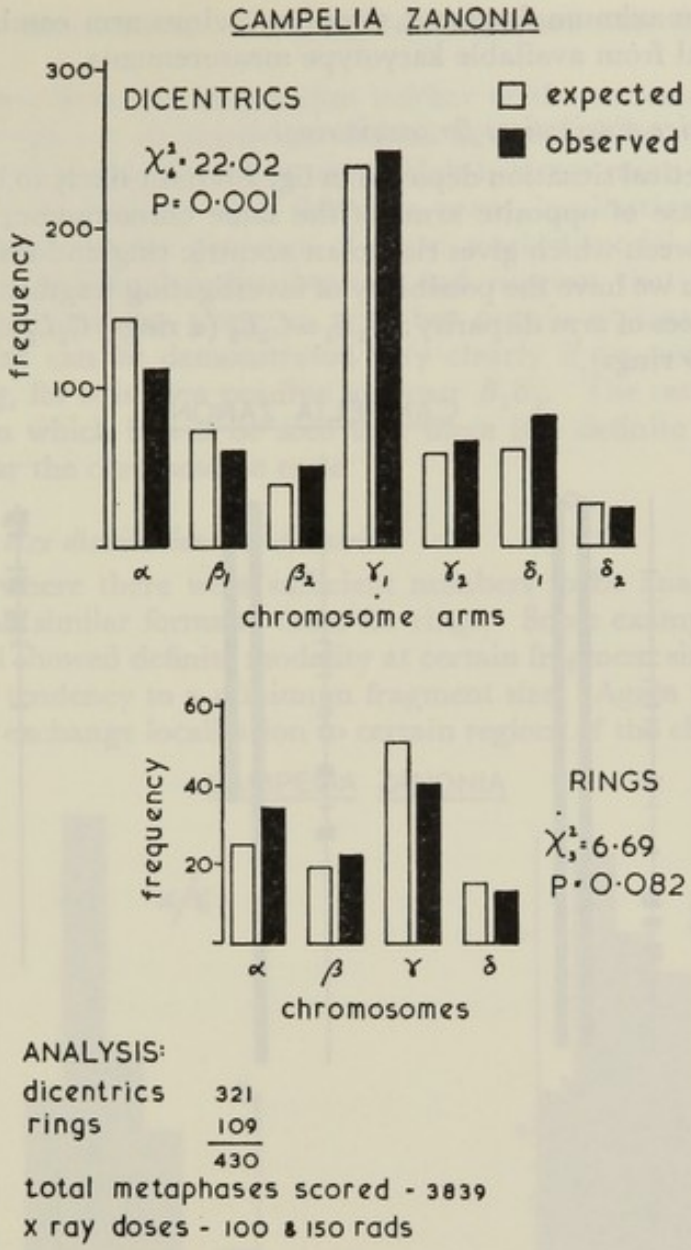


FIG. 3.—Overall participation of arms in chromosome exchanges.

For 100 rads, agreement with expectation is acceptable ($\chi^2_{21} = 29{\cdot}51$; $P = 0{\cdot}10$) but for 150 rads it is very poor ($\chi^2_{21} = 48{\cdot}01$; $P = 0{\cdot}0007$).

4.3 *Participation within arms*

About 30 normal cells from control (unirradiated) slides were selected and the standard arms measured with the same methods as those employed for fragments.

Estimates of s/t were obtained, thus enabling values of s_c to be computed for those cells where β_2 could not be used as a standard arm.

The fragment (in all cases single, since incompleteness of chromosome exchanges in *Campelia* is <1 per cent.) associated with every exchange was measured (l_c) together with the standard arm β_2 ($= s_c$) or its substitute, in the same cell. The normalised fragment length in s units was obtained from l_c/s_c, and expressed to the nearest 0·5 s unit for the purpose of the histograms.

Expected maximum fragment sizes for various arm combinations were also calculated from available karyotype measurements.

4.4 *Fragment size distributions for centric rings*

The theoretical situation depicted in fig. 2 is most likely to be approached only in the case of opposite arms of the same chromosome, asymmetrical exchange between which gives rise to an acentric ring and fragment.

In *Campelia* we have the possibility of investigating fragment distributions for three degrees of arm disparity: $C_1L_1 \simeq C_2L_2$ (α rings) $C_1L_1 < C_2L_2$ (β rings) $C_1L_1 \ll C_2L_2$ (γ rings).

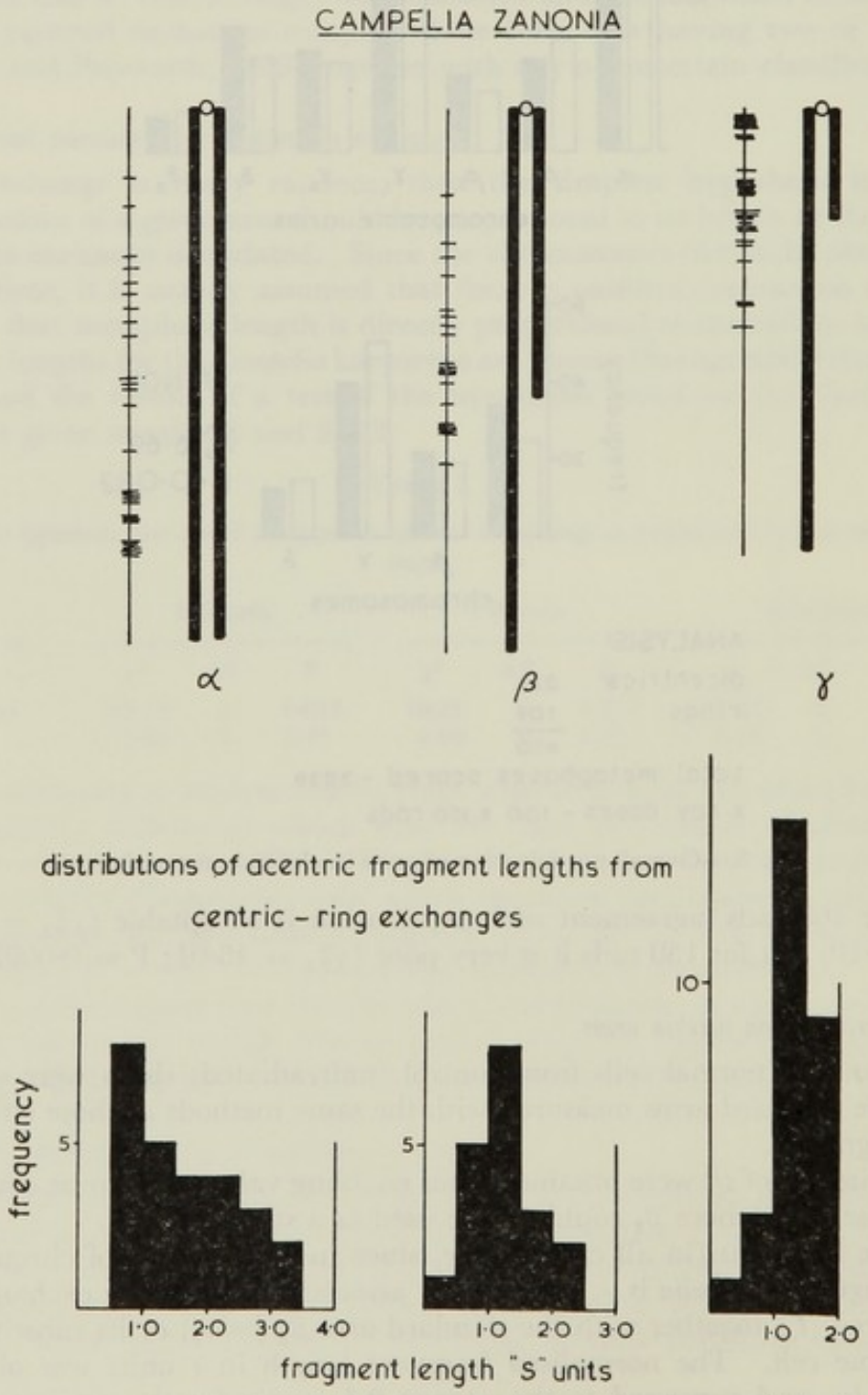


FIG. 4.—Frequency distributions of fragment size for centric rings, and the location of exchange points assuming condition 2 (see text).

Frequency distributions of fragment size are shown for these three ring classes in fig. 4.

It is obvious from the shapes that neither of the extreme conditions is fulfilled, and this was in general confirmed from statistical tests. There is evidence of a minimum fragment size, which becomes more obvious as the arm disparity increases; and therefore some localisation is definitely indicated. However the presence of quite marked modal sizes rules out the idea of a rectangular distribution, and suggests that probability of exchange is not uniform along an arm, but certain regions are favoured. This last point can be demonstrated very clearly if we assume condition 2 is operating, for it is then possible to locate B_1B_2. The results are shown in fig. 4 from which it will be seen that there is a definite preference for exchange near the chromosome ends.

4.5 *Fragment size distributions for dicentrics*

In cases where there were sufficient numbers to be analysed, the distributions had similar forms to those for rings. Some examples are shown in fig. 5. All showed definite modality at certain fragment sizes, and nearly all showed a tendency to a minimum fragment size. Again this is strongly suggestive of exchange localisation to certain regions of the chromosomes.

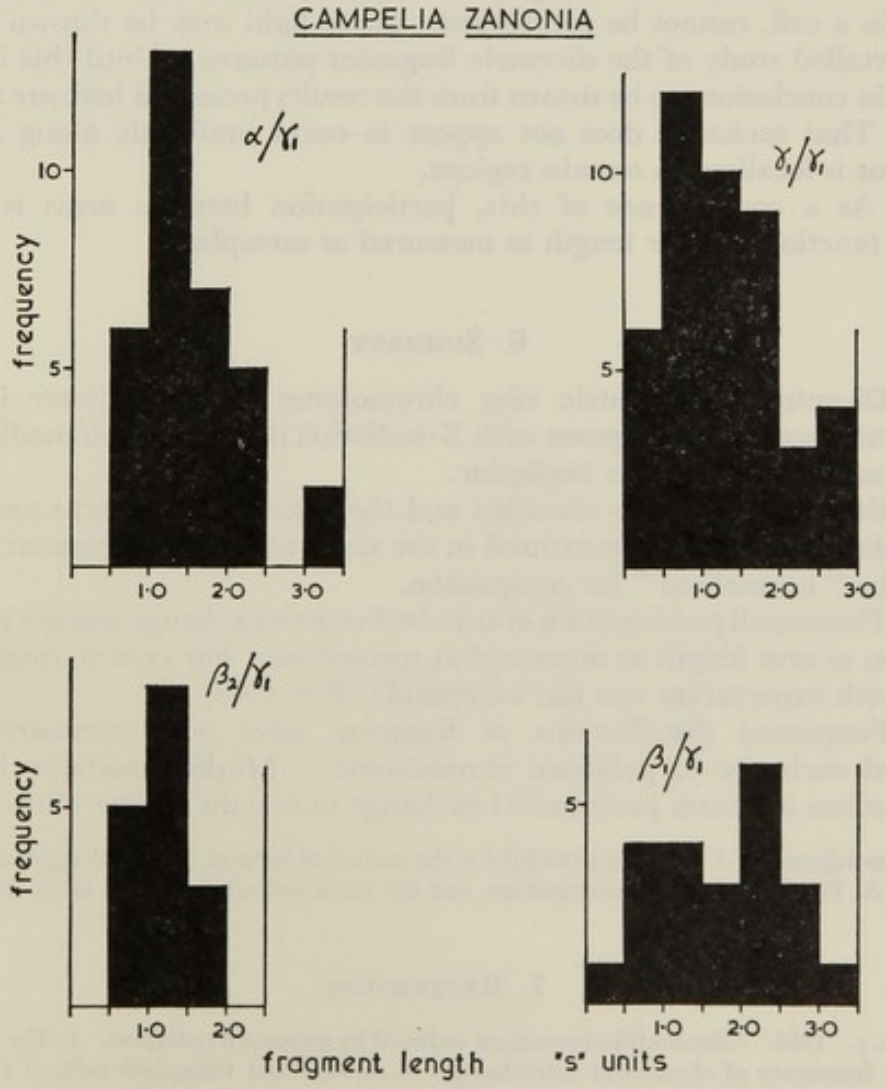


FIG. 5.—Frequency distributions of fragment size for some dicentrics.

5. Discussion and conclusions

Participation between and within arms must of necessity be related. It is not therefore surprising, in view of the finding that participation along a given arm is not uniform, to find that participation between arms is not proportional to length. The agreement between observed and expected is not however very poor and in this respect the data are in line with those of Norman and Sasaki (1966) for *chromosome* exchanges in human leucocytes and in contrast to most of the findings for *chromatid* exchanges.

Campelia (like *Tradescantia*) has no demonstrable heterochromatin so the tendency for localisation of exchanges to certain arm regions cannot be explained on those grounds (*cf. Vicia*, Evans and Bigger, 1961). The possibility that, at least for rings, the preponderance of exchanges occur near the chromosome ends, is in contrast to the findings of Sax (1940) for *Tradescantia* microspores who claims a preferential exchange close to the centromeres. It should be noted however that some of his data was obtained after radiation doses where distortion would be important. In *Hordeum*, Kumar and Natarajan (1966) find the majority of ring sites at the distal ends of the chromosomes, but again the radiation doses used were high.

The possibility that the observed localisation is an artefact produced by non-uniform contraction along chromosome arms, and between chromosomes in a cell, cannot be overlooked. Some light may be thrown on this by a detailed study of the dicentric fragment patterns. Until this is done, the main conclusions to be drawn from the results presented here are:

(1) That exchange does not appear to occur uniformly along a given arm, but is localised in certain regions.

(2) As a consequence of this, participation between arms is not a simple function of their length as measured at metaphase.

6. Summary

1. Dicentric and centric ring chromosome exchanges were induced in *Campelia zanonia* microspores with X-radiation doses at which modification by secondary exchanges is negligible.

2. Each exchange was classified and the associated fragment measured. A standard arm was also measured in the same cell so that fragment lengths could be " normalised " for comparison.

3. The overall participation of arms in dicentric exchange was not a simple function of arm length as measured at metaphase. For centric rings agreement with expectation was just acceptable ($P = 0{\cdot}08$).

4. Frequency distributions of fragment sizes were consistent with localised exchange in polarised chromosomes. Marked modality in these distributions indicates preferential exchange in certain regions of the arms.

Acknowledgments.—I am very grateful for the technical help of Mr D. Wigglesworth and Mrs M. A. Pritchard in this investigation, and the statistical advice of Mr D. G. Papworth.

7. References

Evans, H. J. 1961. Chromatid aberrations induced by gamma irradiation. I. The structure and frequency of chromatid interchanges in diploid and tetraploid cells of *Vicia faba*. *Genetics*, *46*, 257-275.

EVANS, H. J., AND BIGGER, T. R. L. 1961. Chromatid aberrations induced by gamma irradiation. II. Non-randomness in the distribution of chromatid aberrations in relation to chromosome length in *Vicia faba* root-tip cells. *Genetics*, *46*, 277-289.

FOX, D. P. 1966. The effects of X-rays on the chromosomes of locust embryos. II. Chromatid interchanges and the organisation of the interphase nucleus. *Chromosoma*, *20*, 173-194.

KUMAR, S., AND NATARAJAN, A. T. 1966. Kinetics of two-break chromosome exchanges and the spatial arrangement of chromosome strands in the interphase nucleus. *Nature*, *209*, 796-797.

NORMAN, A., AND SASAKI, M. S. 1966. Chromosome exchange aberrations in human lymphocytes. *Int. J. Rad. Biol.*, *11*, 321-328.

REIGER, R., AND MICHAELIS, A. 1959. Vergleichende untersuchungen zur verteilung der verschiedene mutagene induzierter brücke über den Chromosomensatz von *Vicia faba* L. *Chromosoma*, *10*, 163-178.

SAVAGE, J. R. K. 1970. Sites of radiation induced chromosome exchanges. In *Current Topics in Radiation Research* (eds. M. Ebert and A. Howard), *6*, 129-194. North-Holland Publishing Co., Amsterdam.

SAVAGE, J. R. K., AND PAPWORTH, D. G. 1969. Distortion hypothesis: An alternative to a limited number of sites for radiation-induced chromosome exchange. *J. Theoret. Biol.*, *22*, 493-514.

SAVAGE, J. R. K., AND PRITCHARD, M. A. 1969. *Campelia zanonia* (L.) H.B.K.: A new material for the study of radiation-induced chromosomal aberrations. *Radiat. Bot.*, *9*, 133-139.

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

THE INDUCTION OF CHROMOSOMAL ABERRATIONS BY SULPHUR MUSTARD IN MARSUPIAL LYMPHOCYTES

D. SCOTT* and T. R. L. BIGGER

Medical Research Council, Radiobiology Unit, Harwell, Didcot, Berkshire, Great Britain

1. Introduction

When root-tips of *Vicia faba* are treated with maleic hydrazide (Evans and Scott, 1964; Scott, 1968) or the alkylating agents, nitrogen mustard (Evans and Scott, 1969) and sulphur mustard (Bhatt, Revell, Evans and Scott, unpublished), lesions, from which chromatid aberrations are derived, can be induced in cells in all stages of the mitotic cycle. The aberrations are, however, only realised at the time of DNA synthesis and presumably result from replication errors.

The evidence for these conclusions came from experiments in which the chromosome-breaking agents were given in conjunction with tritium-labelled thymidine (^{3}HTdr) in order to partition the asynchronous population into G_2, S and G_1 phases (fig. 1). Observations were then made on the

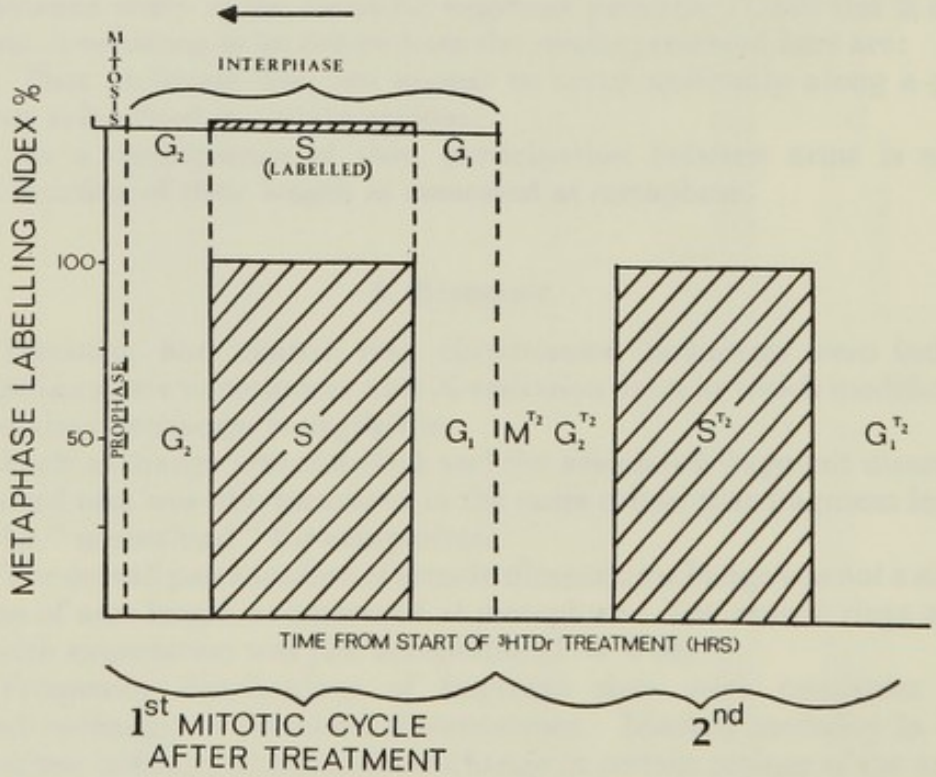


Fig. 1.—Progression through the mitotic cycle of an asynchronous population of cells, pulse-labelled with ^{3}HTdr. It is assumed that all cells pass through the cycle at the same rate and that the duration of metaphase is short. Labelled metaphases ■.

presence or absence of chromosomal abnormalities in labelled and unlabelled mitotic cells, at various times after treatment, in order to determine the time of induction of aberrations in relation to the mitotic cycle.

* Present address: Peterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester.

An important question which arises as a result of these experiments is whether or not the lesions from which aberrations are derived can be repaired before being " fixed " at the time of DNA synthesis. There is now evidence, in mammalian cells as well as in micro-organisms, of repair of alkylation lesions in DNA (Roberts, Brent and Crathorn, 1968; Hahn, Yang and Parker, 1968).

A synchronously developing population of cells is required for such an investigation. Small lymphocytes in the blood are normally all " resting " in the same stage of the cell cycle, G_1 or G_0 (Bender and Prescott, 1962), and can be stimulated with phytohaemagglutinin (Hungerford *et al.*, 1959) to progress, initially in a synchronous manner, through the mitotic cycle. Peripheral blood lymphocytes of the potoroo, or Tasmanian rat-kangaroo (*Potorous tridactylus*), were considered to be a suitable material as these animals have only a small number, $2n = 12$ (♀) or 13 (♂), of readily inter-distinguishable chromosomes (Sharman, McIntosh and Barber, 1950; Shaw and Krooth, 1964).

2. Experiment 1

First it was necessary to determine whether or not similar results to those in *Vicia* would be obtained in potoroo lymphocytes treated in a similar manner with sulphur mustard (SM). Lymphocytes were grown *in vitro* for 48 hr. before treatment, at which time there were expected to be cells in all stages of the mitotic cycle (fig. 2).

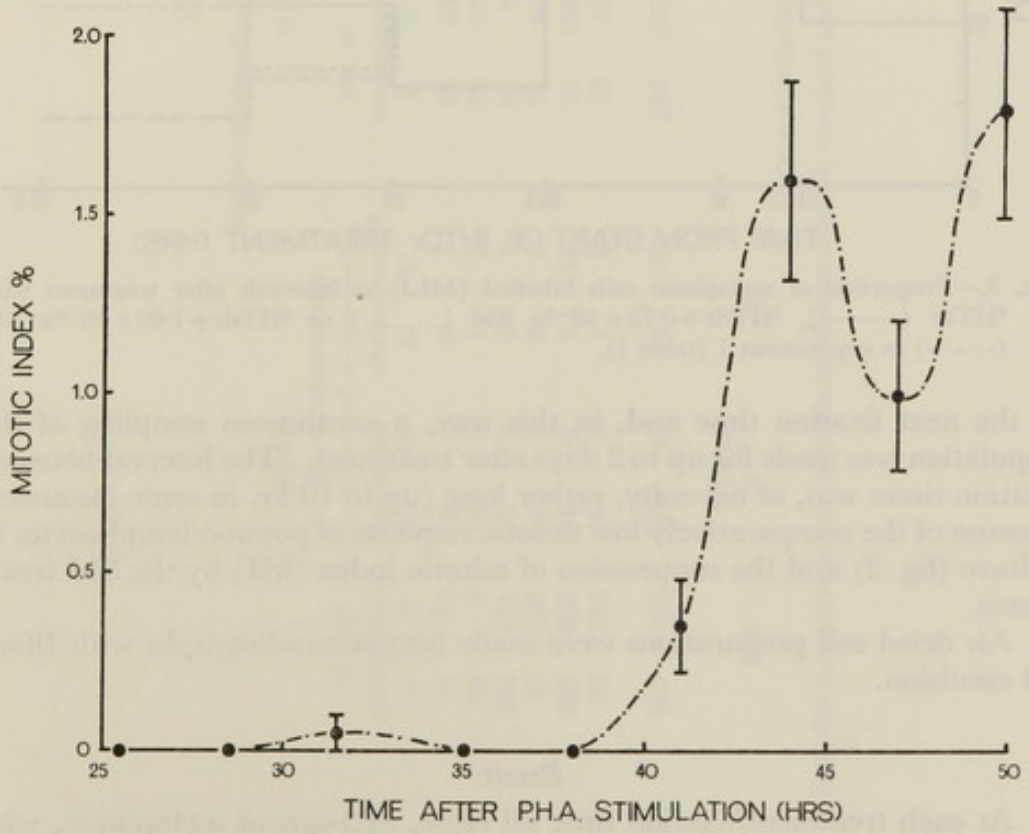


Fig. 2.—Mitotic index in potoroo blood cultures. Cells given 3 hr. colcemid before fixation. 2000 cells scored per point.

Materials and methods

A small incision was made in the lateral tail vein of a male potoroo to obtain 10 ml. of blood. Whole-blood microcultures (except controls) were given ^{3}HTdr (1·0 μCi/ml., SA 3·2 Ci/mM), for 1 hr., 48 hr. after stimulation. Cells were washed, treated with SM (0·75 or $1{\cdot}00 \times 10^{-6}$M) in Hanks B.S.S. for 40 min., then recultured at 37° C. At each fixation time (table 1, fig. 3) colcemid was added to those cultures to be harvested

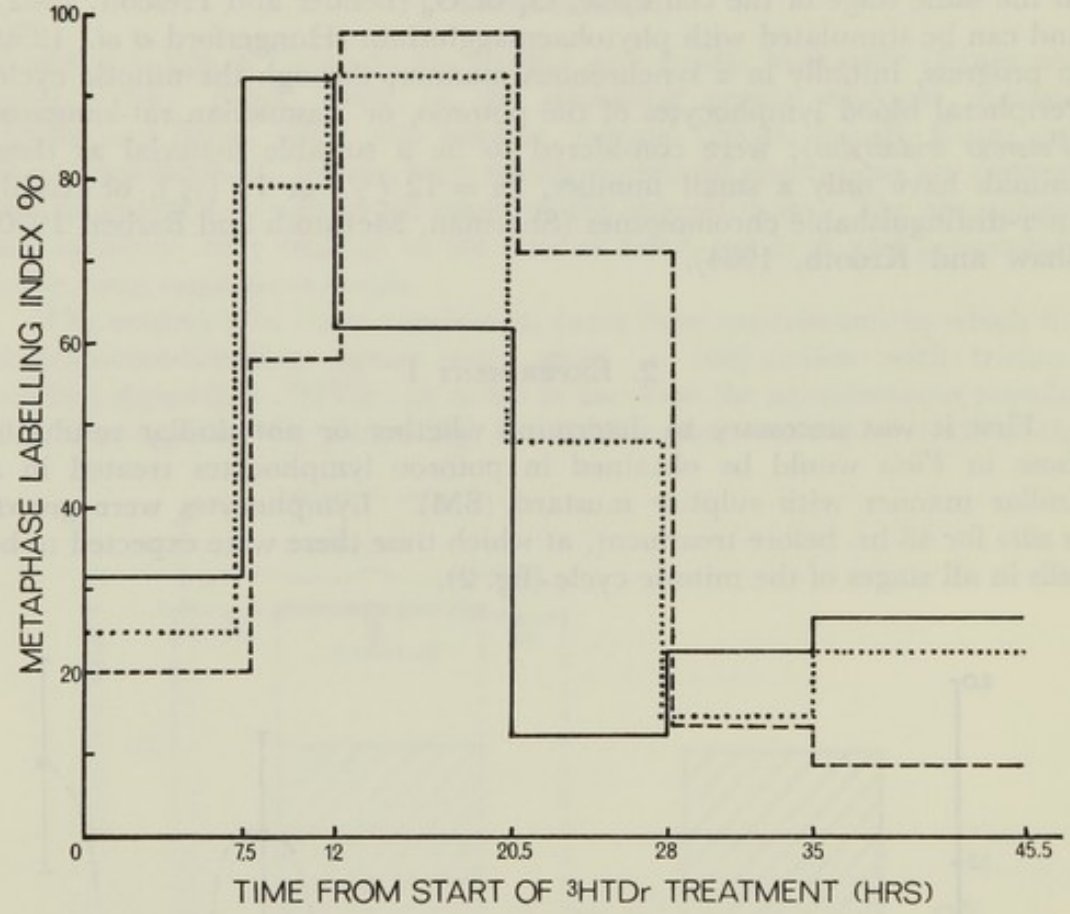


FIG. 3.—Proportion of metaphase cells labelled (MLI) at intervals after treatment with ^{3}HTdr (———), ^{3}HTdr+$0{\cdot}75 \times 10^{-6}$M SM (.....) or ^{3}HTdr+$1{\cdot}00 \times 10^{-6}$M SM (- - - - -) in experiment 1 (table 1).

at the next fixation time and, in this way, a continuous sampling of the population was made for up to 2 days after treatment. The interval between fixation times was, of necessity, rather long (up to 10 hr. in some instances) because of the comparatively low mitotic response of potoroo lymphocytes in culture (fig. 2) and the suppression of mitotic index (MI) by the SM treatments.

Air dried cell preparations were made into autoradiographs with Ilford L4 emulsion.

Results

At each treatment/fixation time all clear, well-spread metaphases, with a complete chromosome complement, were scored for the presence or absence of label and aberrations (table 1).

TABLE 1

Numbers of aberrations in experiment 1 (figs. 3-6)

Fixation time after ^{3}HTdr (hr.)	^{3}HTdr						^{3}HTdr + SM ($0{\cdot}75 \times 10^{-6}$M)						^{3}HTdr + SM ($1{\cdot}00 \times 10^{-6}$M)					
	No. of metaphases		No. of aberrations: chromatid		chromosome*		No. of metaphases		No. of aberrations: chromatid		chromosome		No. of metaphases		No. of aberrations: chromatid		chromosome	
	L	U	L	U	L	U	L	U	L	U	L	U	L	U	L	U	L	U
7½	32	69	0	1	0	1	28	85	3	3	0	0	19	77	2	5	0	0
12	99	8	6	0	1	2	79	21	15	2	2	0	47	34	4	2	0	1
20½	194	121	16	3	3	1	63	5	12	1	1	0	100	2	20	0	0	0
28	47	336	6	7	0	2	77	83	38	28	0	7	71	29	27	14	1	1
35	202	695	34	16	9	12	23	138	25	76	1	7	63	401	54	187	4	10
45½	93	261	14	8	1	2	13	45	3	10	0	3	12	133	8	38	1	5
Totals	667	1490	76	35	14	20	283	377	96	120	4	17	312	676	115	246	6	17

Control

158 | 4 | 3

Gaps not included in aberration yields.

* Includes symmetrical and asymmetrical interchanges, terminal and interstitial deletions. Types of chromatid aberrations shown in table 3.

(1) *The mitotic cycle*

After a pulse label of ^{3}HTdr the first cells to arrive at mitosis are unlabelled (U) cells that were in G_2 at the time of treatment. These are followed by labelled (L) mitotic cells that were in S and then by U cells that were in G_1. The UG_2 cells then divide again (T_2 mitosis) followed by the LS cells again ... etc. Fig. 1 shows the expected changes in the proportion of mitotic cells labelled (metaphase labelling index—MLI) in an asynchronous population in which all cells progress at the same rate through the cycle. Not all cells do move at the same rate, however, and fig. 3 shows the actual MLIs after ^{3}HTdr and ^{3}HTdr+SM treatments in this experiment, from which we see that SM causes a dose-dependent protraction of the cell cycle which appears to result mainly from an elongation of the S phase, as after maleic hydrazide (MH) and nitrogen mustard (NM) in *Vicia*.

(2) *Chromosomal aberration yields after:*

(*a*) *^{3}HTdr only*

(i) The frequency of chromosome-type aberrations (0·016 per cell, overall), which did not vary significantly with sampling time ($\chi^2_5 = 9{\cdot}27$, $P = 0{\cdot}10$) was no higher than the frequency (0·019/cell) in untreated controls ($P = 0{\cdot}74$; Exact test, Rao, 1952).

(ii) The frequency of chromatid aberrations in U cells (fig. 4) did not vary with sampling time ($\chi^2_5 = 1{\cdot}3$, $P = 0{\cdot}94$) and the mean yield (0·023/cell) was, again, no higher than the control value of 0·025/cell ($P = 0{\cdot}79$).

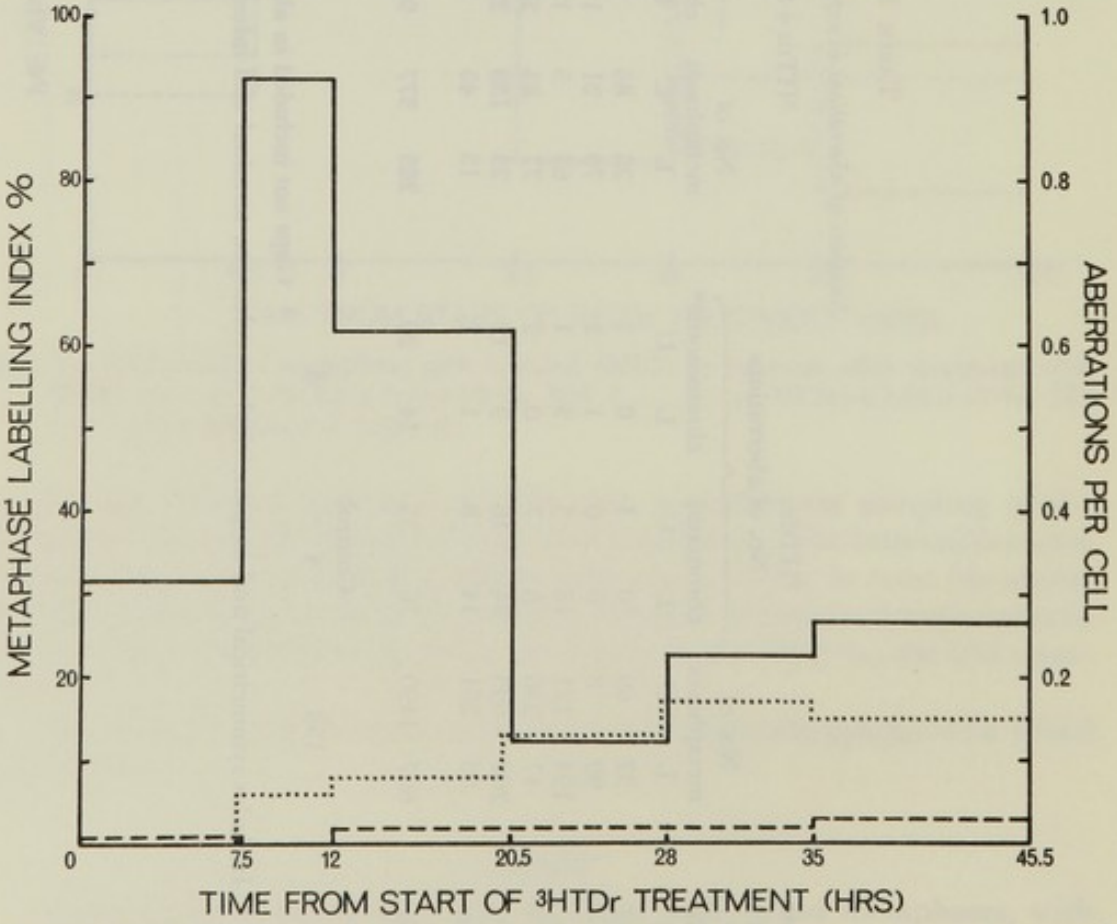


FIG. 4.—MLI (———) and the frequency of chromatid aberrations in labelled (.....) and unlabelled (- - - -) cells at intervals after ^{3}HTdr in experiment 1 (table 1).

(iii) The chromatid aberration yield in L cells (fig. 4) did however, increase with increasing time after treatment ($\chi^2_5 = 17{\cdot}7$, $P = 0{\cdot}0032$) and was higher than the control value at all except the first two fixation times.

It would appear that the ^{3}HTdr induced a low frequency of chromatid aberrations in L cells. This has been observed in many cell systems (for references see Cleaver, 1967).

(b) ^{3}HTdr+SM (table 1)

(i) The overall yield of chromosome-type aberrations (0·032/cell) after the lower concentration of SM was greater than after ^{3}HTdr alone (0·016/cell; $\chi^2_1 = 6{\cdot}67$, $P = 0{\cdot}0098$) but the difference was not significant after the higher SM concentration (0·023/cell; $\chi^2_1 = 2{\cdot}11$, $P = 0{\cdot}15$). However, these results do not mean that chromosome aberrations were in fact induced. " Apparent " chromosome-type aberrations can be derived from chromatid aberrations appearing at a previous mitosis (Evans, 1970) and in experiments 3 and 4, where only T_1 cells were examined, there was no evidence that SM can induce chromosome-type aberrations. Several agents are now known which will induce chromatid aberrations only (Kihlman, 1966).

(ii) The frequencies of chromatid aberrations in L and U metaphases after SM are given in figs. 5 and 6. The yields have been reduced by an amount equal to that induced by ^{3}HTdr only, and, where the frequency at any given sampling time was found, by an Exact test, to be no higher than after ^{3}HTdr alone (fig. 4) the yield is shown as zero.

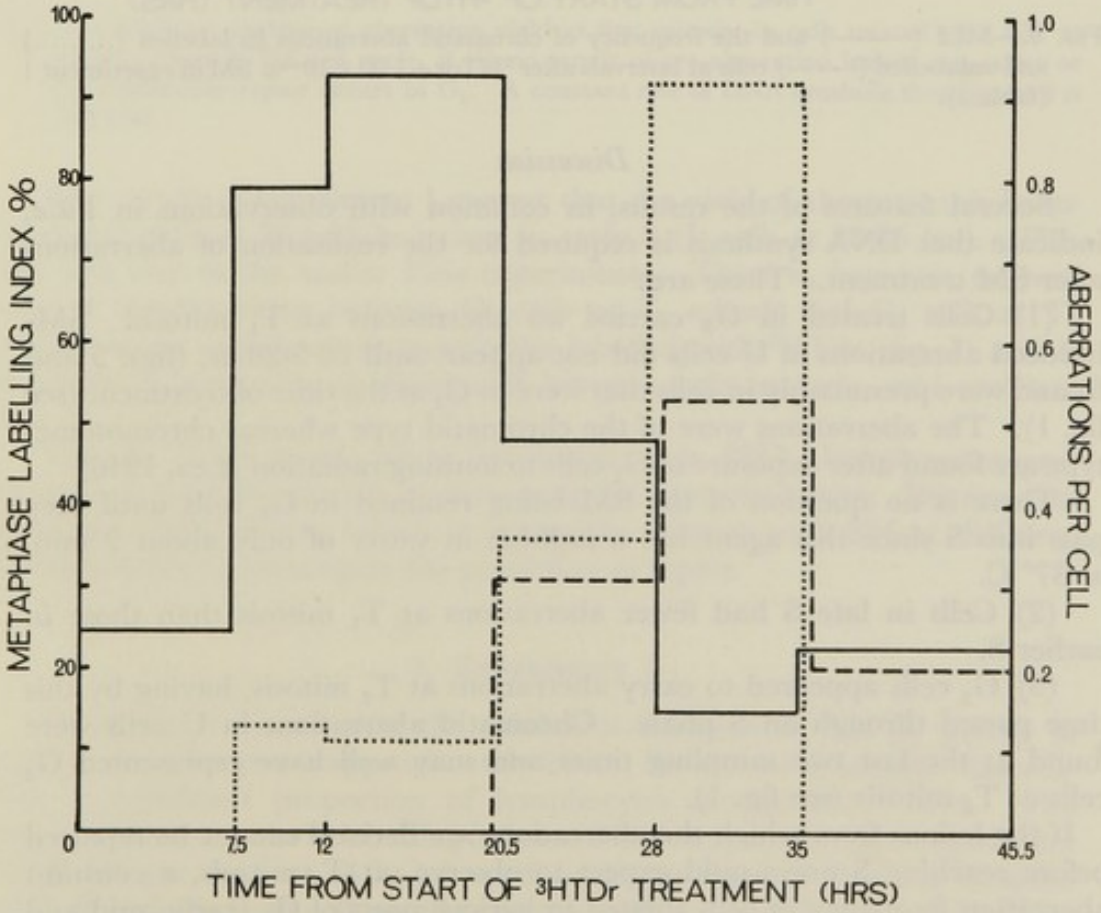


FIG. 5.—MLI (———) and the frequency of chromatid aberrations in labelled (.....) and unlabelled (- - - -) cells at intervals after ^{3}HTdr + $0{\cdot}75 \times 10^{-6}$M SM in experiment 1 (table 1).

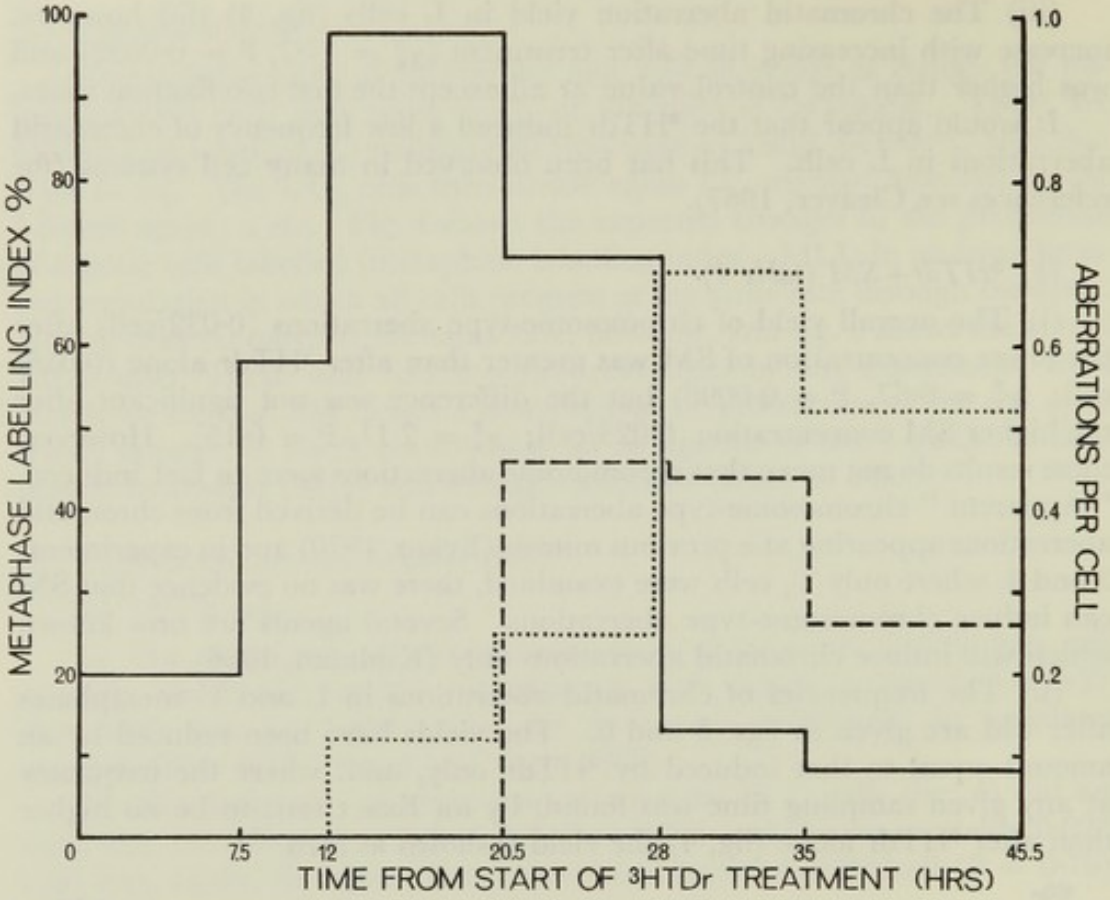


FIG. 6.—MLI (———) and the frequency of chromatid aberrations in labelled (.....) and unlabelled (- - - -) cells at intervals after ^{3}HTdr + $1{\cdot}00 \times 10^{-6}$M SM in experiment 1 (table 1).

Discussion

Several features of the results, in common with observations in *Vicia*, indicate that DNA synthesis is required for the realisation of aberrations after SM treatment. These are:

(1) Cells treated in G_8 carried no aberrations at T_1 mitosis. SM-induced aberrations in U cells did not appear until 20·5-28 hr. (figs. 5 and 6) and were presumably in cells that were in G_1 at the time of treatment (see fig. 1). The aberrations were of the chromatid type whereas chromosome-type are found after exposure of G_1 cells to ionising radiation (Lea, 1946).

There is no question of the SM being retained in G_1 cells until they pass into S since this agent has a half-life in water of only about 2 min. at 37° C.

(2) Cells in late S had fewer aberrations at T_1 mitosis than those in earlier S.

(3) G_2 cells appeared to carry aberrations at T_2 mitosis, having by this time passed through an S phase. Chromatid aberrations in U cells were found at the last two sampling times and may well have represented G_2 cells at T_2 mitosis (see fig. 1).

If the lesions from which the aberrations are derived cannot be repaired before reaching S one would expect to observe, at T_1 mitosis, a constant aberration frequency in cells treated in various parts of G_1 (early, mid and late) and a decreasing yield in S cells, the rate of decrease being determined by the amount of unreplicated DNA present at the different stages of S

(fig. 7). Alternatively if repair of pre-aberration lesions *is* possible one might expect to find that cells furthest away from S (*i.e.* in early G_1) would have less aberrations than those nearer to S (late G_1), since the former will have had more time to repair the damage before it is " fixed " at S (fig. 7).

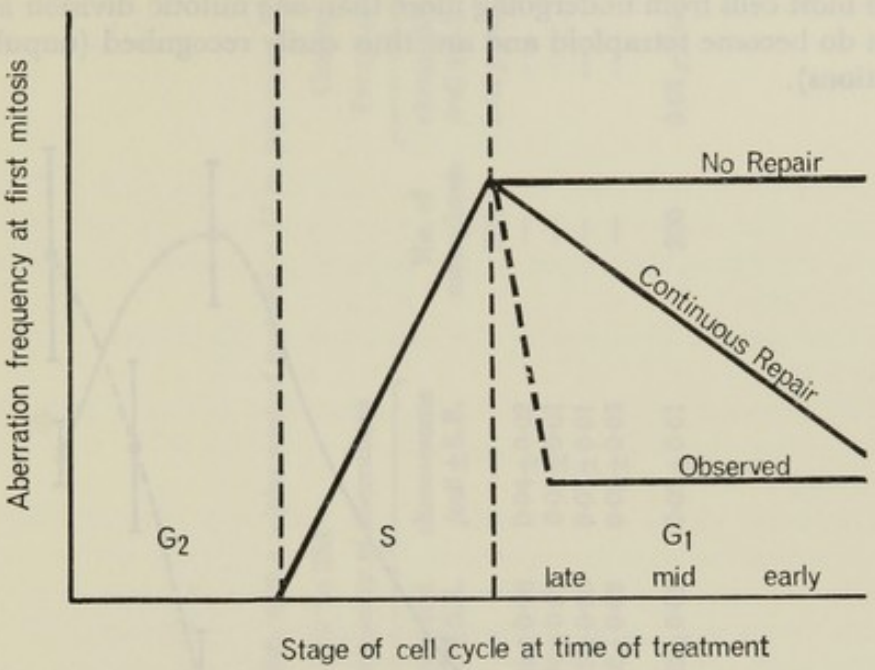


FIG. 7.—Expected pattern of aberration yield at first mitosis, in cells treated with SM at various stages of the cell cycle, if (1) no repair of pre-aberration lesions can occur or (2) continuous repair occurs in G_1. A constant rate of DNA synthesis throughout S is assumed.

The results of experiment 1 suggest that the yield of aberrations is lower in G_1 (U cells at 20·5-28 hr.) than in early S (L cells at 28-35 hr.). This was also true in the earlier *Vicia* experiments. There is, however, no sure way of distinguishing between G_1 cells at T_1 mitosis and G_2 cells at T_2 mitosis in an asynchronous population labelled with ^{3}HTdr (fig. 1) and it is certainly impossible to distinguish between cells in early, mid and late G_1.

On the other hand, when stimulated with PHA, lymphocytes pass through a long G_1 period as they progress towards mitosis (Bender and Prescott, 1962), during which it is possible to treat with SM at different times in order to investigate the possibility of repair.

3. EXPERIMENT 2

From fig. 8 we see that the minimum duration of this G_1 period in potoroo lymphocytes is approximately 20 hr., for at later times after stimulation a significant proportion of lymphocytes incorporate ^{3}HTdr. The absence of a sharp rise in labelling index and the comparatively low maximum value (11 per cent. at 44 hr.) is attributable to the fact that the duration of G_1 is variable from cell to cell (minimum 20 hr.) and that not all lymphocytes respond to the PHA by undergoing DNA synthesis (Sasaki and Norman, 1966).

The MI in fig. 8, which was determined after the addition of colcemid to cultures at 24 hr. (Sasaki and Norman, 1966) was found to be higher than after short term colcemid treatments (compare with fig. 2) and it was found to be necessary to use this procedure in subsequent experiments to obtain sufficient mitotic cells for analysis. Continuous colcemid treatment prevents most cells from undergoing more than one mitotic division and the few that do become tetraploid and are thus easily recognised (unpublished observations).

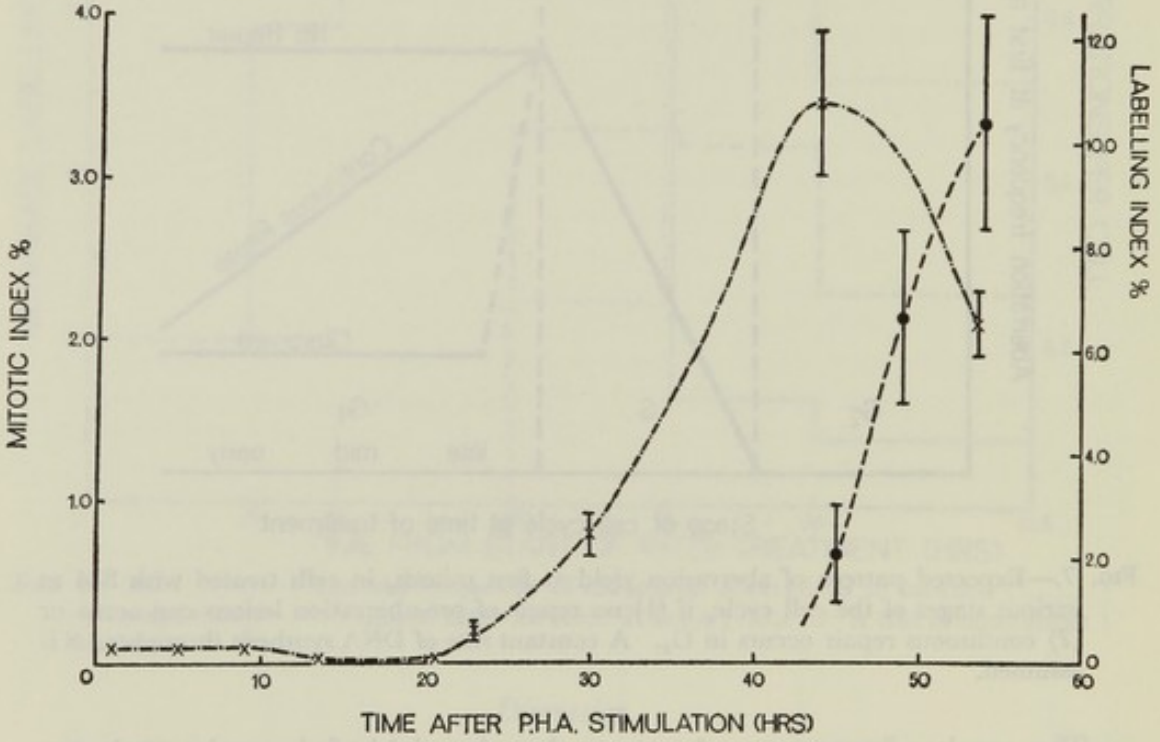


FIG. 8.—Proportion of mononuclear leucocytes labelled after a 1 hr. treatment with ^{3}HTdr before fixation at various intervals after culture initiation (X); 1500 cells scored per point. Mitotic index after addition of colcemid at 24 hr. (●); 750 cells scored per point. Experiment 2.

4. EXPERIMENT 3

This experiment was designed to investigate the effect of fixation time on the aberration frequency of cells treated with SM at a single time in G_1 (17 hr. after stimulation). If the difference between cycle times, of those cells which arrive at first mitosis at different intervals after stimulation, is mainly attributable to variations in the duration of G_1 (as the data of Sasaki and Norman, 1966, indicate) and if a repair process of the type shown in fig. 7 can occur, then one would expect a decreasing yield of aberrations with increasing fixation time. This follows since cells which arrive early at T_1 mitosis will, in effect, have been treated later in their G_1 period than those which arrive later.

Materials and methods

Preliminary G_1 treatments with SM, to find a suitable concentration for subsequent experiments, showed that:

(1) concentrations $> 2 \times 10^{-6}$M almost completely suppressed mitotic activity whereas those of less than $0{\cdot}3 \times 10^{-2}$M had little effect upon MI

TABLE 2

Aberration frequencies at various fixation times after stimulation, with a sulphur mustard treatment at 17 hr. (experiment 3)

Time after stimulation (hr.)	$1{\cdot}00\times10^{-6}$M SM			$1{\cdot}50\times10^{-6}$M SM			Control		
		Frequency of aberrations			Frequency of aberrations			Frequency of aberrations	
	No. of metaphases	chromatid /cell ± S.E.	chromosome /cell ± S.E.	No. of metaphases	chromatid /cell ± S.E.	chromosome /cell ± S.E.	No. of metaphases	chromatid /cell ± S.E.	chromosome /cell ± S.E.
47	—	—	—	—	—	—	200	0·01 ± 0·01	0·04 ± 0·01
65	183	0·13 ± 0·03	0·01 ± 0·01	82	0·26 ± 0·06	0·04 ± 0·02	—	—	—
71	165	0·16 ± 0·03	0	106	0·24 ± 0·05	0·01 ± 0·01	—	—	—
77	111	0·15 ± 0·04	0	72	0·19 ± 0·05	0·01 ± 0·01	—	—	—
88	46	0·15 ± 0·06	0	35	0·26 ± 0·09	0·03 ± 0·03	—	—	—
Totals	505	0·15 ± 0·02	0·004 ± 0·003	295	0·23 ± 0·03	0·02 ± 0·01	200	0·01 ± 0·01	0·04 ± 0·01

but induced very few chromosomal aberrations. The workable range appeared to be from 0·5 to $1{\cdot}5 \times 10^{-6}$M.

(2) the time of onset of mitosis in cultures given SM in G_1 was considerably delayed compared with untreated controls, probably because of a protraction of the S phase (Roberts, Brent and Crathorn, 1968).

In the present experiment, cells were given SM at 1·00 or $1{\cdot}50 \times 10^{-6}$M, 17 hr. after stimulation, and colcemid (1·5 μg/ml.) at 24 hr. and again at 48 hr.

Results (table 2)

(1) Before 65 hr. and later than 88 hr. after stimulation the MI in those cultures given SM was extremely low and no tetraploid cells were observed. It appears, therefore, that most of the mitotic population was examined, and at T_1 mitosis.

(2) There was no evidence of the induction of chromosome-type aberrations by SM.

(3) The frequency of chromatid aberrations did not vary with fixation time after either the lower SM concentration ($\chi^2_3 = 0{\cdot}72$, $P = 0{\cdot}87$) or the higher ($\chi^2_3 = 0{\cdot}73$, $P = 0{\cdot}87$).

(4) The mean yields of chromatid aberrations were considerably lower than the frequencies in, what were assumed to be, G_1 cells in experiment 1 (*i.e.* U cells between 20·5 and 28 hr.). This suggests that these samples in experiment 1 were not a pure G_1 population and emphasises the difficulty of interpreting such results obtained after the treatment of an asynchronous population.

(5) The chromatid aberration yields were markedly lower than those in early S cells (L cells at 28-35 hr.) in experiment 1.

Discussion

The lower yield of aberrations in G_1 than in early S cells suggests repair, but the absence of a fall-off in yield with increasing fixation time indicates that a continuous repair process in G_1 (fig. 8) does not occur. Perhaps repair of the lesions is a short-lived process.

5. Experiment 4

Materials and methods

Cells were treated with SM ($1{\cdot}25 \times 10^{-6}$M) before, and at several times after stimulation (fig. 9). Colcemid was added at 24 and 48 hr. except to those cells given SM at 54 hr., in which case it was added after the SM treatment. Controls (no SM) were fixed at 50 hr. and the rest between 70 and 76 hr.

Results (table 3, fig. 9)

(1) The frequency of chromosome-type aberrations did not vary with treatment time ($\chi^2_6 = 7{\cdot}57$, $P = 0{\cdot}27$) and the mean yield (0·021/cell) was no higher than the control value of 0·016/cell ($\chi^2_1 = 0{\cdot}54$, $P = 0{\cdot}46$).

(2) The yield of chromatid aberrations (excluding gaps) was constant in G_1 (*i.e.* at the 1·5, 5·5, 9·5 and 14 hr. treatments) with $\chi^2_3 = 1{\cdot}17$ and $P = 0{\cdot}76$, the mean frequency being 0·114/cell.

TABLE 3

Numbers of aberrations in experiment 4 (fig. 9)

Treatment time after stimulation (hr.)	Metaphases	Iso-chromatid gaps	Chromatid gaps	Chromatid-type aberrations					Chromosome-type aberrations
					Intrachanges				
				Inter-changes	Iso-chromatid deletions	Chromatid deletions	Other intra-changes	Triradials	
Pre-stimulation	437	9	18	2	32	9	17	0	3
1½	320	2	11	3	15	4	9	0	6
5½	525	6	25	4	36	9	10	1	11
9½	683	6	17	8	49	9	16	1	14
14	691	12	26	7	49	9	12	1	19
21	585	10	17	9	52	12	12	0	14
54	84	1	4	4	16	5	4	0	1
Totals	3325	46	118	37	249	57	80	3	68
Control	794	9	16	0	0	4	6	0	13

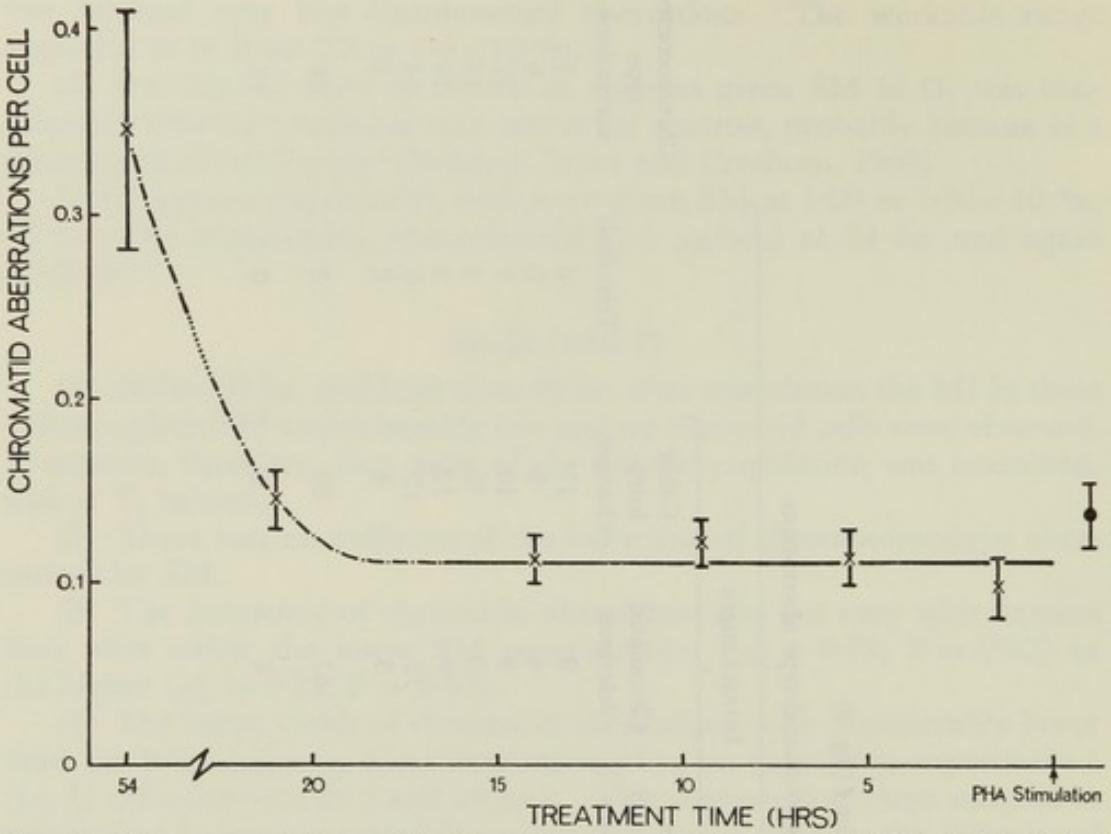


FIG. 9.—Frequency of chromatid aberrations in cells treated with SM ($1{\cdot}25 \times 10^{-6}$M) before, and at various times after stimulation (experiment 4, table 3). Scale on abscissa reversed for comparison with fig. 7.

(3) The yield in cells treated before stimulation was a little higher (0·137/cell) than the G_1 yield, but not significantly so ($\chi_1^2 = 1{\cdot}75$, $P = 0{\cdot}19$).

(4) The yield after the 21 hr. treatment (0·145/cell) was higher than that in G_1 ($\chi_1^2 = 3{\cdot}88$, $P = 0{\cdot}05$) probably because some cells had entered S at this time (fig. 9).

(5) At 54 hr. the yield (0·345/cell) was markedly higher than in G_1 ($\chi_1^2 = 35{\cdot}6$, $P = 0$) and, judging from the results of experiment 1, included a high proportion of S cells.

6. General Discussion

These experiments have shown that the frequency of chromatid aberrations in cells treated in G_1 is constant, but is lower than the frequency in early S cells (fig. 7). If the low G_1 frequency is the result of repair of pre-aberration lesions, before they reach S, it cannot be a continuous repair process as envisaged in fig. 7, or the yield would have risen throughout G_1. It may, however, be a short-lived process, taking place for a limited time after treatment. In this respect it would differ from the continuous repair of pre-mutational damage in G_1 cells of *Paramecium* treated with triethylene melamine (Kimball, 1965) and from the repair of SM-induced lesions which cause a depression in the rate of DNA synthesis in HeLa cells (Roberts, Brent and Crathorn, 1968).

We have assumed, throughout, that the lesions from which aberrations are derived involve the DNA, since aberrations are formed at the time of DNA synthesis, and that the degree of alkylation of DNA is equal at all

stages of the cell cycle (Roberts, Brent and Crathorn, 1968). A puzzling aspect of these results, however, and those in *Vicia*, is the appearance of a considerable frequency of aberrations, at T_2 mitosis, in cells that were in G_2 (and late S, Evans and Scott, 1969) when the agent was given. This suggests that less repair (or perhaps no repair) can take place in chromosomes or chromosome regions which have completed their DNA synthesis at the time of treatment than in prereplication chromosomes. It also suggests that if repair does not take place shortly after treatment it does not occur at all (*e.g.* in the following G_1 period), again indicating a short-lived repair process. This apparent reduction or absence of repair in post-replication chromosomes may be because the lesions at this stage of the cycle are predominantly DNA-protein cross-links rather than being confined to the DNA (Evans and Scott, 1969), and are either irreparable or simply much more frequent than in prereplication chromatin.

It is of course possible that the aberrations result from errors in repair, rather than errors of replication at S, and that there are no aberrations in G_2 cells because no repair, and consequently no misrepair, occurs at this stage.

The nature of the pre-aberration lesions is obviously still obscure. We are currently investigating the possible relationship between the repair of these lesions and the phenomenon of " repair replication " or " unscheduled DNA synthesis " (Rasmussen and Painter, 1964) after treatment of cells with alkylating agents (Hahn, Yang and Parker, 1968; Roberts, Brent and Crathorn, 1968).

7. Summary

1. When marsupial lymphocytes were exposed to sulphur mustard *in vitro*, lesions, from which chromatid aberrations were derived, were induced in cells in all stages of the cell cycle. The aberrations were not realised, however, until the time of DNA synthesis. This confirmed earlier observations in *Vicia*.

2. The frequency of aberrations in cells treated in G_1 was constant throughout this phase and considerably lower than that in early S cells. This was taken to indicate that repair of pre-aberration lesions can take place in G_1 before they are " fixed " at S and that the repair process is short-lived.

Acknowledgments.—We would like to sincerely thank the Animals and Birds Protection Board of Tasmania for supplying the potoroos and Mr P. C. Bates for skilful maintenance of the animals. We are also grateful to Dr A. Loveless for preparing the sulphur-mustard, Mr G. Wilkins for photographic services and Mr D. G. Papworth for statistical advice and computation.

8. References

BENDER, M. A., AND PRESCOTT, D. M. 1962. DNA synthesis in cultures of human peripheral leucocytes. *Exp. Cell Res.*, *27*, 221-229.

CLEAVER, J. E. 1967. *Thymidine Metabolism and Cell Kinetics* (eds. A. Neuberger and E. L. Tatum), 96. North-Holland Publishing Co., Amsterdam.

EVANS, H. J. 1970. Population cytogenetics and environmental factors. In *Human Population Cytogenetics* (eds. P. A. Jacobs, W. H. Price and P. Law), 197. Pfizer Medical monographs 5, University of Edinburgh.

EVANS, H. J., AND SCOTT, D. 1964. Influence of DNA synthesis on the production of chromatid aberrations by X-rays and maleic hydrazide in *Vicia faba*. *Genetics*, *49*, 17-38.

EVANS, H. J., AND SCOTT, D. 1969. The induction of chromosome aberrations by nitrogen mustard and its dependence on DNA synthesis. *Proc. Roy. Soc. B., 173,* 491-512.

HAHN, G. M., YANG, S-J., AND PARKER, V. 1968. Repair of sublethal damage and unscheduled DNA synthesis in mammalian cells treated with monofunctional alkylating agents. *Nature, 220,* 1142-1144.

HUNGERFORD, D. A., DONNELLY, A. J., NOWELL, P. C., AND BECK, S. 1959. The chromosome constitution of a human phenotypic intersex. *Amer. J. Hum. Genet., 11,* 215-236.

KIHLMAN, B. A. 1966. *Actions of Chemicals on Dividing Cells.* Prentice-Hall, New Jersey.

KIMBALL, R. F. 1965. The induction of reparable premutational damage in *Paramecium aurelia* by the alkylating agent triethylene melamine. *Mutation Res., 2,* 413-425.

LEA, D. E. 1946. *Actions of Radiations on Living Cells.* University Press, Cambridge.

RAO, C. R. 1952. Advanced statistical methods in biometric research, 206. John Wiley, New York. Chapman and Hall, London.

RASMUSSEN, R. E., AND PAINTER, R. B. 1964. Evidence for repair of ultraviolet damaged deoxyribonucleic acid in cultured mammalian cells. *Nature, 203,* 1360-1362.

ROBERTS, J. J., BRENT, T. P., AND CRATHORN, A. R. 1968. The mechanism of the cytotoxic action of alkylating agents on mammalian cells. In *The Interaction of Drugs and Subcellular Components in Animal Cells* (ed. P. N. Campbell). J. and A. Churchill Ltd., London, 5-27.

SASAKI, M. S., AND NORMAN, A. 1966. Proliferation of human lymphocytes in culture. *Nature, 210,* 913-914.

SCOTT, D. 1968. The additive effect of X-rays and maleic hydrazide in inducing chromosomal aberrations at different stages of the mitotic cycle in *Vicia faba. Mutation Res., 5,* 65-92.

SHARMAN, G. B., MCINTOSH, A. J., AND BARBER, H. N. 1950. Multiple sex chromosomes in the marsupials. *Nature, 116,* 996.

SHAW, M. W., AND KROOTH, R. S. 1964. The chromosomes of the Tasmanian rat-kangaroo (*Potorous tridactylis apicalis*). *Cytogenetics, 3,* 19-33.

CYTOGENETICS OF A GROUP OF MORABINE GRASSHOPPERS WITH XY and X_1X_2Y MALES*

M. J. D. WHITE and J. CHENEY
Department of Genetics, University of Melbourne

1. Introduction

In previous papers on the cytogenetics of various species of Eumastacid grasshoppers belonging to the endemic Australian subfamily Morabinae, the role of chromosomal rearrangements in the differentiation of populations, races and species has been discussed from various angles (White, 1956, 1957, 1966*a*, *b*, 1968, 1969, 1970*b*; White, Blackith, Blackith and Cheney, 1967; White, Carson and Cheney, 1964; White and Cheney, 1966; White, Cheney and Key, 1963; White, Key, André and Cheney, 1969; White and Webb, 1968). The rearrangements which are easily recognisable are fusions (leading to a decrease in chromosome number), dissociations (producing evolutionary increases in chromosome number) and pericentric inversions. In a few instances translocations other than fusions and dissociations have succeeded in establishing themselves in local populations or races of these insects (*e.g.* in the Translocation Race of " P24 ", White *et al.*, 1967). Other types of rearrangements such as paracentric inversions, duplications and deletions, must also have occurred in the Morabine grasshoppers, but are difficult to detect by ordinary techniques and evidence for them is indirect.

We now know of the existence of approximately 240 species of Morabinae, all except about 40 being undescribed. Pending description of the remainder by Dr K. H. L. Key (Division of Entomology, C.S.I.R.O.) they will be designated by the code-numbers (P1, P2...) used in the Australian National Insect Collection. A numerical system for designating the chromosomal fusions and dissociations which have occurred in the Morabinae was introduced by White and Webb (1968) and will be continued in the present paper. The great majority of the species of Morabinae have distinguishable, *i.e.* unique, karyotypes, and in numerous instances a species consists of two or more chromosomal races. The probability that chromosomal rearrangements have in many instances played some kind of direct role in speciation in this group of insects, by generating post-mating isolating mechanisms, has been discussed by White *et al.* (1967) and White (1968, 1969, 1970*c*).

Most species of morabine grasshoppers have XO males, and this was clearly the original condition in the subfamily. However, neo-XY mechanisms have arisen by fusions between the original X and one of the autosomes on 11 different occasions in the phylogeny of the group. And in five of these instances a further fusion between the neo-Y and a second autosome has given rise to an X_1X_2Y system. In the *viatica* group of species, three taxa have independently acquired neo-XY sex chromosome mechanisms (White *et al.*, 1967) and in a group of species from central New South Wales both XY and X_1X_2Y mechanisms occur (White and Cheney, 1966). The present paper deals with the " P52 " group, in this case from central

* Supported by Public Health Service Grant No. GM-07212 from the United States National Institutes of Health and by a grant from the Australian Research Grants Committee.

Queensland, which have XY and X_1X_2Y males. This group includes four species, designated P52*a*, P52*b*, P85 and P53.

2. Distribution and ecology of the species

The distribution of the species, as far as known, is shown in fig. 1, where all the localities at which they have been collected are indicated. It will

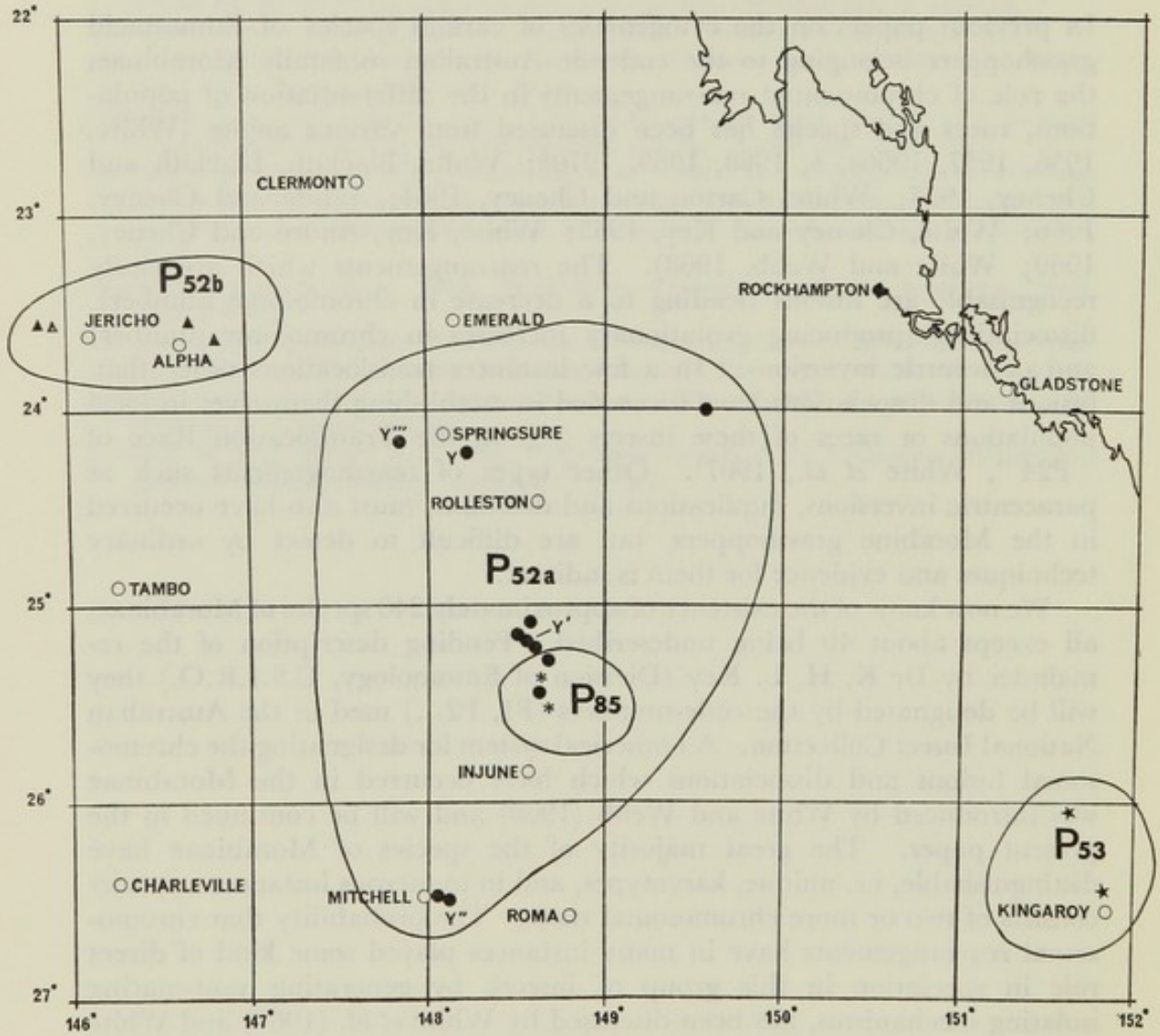


Fig. 1.—Map of south-central Queensland, showing the distribution, as far as known, of the members of the P52 species-group. Towns indicated by hollow circles. In the case of P52*a* the distribution of the various types of Y-chromosomes is indicated.

be seen that P53, P85 and P52*b* have only been collected in small, widely separated areas. P52*a* is a relatively widespread species (for a morabine grasshopper), which occurs from Mitchell in the south to the vicinity of Duaringa in the north. It is likely that the actual distribution ranges of all the species are considerably wider than is indicated in fig. 1, since there are very large areas of central Queensland remote from roads, which have not been visited by collectors of morabine grasshoppers. Whether there are any areas of sympatry between P52*a* and either P52*b* or P53 must remain conjectural for the present. The actual localities at which the cytological material was collected are listed in table 1.

TABLE 1

Localities at which the cytological material was collected
(all in Queensland)

P52a

11 miles SE of Springsure; 16 miles WSW of Springsure; 10 miles SW of Lake Nuga Nuga; 16 miles SSW of Lake Nuga Nuga; 18 miles S of Lake Nuga Nuga; 17 miles WNW of Arcadia H.S., near Injune; 13 miles WSW of Arcadia H.S.; 22 miles NNE of Mt. Hutton H.S.; 20 miles SSE of Wyseby H.S.; 12 miles ENE of Westgrove H.S.; 4 and 5 miles E of Mitchell (also known from 21 miles SSW of Duaringa, but material from that locality has not been examined cytologically).

P52b

5 miles E of Alice; 6 miles E of Alice; 6 miles E of Alpha; 8 miles NNE of Alpha.

P85

Carnarvon Range, 3 miles N of the north fork of the Dawson River.

P53

10 miles NNW of Hivesville, near Wondai (also known from 6 miles N of Kingaroy, but material from that locality has not been examined cytologically).

All species of the P52 group are shrub-feeders, but they are not particularly associated with any one species or genus of shrubs. P52*a* has been found on *Cassinia* sp. at several localities in the Carnarvon Ranges, and near Mitchell it was living among grass and *Helichrysum* sp., undoubtedly feeding on the latter. While certainly not confined to a diet of Compositae, it does seem to feed on these genera in nature, to a considerable extent. To the west of its distribution area (*i.e.* between Tambo and Alpha) P52*a* seems to be replaced on the *Cassinia* bushes by a member of another Morabine group, P143.

3. KARYOTYPES

Measurements of the percentage chromosome lengths of the four species are given in table 2. These are based on metaphases in female somatic cells, following colcemid injection and squashing. The three neo-XY species P52*a*, P85 and P53 all show $2n♂♀ = 16$, while P52*b* has $2n♂ = 15$, $2n♀ = 16$, as a result of a fusion between the neo-Y and an autosome, which has converted the neo-XY mechanism into an X_1X_2Y one. Male karyotypes have been studied in spermatogonial metaphases in sectioned material (figs. 2, 3, 4, 10).

All the members of the P52 group have a large equal-armed metacentric " AB " autosome which forms about 27·5 to 30 per cent. of the total karyotype. The " CD " autosome constitutes approximately 15 to 17 per cent. of the total chromosome length. It is an unequal-armed metacentric in all the species except P53, in which it has been converted into an acrocentric, presumably by a pericentric inversion. The metacentric AB and CD elements are present in many species groups of the Morabine grasshoppers, and may be regarded as forming part of the primitive karyotype of the whole subfamily (White, 1969).

In P52*a*, P85 and P53 the longer limb of the neo-X represents the original sex chromosome which has become fused to one of the small autosomes, the homologue of this autosome becoming the neo-Y, now confined

TABLE 2

Percentage chromosome and arm lengths of the four species

Chromosome	*P52a* (A)*	*P52a* (B)†	*P52b*	*P85*	*P53*
AB‡	14·20	14·53	13·72	14·26	14·98
	14·20	14·53	13·72	14·26	14·98
CD	10·20	9·78	9·02	9·27	16·27
	7·02	6·23	5·59	6·02	—
X	14·24	14·53	13·34	14·20	15·82
	8·96	8·60	8·01	9·63	9·60
(1)	5·38	5·43	5·08	6·14	7·02
	4·34	3·85	4·32	2·29	1·07
(2)	7·02	6·92	X_2 10·42	6·02	6·67
	—	—	0·38	1·20	0·18
(3)	5·48	6·52	6·86	5·05	5·69
	—	—	0·26	1·32	0·18
(4)	4·82	5·83	5·08	4·81	4·53
	—	—	0·13	0·96	—
(5)	4·14	3·26	2·54	3·25	3·02
	—	—	1·52	1·32	—
	100·00	100·01	99·99	100·00	100·01

* Measurements by M. J. D. W. on material from 10 miles SW of Lake Nuga Nuga.
† Measurements by J. C. on material from 5 miles E of Mitchell.
‡ Lengths of the two arms indistinguishable, hence averaged, in each case.

to the male line. Following the numbering system we have adopted for the fusions that have occurred in the phylogeny of the Morabine grasshoppers (White and Webb, 1968), this one may be designated F14. The additional fusion between the neo-Y and a second autosome, which has occurred in P52*b*, is here designated F15.

P52*a*, P85 and P53 all have five pairs of small autosomes, one of the six present in the primitive karyotype having been "used up" in F14; P52*b* has only four pairs of small autosomes (a further one having been "used up" in F15). There are certain difficulties in determining just which of the six small autosomal pairs in the primitive karyotype were involved in F14 and F15. Since the XO ancestor of the P52 group is unknown and presumably extinct, the first question is perhaps unanswerable. The data of table 2 suggest that it was chromosome No. 2 (probably the third of the original six small elements) which was involved in F15. We may now consider the karyotypes of the individual species in detail.

P52a: The karyotype of this species has already been figured and described, as it appears in the spermatogonial metaphases (White, 1970*b*). Two sets of measurements of the female karyotype, based on material from different localities and measured by different observers, are given in table 2. The discrepancies are very minor and almost certainly non-significant. The equal-armed AB chromosome is 28-30 per cent. of the total chromosome length and the CD is 16-17 per cent. of the T.C.L. The largest of the small autosomes (No. 1) is a metacentric, with subequal arms. A similar metacentric occurs in P52*b*, but in P85 and P53 the arms of this chromosome are more markedly unequal. The four remaining autosomes of P52*a* (Nos. 2, 3, 4 and 5) are all strictly acrocentric, *i.e.* their short arms are undetectable

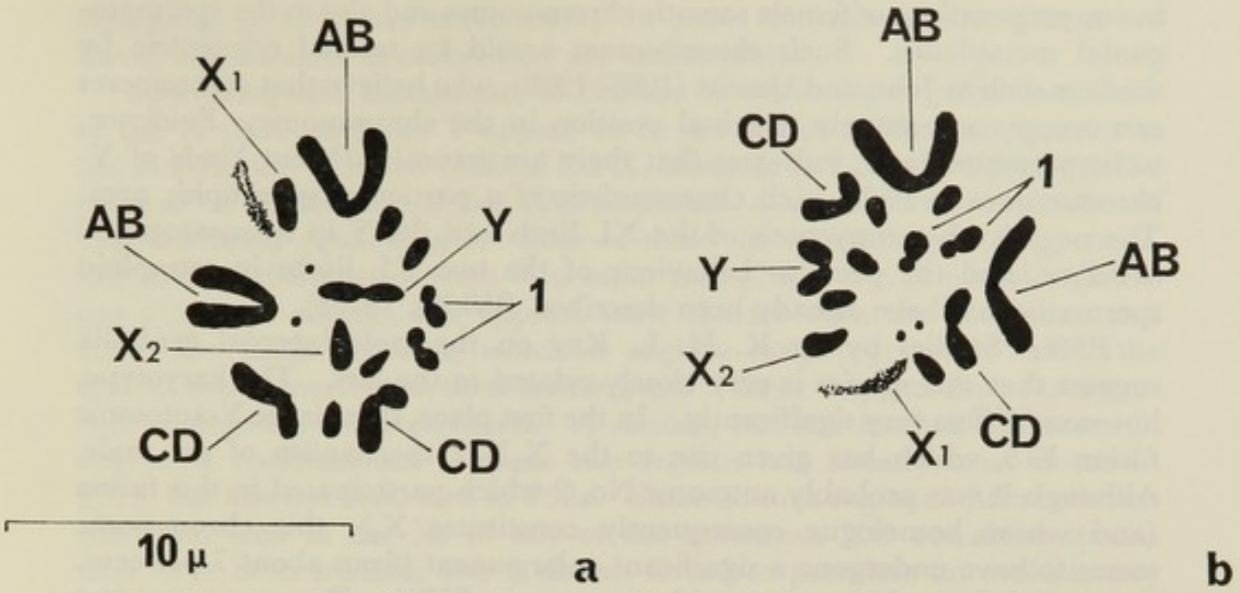


FIG. 2.—Spermatogonial metaphases of P52*b* (material from 8 miles NNE of Alpha).

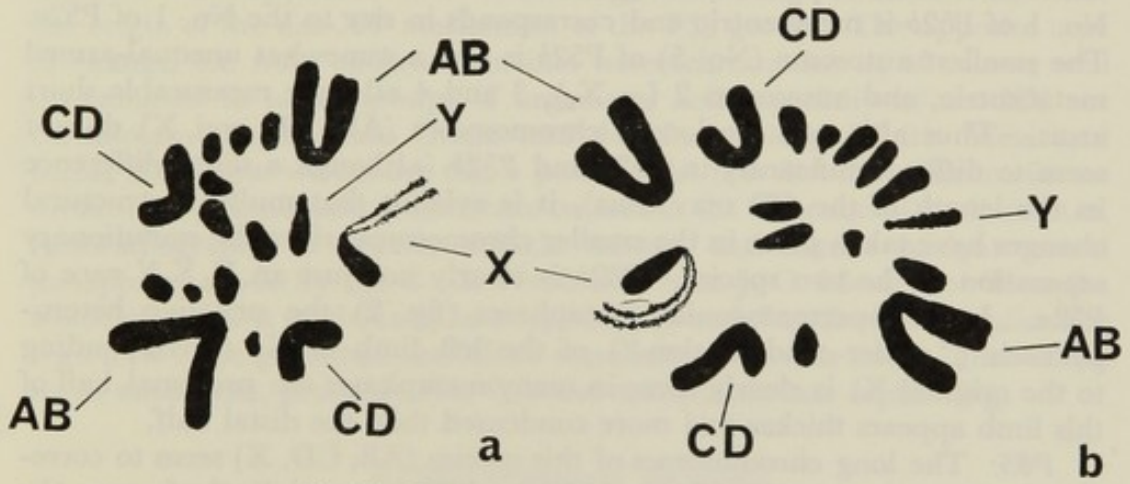


FIG. 3.—Spermatogonial metaphases of P85.

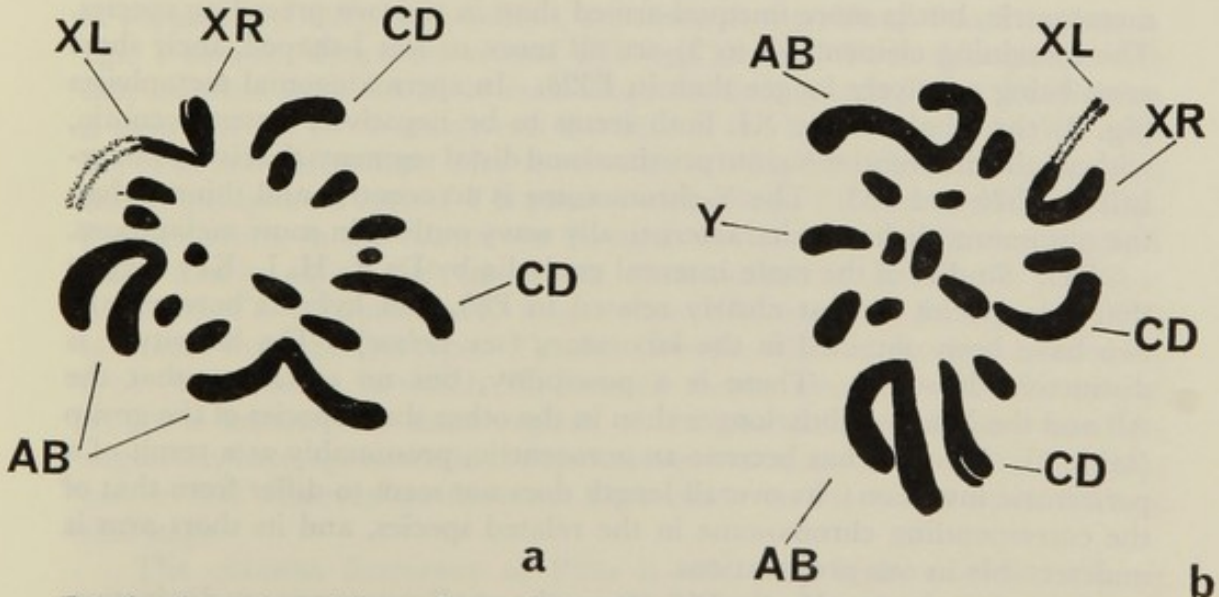


FIG. 4.—Spermatogonial metaphases of P53, showing the characteristic appearance of the X-chromosome.

in our preparations of female somatic chromosomes and also in the spermatogonial metaphases. Such chromosomes would be termed telocentric by workers such as John and Hewitt (1966, 1968), who believe that centromeres can occupy a genuinely terminal position in the chromosome. Evidence, to be presented later, indicates that there are several different kinds of Y-chromosomes in P52*a*, each characteristic of a particular geographic area. The negative heteropycnosis of the XL limb and the Y in spermatogonial divisions, and the peculiar behaviour of the two XL limbs in tetraploid spermatogonia have already been described (White, 1970*b*).

P52b: Studies by Dr K. H. L. Key on the male internal genitalia suggest that this species is very closely related to the last. The karyotype, however, differs very significantly. In the first place, there is the Y-autosome fusion F15, which has given rise to the X_1X_2Y mechanism of the male. Although it was probably autosome No. 2 which participated in this fusion (and whose homologue consequently constitutes X_2), this chromosome seems to have undergone a significant enlargement (from about 7 per cent. of the T.C.L. in P52*a* to over 10 per cent. in P52*b*). One must presume that one or more duplications of genetic material have occurred. Autosome No. 1 of P52*b* is metacentric and corresponds in size to the No. 1 of P52*a*. The smallest autosome (No. 5) of P52*b* is also a somewhat unequal-armed metacentric, and autosomes 2 (= X_2), 3 and 4 all have measurable short arms. Thus although the longer chromosomes (AB, CD and X) do not seem to differ significantly in P52*a* and P52*b* (although a slight difference in the length of the CD may exist), it is evident that multiple structural changes have taken place in the smaller chromosomes since the evolutionary separation of the two species. P52*b* is clearly not just an X_1X_2Y race of P52*a*. In the spermatogonial metaphases (fig. 2) the negative heteropycnosis (" under-condensation ") of the left limb of X_1 (corresponding to the original X) is clearly seen; in many metaphases the proximal half of this limb appears thicker and more condensed than the distal half.

P85: The long chromosomes of this species (AB, CD, X) seem to correspond very well in length to those of P52*a* and P52*b*, especially the former. On the other hand, the five pairs of small autosomes are distinctive. No. 1 is a metacentric, but is more unequal-armed than in the two preceding species. The remaining elements (2 to 5) are all more or less J-shaped, their short arms being relatively longer than in P52*b*. In spermatogonial metaphases (fig. 3) the whole of the XL limb seems to be negatively heteropycnotic, without the differentiation into proximal and distal segments that is characteristic of P52*b* and P53. The Y-chromosome is acrocentric and thinner than the autosomes; it has a characteristically wavy outline in some metaphases.

P53: Studies of the male internal genitalia by Dr K. H. L. Key suggest that this species is most clearly related to P85, and hybrids between the two have been obtained in the laboratory (see below). The karyotype is distinctive, however. There is a possibility, but no certainty, that the AB and the X are slightly longer than in the other three species of the group (table 2). The CD has become an acrocentric, presumably as a result of a pericentric inversion; its overall length does not seem to differ from that of the corresponding chromosome in the related species, and its short arm is undetectable in our preparations.

As seems to be usual in the P52 group, the small autosomes are distinctive. No. 1 is even more unequal-armed than in P85. The remaining small

autosomes (2 to 5) are acrocentric; in some of them the short arms are barely measurable, in others they are undetectable.

Spermatogonial metaphases of P53 are shown in fig. 4. The short limb of the X (XR) and the Y appear to be condensed to the same extent as the autosomes. The left limb of the X, however (which corresponds to the original X chromatin of the XO ancestor), consists of two segments of approximately equal length. Both of these show negative heteropycnosis, but to very different degrees. In some metaphases the proximal segment has a smooth outline, but appears more slender than the autosomes and XR; the distal segment, which is slightly longer, generally shows the typical under-condensed, faint-staining and " fuzzy " appearance characteristic of negative heteropycnosis. A similar, but less marked difference in degree of condensation of these two regions has already been noted in P52*b*, but has not been seen in P52*a*. Since no such regional differentiation has been seen in the Xs of any of the numerous XO morabine species that have been studied, one is almost forced to postulate an evolutionary transformation of heterochromatin which originally manifested typical negative heteropycnosis into something resembling euchromatin, since the origin of the neo-XY mechanism of the P52 group. The opposite type of change (*i.e.* from euchromatin into heterochromatin) is, of course, well documented in many groups of organisms and especially in neo-XY sex chromosome mechanisms (Mesa and de Mesa, 1967; White, Mesa and Mesa, 1967), but we believe this is possibly the first definite evidence for an evolutionary conversion of heterochromatin in the direction of euchromatin. Autoradiographs of ovarian follicle mitoses and female midgut caecal metaphases made by Mr G. C. Webb and Miss J. C. Meek show that the whole of the X is moderately late-replicating, and that the latest-replicating regions are the proximal and distal segments of the XL limb. One of the small autosomes, probably No. 4, is also moderately late-replicating.

4. Male meiotic divisions

P52a: First metaphases of this species, drawn from material collected at a number of different localities, are shown in figs. 5 and 6 and in plate I, figs. 1 and 2. There are several different kinds of Ys in this species, but only one has been found at each locality; their geographical distribution is shown in fig. 1. We may call the limb of the Y which is associated with the X at first metaphase, YL; the free limb being YR. In an area 11 miles SE of Springsure a type of Y is present with a long YL and a very short YR. Farther south, in the Carnarvon Ranges, the YL and YR limbs are much more nearly equal and the whole chromosome is shorter (Y′). Near Mitchell, YL is considerably longer than YR, but the chromosome (Y″) is smaller than the first type. Finally, 16 miles WSW of Springsure a fourth type (Y‴) occurs, in which YL is much shorter than YR. In the case of Y″, it is known that some regions of the chromosome, at any rate, are negatively heteropycnotic in the spermatogonial metaphases (White, 1970*b*); but critical observations have not been made on the other types of Ys at this stage.

The chiasma frequency of P52*a* is relatively high, and there is no particular localisation of chiasmata. The AB bivalent shows 2-4 chiasmata, the CD almost invariably 2, bivalent No. 1 shows 1-2, the other bivalents

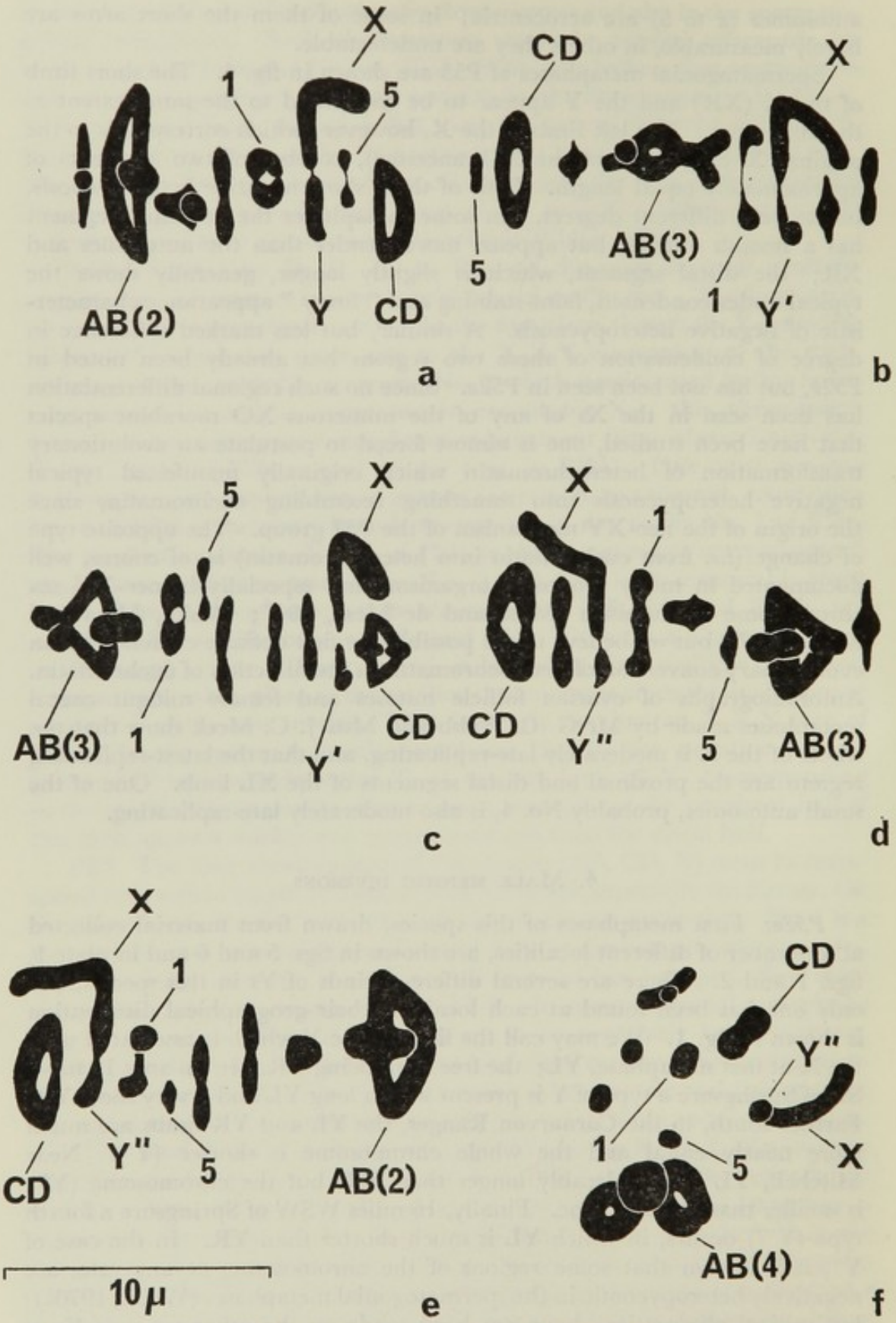


FIG. 5.—First metaphases, in side view (expect *f*, which is a polar view) of P52*a* from various localities. *a*, from 11 miles SE of Springsure; *b* and *c* from 17 miles WNW of Arcadia H.S.; *d-f* from 5 miles E of Mitchell. In the case of the AB bivalent the number of chiasmata is indicated thus: AB(3). The various types of Y-chromosomes are indicated.

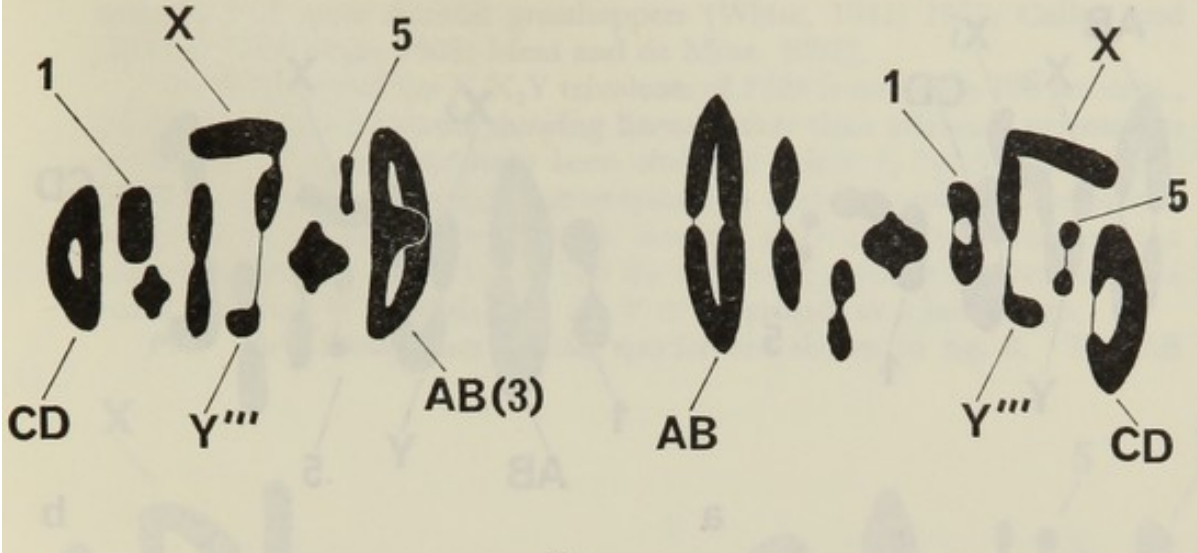


FIG. 6.—First metaphases, in side view, of P52*a* from 16 miles WSW of Springsure, showing the Y''' type of neo-Y.

invariably one only. Whatever the type of Y, the association between it and the XR limb is invariably terminal at first metaphase. The overall chiasma frequency varies only slightly between the different populations sampled and is always about 11·4.

P52b: First metaphases of this species are shown in fig. 7 and plate I, figs. 3-5. The AB and CD bivalents usually show a chiasma in each arm, completely terminalised at metaphase; occasionally one or both of these bivalents shows only a single chiasma (fig. 7, *c-f*). The largest of the small chromosomes (No. 1) frequently shows two chiasmata, at least in the material from near Alice (in individuals from 8 miles NNE of Alpha it almost invariably forms a single chiasma). Autosomal bivalents 3 and 4 are not easily distinguishable; one of them often forms a proximal chiasma. The little No. 5 bivalent is quite distinctive on account of its metacentric shape; it never forms more than a single chiasma.

The X_1X_2Y trivalent is very characteristic. The Y is a metacentric with almost exactly equal arms. It is connected at first metaphase by two terminalised chiasmata to the metacentric X_1 (equivalent to the X of P52*a*, but possibly a little shorter) and the acrocentric X_2.

The overall chiasma frequency per nucleus is approximately 8·8 in material from 8 miles NNE of Alpha; it would be slightly higher in populations near Alice. Because of the extreme development of distal localisation of its chiasmata, in the longer chromosomes, P52*b* must be regarded as a species with a low level of genetic recombination (unless, indeed, the pattern of chiasma distribution in the female is quite different.)

In general, the X_1X_2Y trivalents in Morabine grasshoppers are extremely efficient, *i.e.* the X_1 and X_2 regularly pass to one pole at first anaphase, the Y going to the opposite pole (White and Cheney, 1966). This efficiency results from two causes—regularity of synapsis (and presumably also of chiasma formation in the terminal regions of the chromosomes, cruciform chiasma-configurations not being visible) and regularity of alternate orientation of the three centromeres on the spindle at premetaphase. It contrasts with the relative inefficiency of similar sex chromosome trivalents in many

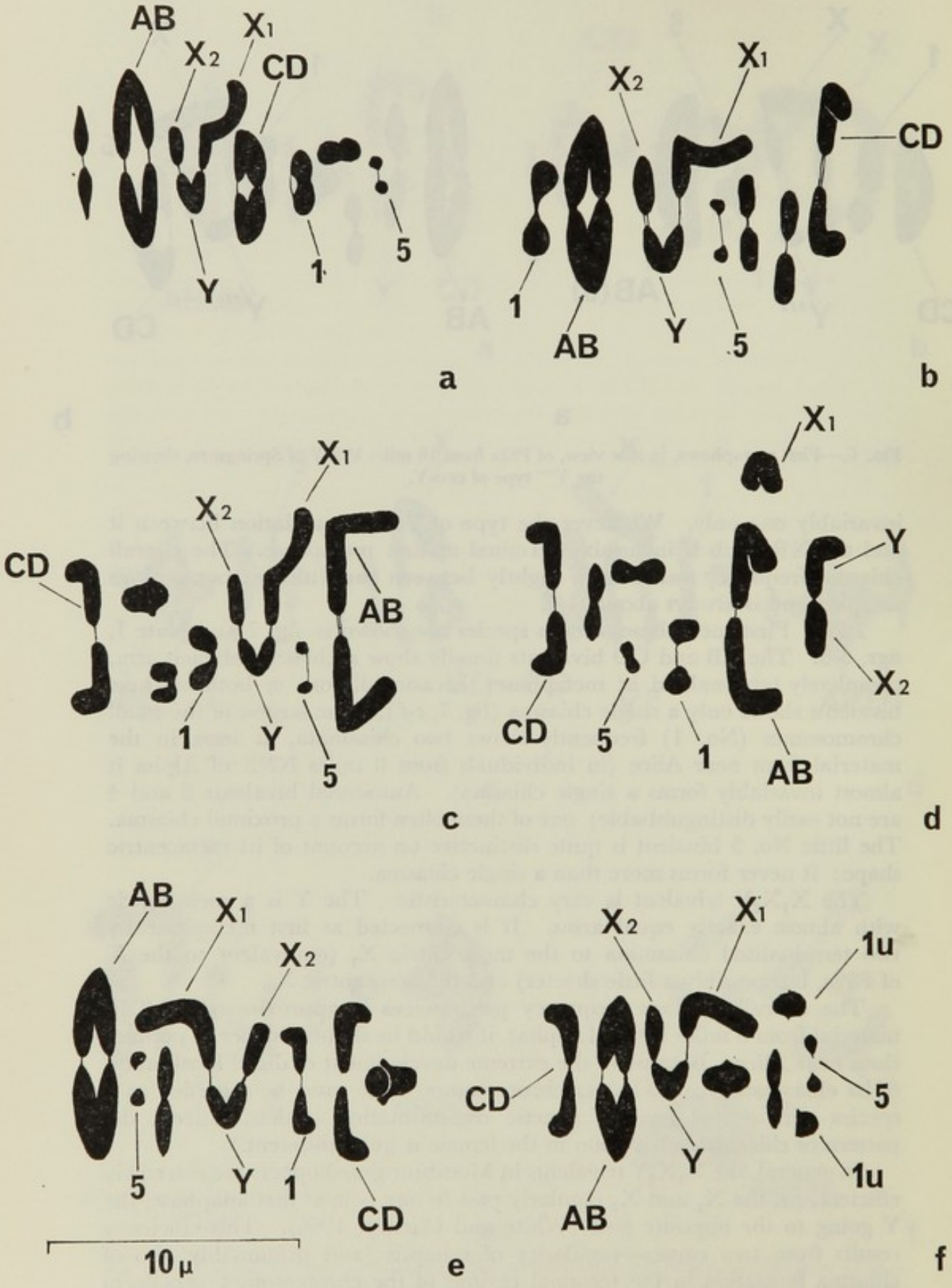


FIG. 7.—First metaphases, in side view, of P52*b*; *a*, from 5 miles E of Alice, *b-f* from 8 miles NNE of Alpha. In *d* the X_1 chromosome is asynaptic and in *f* the No. 1 pair is represented by two univalents.

mantids and some Acridid grasshoppers (White, 1941, 1965; Callan and Jacobs, 1957; Mesa, 1963; Mesa and de Mesa, 1967).

The efficiency of the X_1X_2Y trivalents of P52*b* is not quite 100 per cent., however. Some trivalents showing linear rather than alternate orientation of the three centromeres have been observed (plate I, fig. 5); it is quite possible that these represent premetaphase or early metaphase orientations which may undergo re-orientation into an alternate configuration at a slightly later stage. And in fig. 7, *d* the X_1 chromosome is a univalent, as a result of failure of synapsis with the Y or desynapsis at a later stage.

P85: First metaphases of this species are shown in fig. 8. The AB

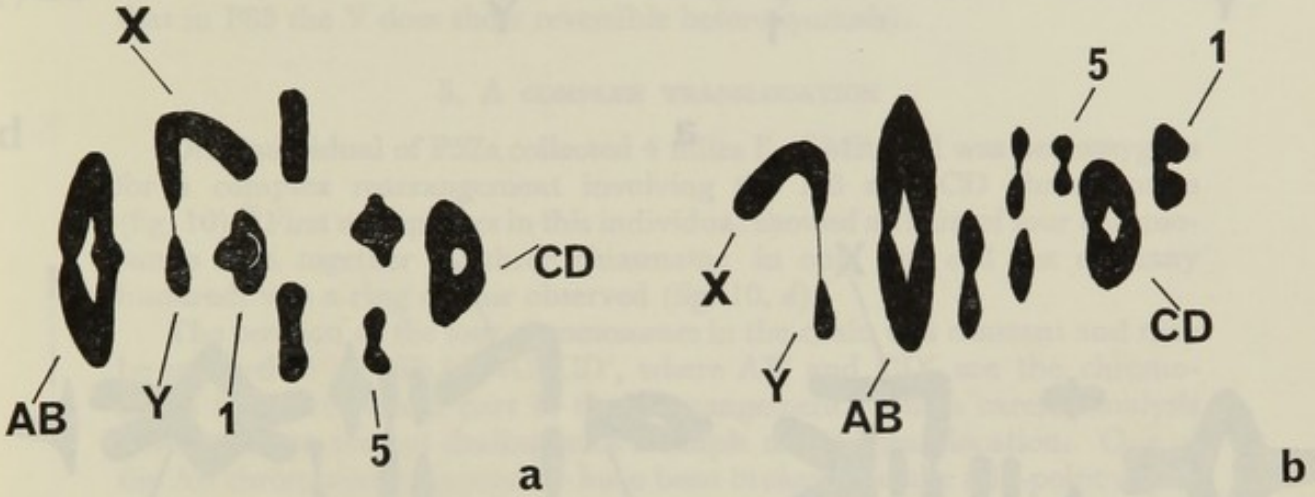


FIG. 8.—First metaphases of P85.

and CD bivalents usually show a single chiasma in each arm; there are never more, but occasionally either the AB or the CD bivalent has only a single chiasma. In general, the chiasmata in these bivalents are completely terminal at first metaphase, but sometimes one of them is subterminal. The association between the X and the acrocentric Y is invariably a terminal one. Of the five small bivalents, the largest generally seems to form two chiasmata, the others one only. The chiasma frequency is thus very slightly under 11·0.

P53: The AB bivalent usually shows two chiasmata, never less but sometimes three or four. When there are two chiasmata, these are always in opposite arms, but apart from this there does not seem to be any chiasma localisation in the AB bivalent, *i.e.* the chiasmata may be either proximal or distal in position. As already stated, the CD chromosome is an acrocentric. This is a very unusual condition for this chromosome in a morabine grasshopper, known elsewhere only in *Keyacris scurra* when either the Blundell or the Snowy inversion is present (White, 1956, 1958). At meiosis in P53 the CD bivalent usually forms a single proximal chiasma, or a proximal and a distal one (very rarely there are three chiasmata). The five small bivalents all invariably form a single chiasma which may be either proximal or distal. The total chiasma frequency per nucleus is 9·6. Because of the absence of any distinct chiasma localisation, P53 must be regarded as a species with a high level of genetic recombination, although not quite as high as that of P52*a*, which has a higher chiasma frequency. Although P53 has a lower chiasma frequency than P85, it probably has a higher level of genetic recombination, because of the much larger number of interstitial chiasmata.

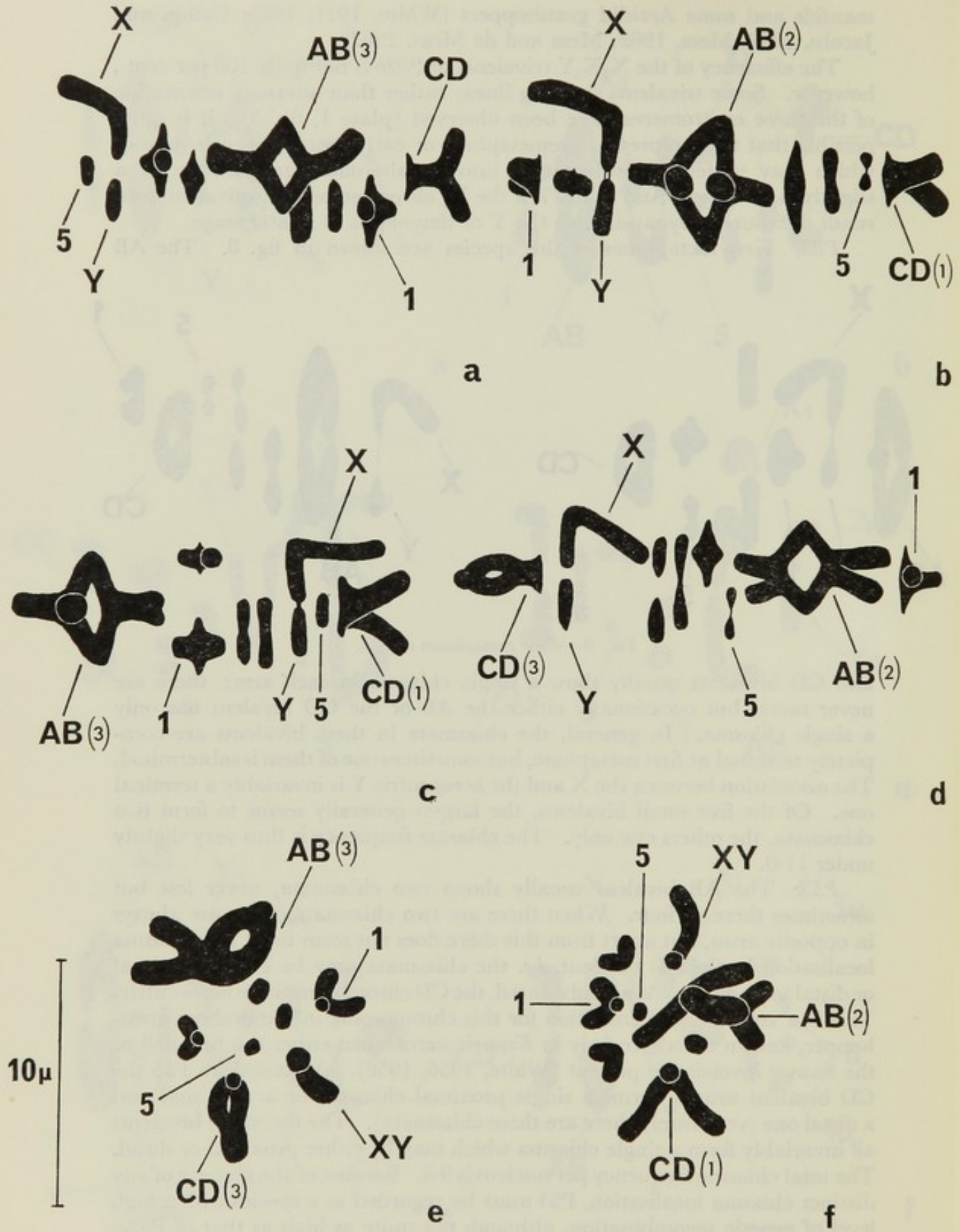


FIG. 9.—First metaphases of P53. *a-d* in side view, *e-f* in polar view. The number of chiasmata in the AB and CD bivalents is indicated by the figures in parentheses.

As in P52*a* and P85, the neo-X and neo-Y are attached by a terminal chiasma at first metaphase (fig. 9). The acrocentric neo-Y is not significantly different in length from the XR limb. It is interesting to note that although neither Y nor XR show any noticeable heteropycnosis in the spermatogonial divisions (fig. 4) they both show approximately the same degree of positive heteropycnosis as XL in diplotene nuclei. Thus, whereas the " original " X chromatin (or at least the distal segment of XL) shows the reversible type of heteropycnosis that is so characteristic of X-chromosomes throughout the Caelifera, the chromatin of the originally autosomal XR limb and the Y exhibit the non-reversible type of heteropycnosis that is normally characteristic of autosomal heterochromatin in this group of insects (it will be recalled that in P85 the Y does show reversible heteropycnosis).

5. A complex translocation

One individual of P52*a* collected 4 miles E of Mitchell was heterozygous for a complex rearrangement involving the AB and CD chromosomes (fig. 10). First metaphases in this individual showed a chain of four chromosomes held together by three chiasmata; in only one cell out of many hundreds was a ring of four observed (fig. 10, *d*).

The position of the four chromosomes in the chain was constant and may be referred to as CD-AB′-AB-CD′, where AB′ and CD′ are the chromosomes that have taken part in the rearrangement. But a careful analysis shows that we are not dealing with a simple mutual translocation. One of the AB chromosomes appears to have been broken near the mid-point of one of its limbs and one of the CD chromosomes has undergone breakage in the longer limb (C). But the D limbs of the CD and CD′ chromosomes, which are " free " at the two ends of the chain and do not have any chiasmata, are unequal in length—that of CD′ is shorter than that of CD.

The most satisfactory interpretation of this rearrangement is that it is a three-break one. Two of the breaks would have been in the CD chromosome, one in each limb, and the third in the AB. In the course of this rearrangement the segment between the two breaks in the CD underwent a pericentric inversion. Such a rearrangement explains (1) why the " free " limbs of CD and CD′ are unequal in length, and (2) why they are unsynapsed, *i.e.* why a chain is formed rather than a ring. The extreme tips of the D limbs are, of course, homologous and uninverted with respect to one another, so that a ring *can* be formed; but the homologous segment must be so short that this happens only very rarely.

" Disjunctional " and " non-disjunctional " orientations of the chain of four occur with approximately equal frequency. The fecundity of the individual must consequently have been very severely reduced. The rearrangement was presumably a newly arisen one which could not have been expected to establish itself in the population.

This rearrangement was illustrated (White, 1970*a*) in a textbook chapter in which it was not pointed out that it was more complex than a simple reciprocal translocation.

6. Hybridisation studies

All members of the P52 group were bred without difficulty in our Melbourne insectary and numerous offspring were invariably obtained

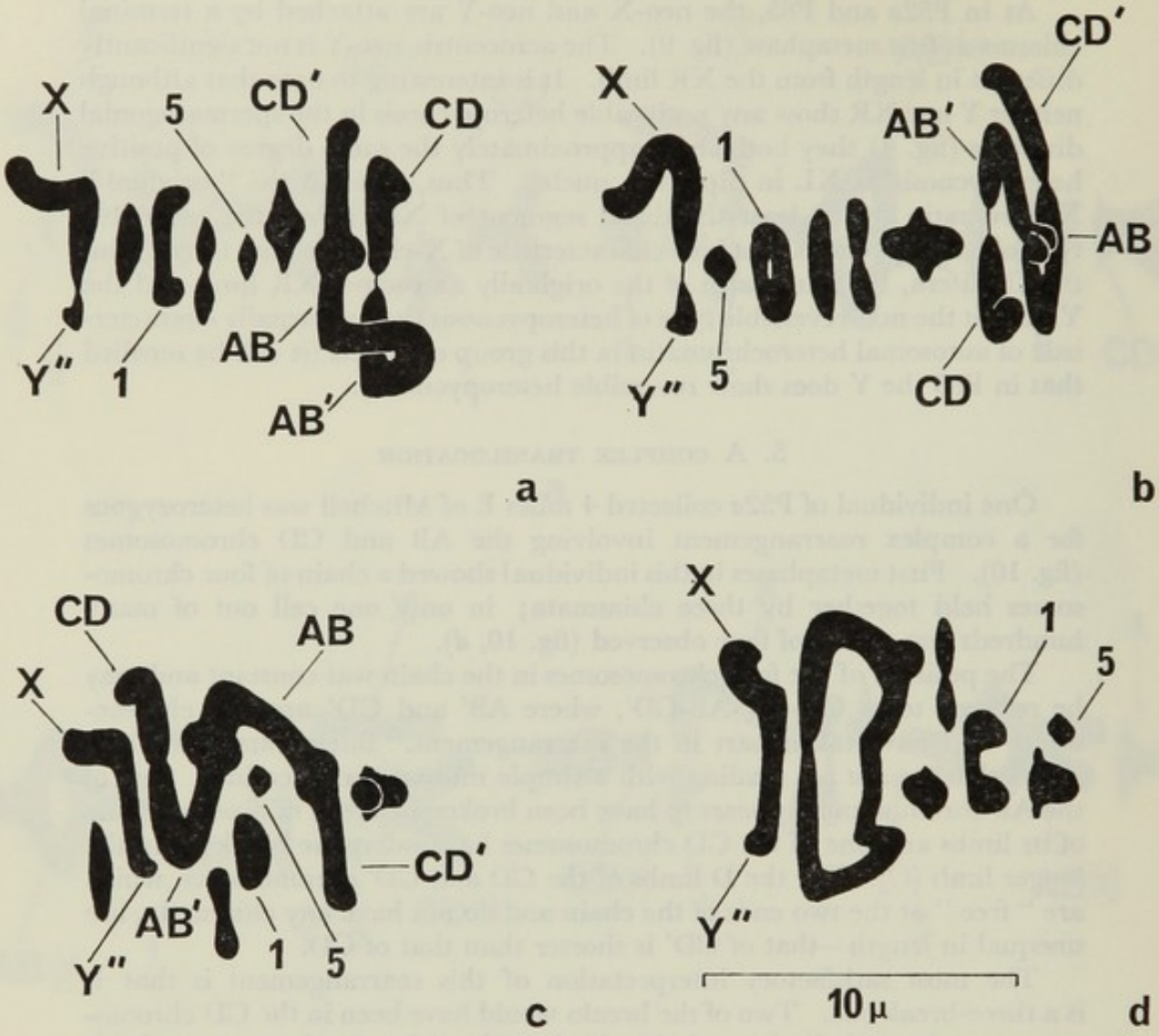
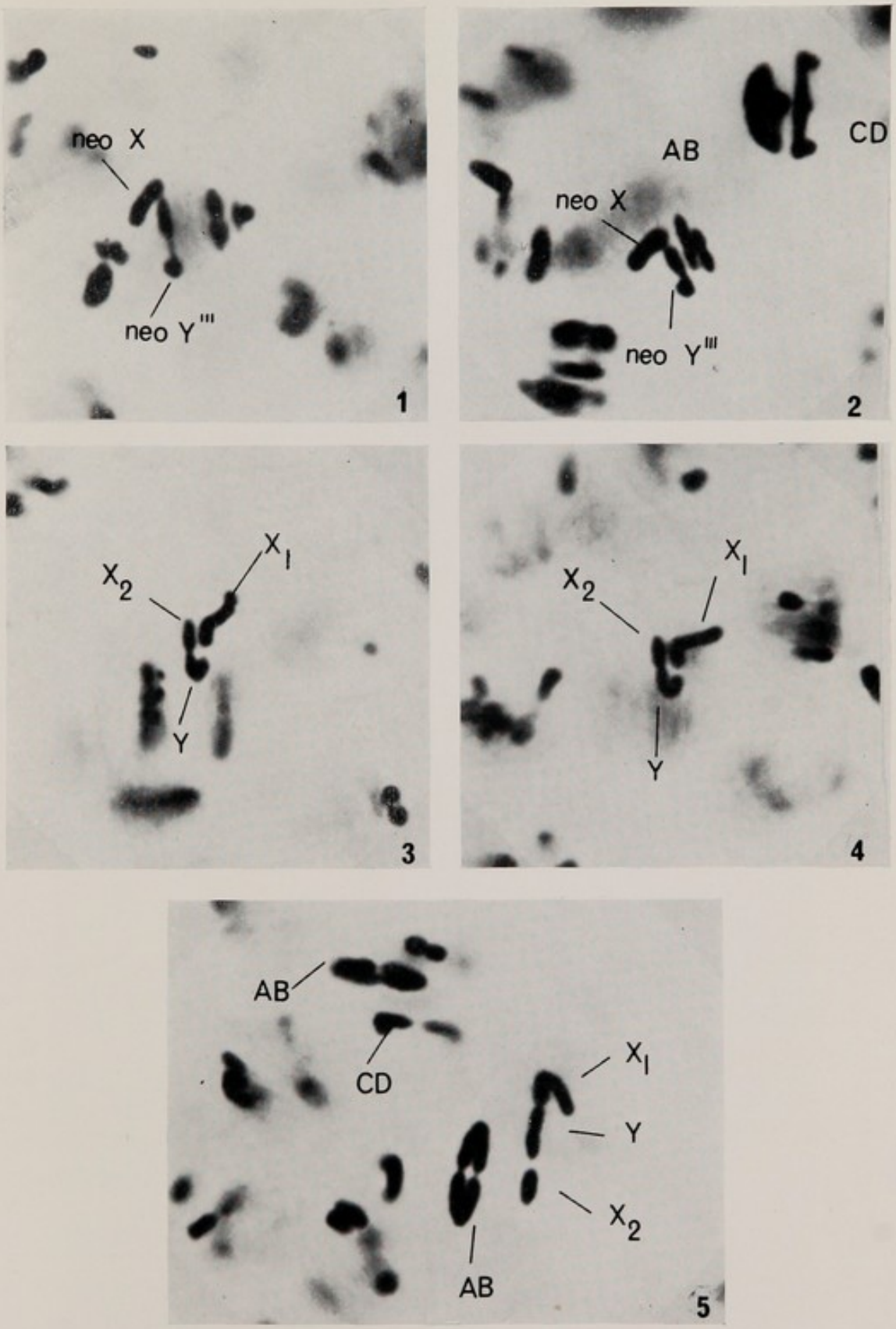


FIG. 10.—First metaphases, in side view, from an individual of P52*a* collected 4 miles E of Mitchell, which was heterozygous for a complex translocation involving the AB and CD chromosomes. In figs *a-c* a chain-of-four configuration (CD-AB′-AB-CD′) is present, while in fig. *d* there is a ring of four chromosomes. Figs. *b* and *c* represent " disjunctional " configurations of the quadrivalent, *a* and *d* " non-disjunctional " configurations.

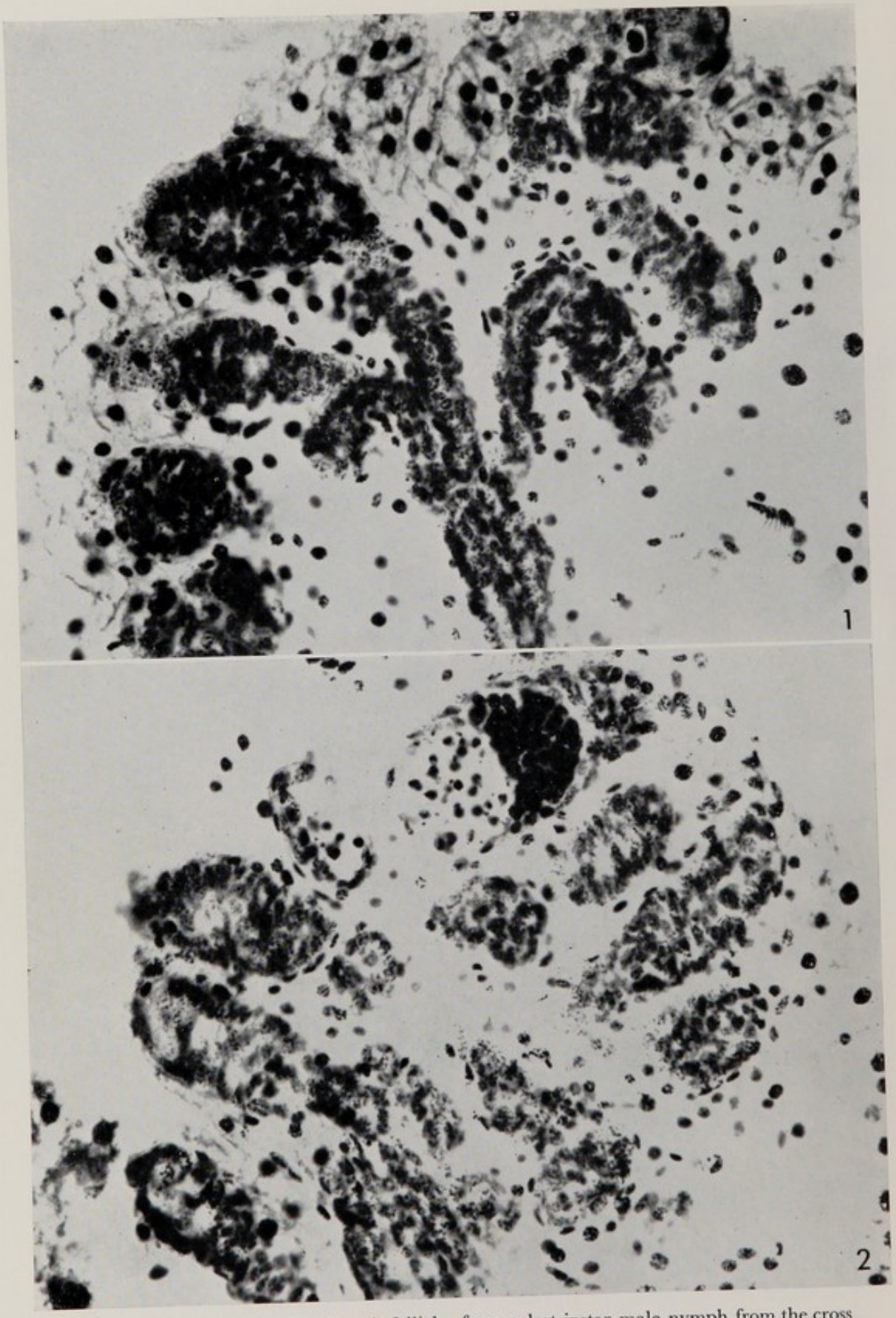
in the stock cages. A number of hybridisation experiments were set up and in all cases copulation occurred between the different species. Thus far, however, the only hybrid offspring obtained have been from the reciprocal crosses P53♂ × P85♀ and P85♂ × P53♀. The sex ratio was apparently normal in the progenies from these crosses and both male and female individuals eventually reached the imaginal stage. They copulated *inter se*, although, since the males must be completely sterile (see below) no F_2 offspring could be expected. Repeated attempts to perform the crosses P52*a*♂ × P53♀ and P53♂ × P52*a*♀ yielded no offspring.

The testes of the F_1 males were very retarded in their development (plate 2, figs. 1 and 2). This seems to result primarily from a failure of the primordial spermatogonia to divide. The photographs in plate 2 were taken from last instar nymphs, but an exactly similar histological picture was found in the testes of some young adults. In some older adults the testes were somewhat larger (although still smaller than normal) and a



FIGS. 1 and 2.—First metaphases of P52*a* from 16 miles WSW of Springsure showing the neo-XY bivalent. The neo-Y is of the Y'''' type.

FIGS. 3-5.—First metaphases of P52*b* from 8 miles NNE of Alpha, showing the X_1X_2Y sex trivalent. In fig. 5 the orientation of the trivalent is linear.



FIGS. 1 and 2.—Undeveloped testis follicles from a last-instar male nymph from the cross P85♂ × P53♀.

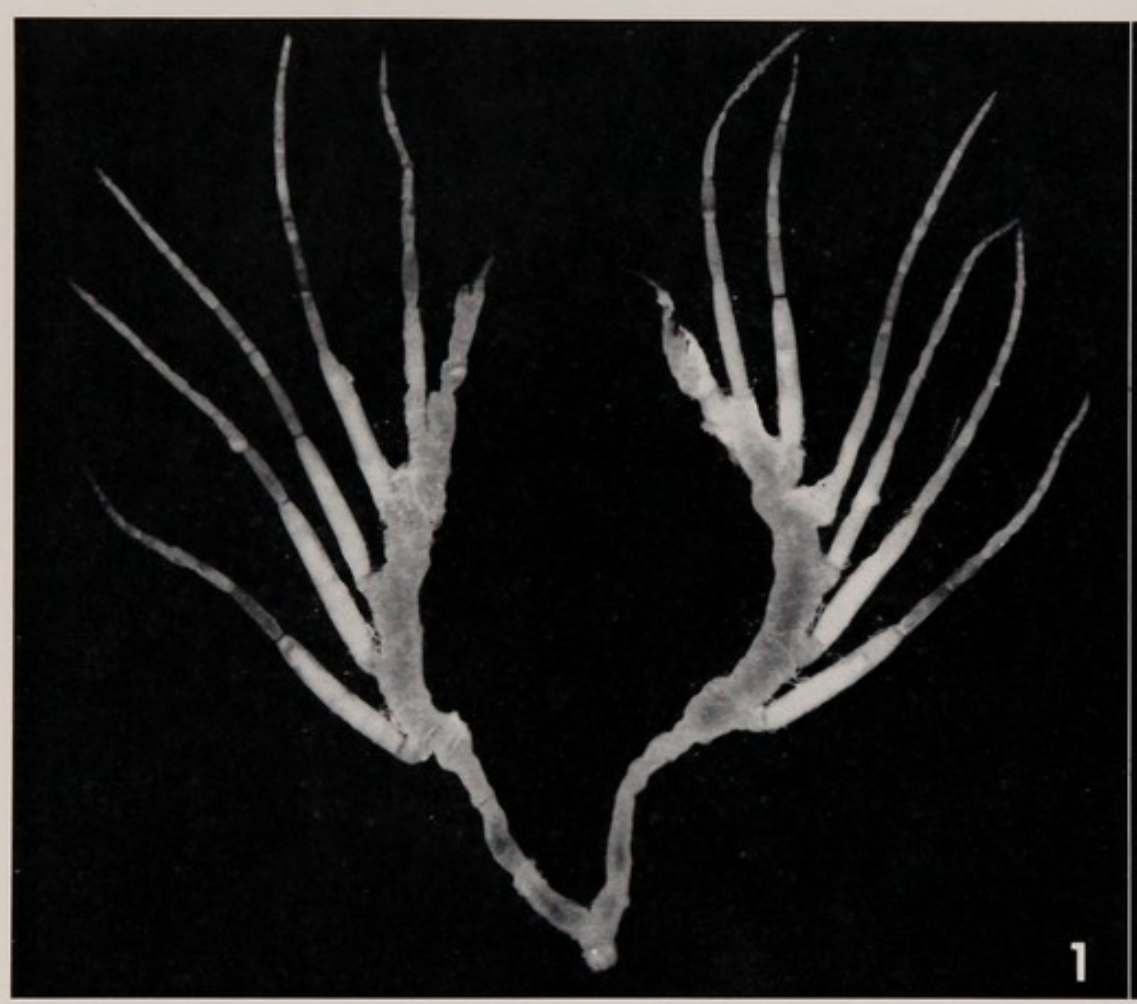


FIG. 1.—Whole ovary of adult P53, dissected and spread out.
FIG. 2.—Ovary of adult F_1 hybrid from the cross P53♂ × P85♀.

highly abnormal spermatogenesis was occurring. In these older testes the spermatogonial mitoses appear to be proceeding normally (fig. 11) and the heteropycnosis of the sex chromosomes is what we would expect in view of their origin; thus in fig. 11, *d* the distinction between the proximal and distal segments of the P53 XL limb is clearly seen, while in fig. 11, *b* the negative heteropycnosis of the P85 Y-chromosome is evident.

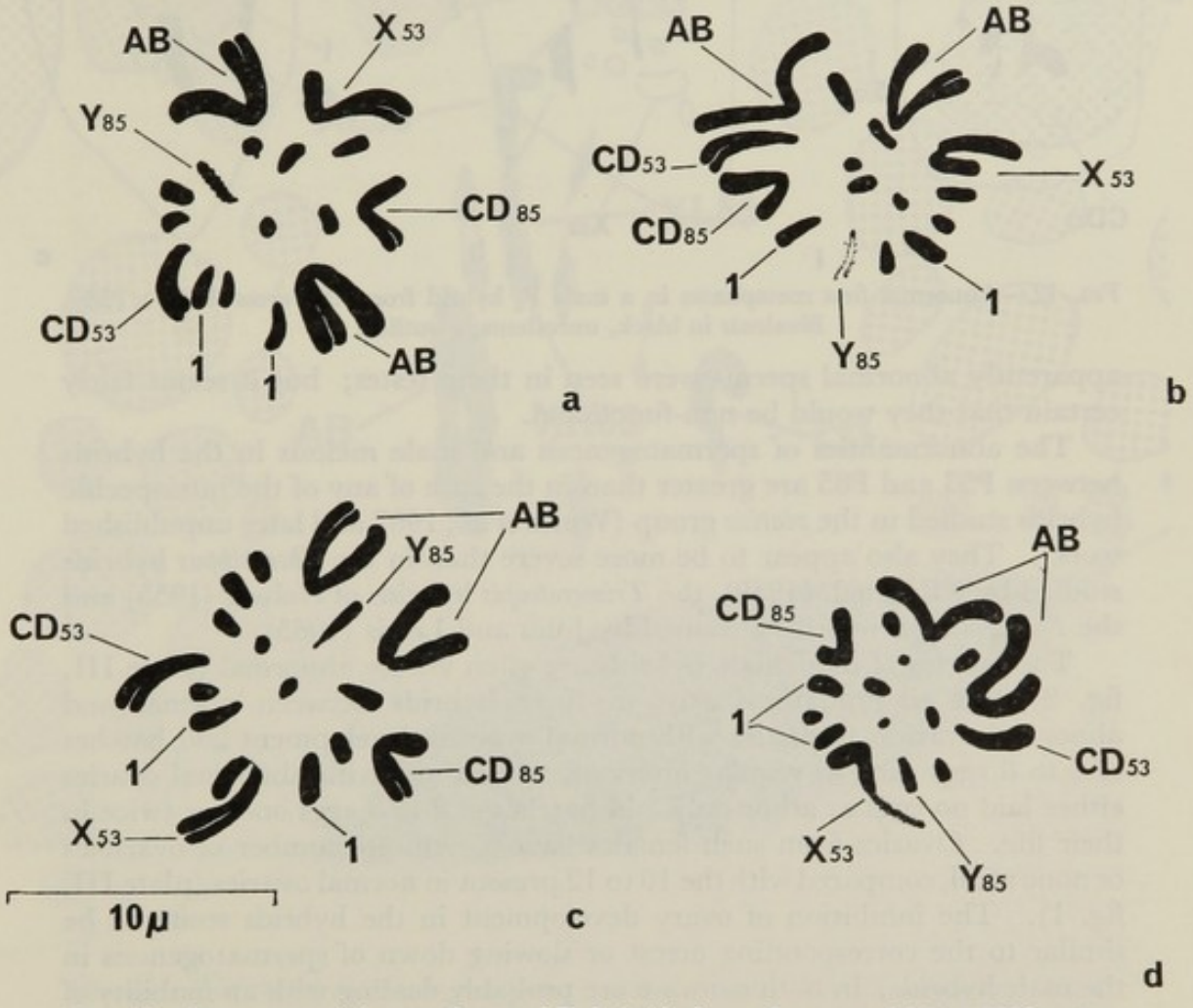


FIG. 11.—Spermatogonial metaphases of F_1 hybrids from the cross P85♂ × P53♀. Note especially the negative heteropycnosis of the Y (obscurely indicated in *a* and *c* but clearly shown in *b*). In *d* the negative heteropycnosis of the distal region of XL is clearly seen.

No significant differences in the degree of retardation and abnormality of spermatogenesis could be detected between the males from the reciprocal crosses. In the larger testes a few cysts of highly abnormal first metaphases were encountered. Some of these peculiar divisions are shown and, to some extent, interpreted in fig. 12. A few of the chromosomes have formed bivalents and appear to possess chiasmata; but their shapes are irregular and they tend to fuse or clump together. It seems to be unusual for more than three or four bivalents (out of a potential eight) to be present. The remaining chromosomes are univalents, sometimes irregular and abnormal in shape; most of them are orientated syntelically on the spindle, but one or two in each cell are often orientated amphitelically. A few bundles of

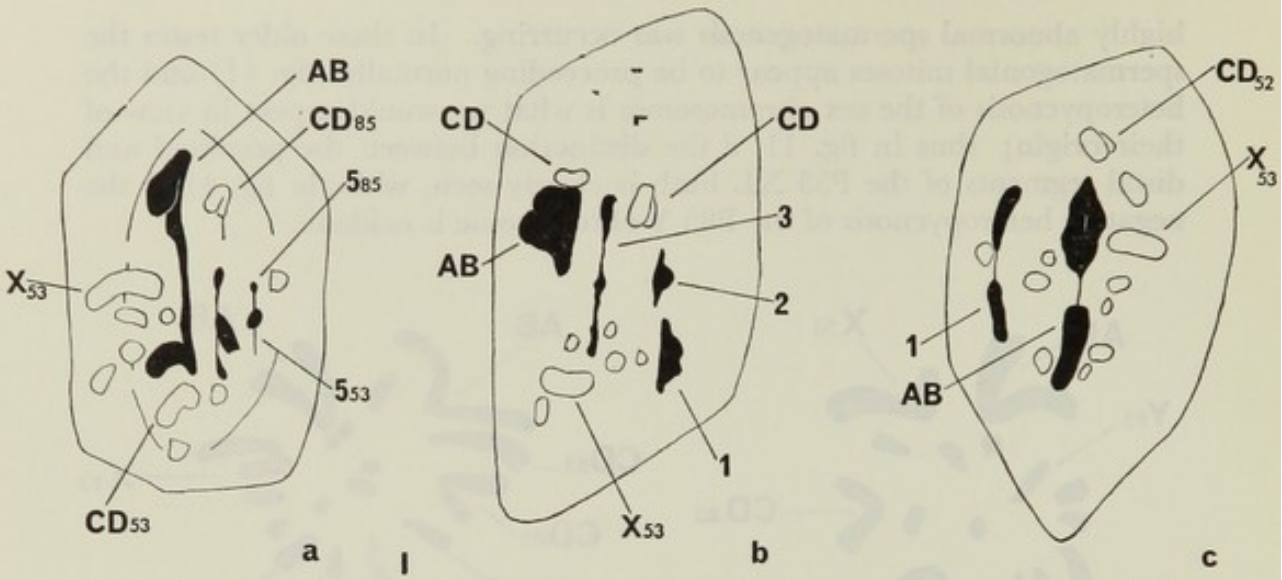


FIG. 12.—Abnormal first metaphases in a male F_1 hybrid from the cross P85♂ × P53♀. Bivalents in black, univalents in outline.

apparently abnormal sperms were seen in these testes; but it seems fairly certain that they would be non-functional.

The abnormalities of spermatogenesis and male meiosis in the hybrids between P53 and P85 are greater than in the case of any of the interspecific hybrids studied in the *viatica* group (White *et al.*, 1967 and later unpublished work). They also appear to be more severe than in the *Chorthippus* hybrids studied by Klingstedt (1939), the *Trimerotropis* hybrids of Helwig (1955) and the *Eyprepocnemis* hybrids obtained by John and Lewis (1965).

The ovaries of the female hybrids are often visibly abnormal (plate III, fig. 2), but all gradations exist in these hybrids between normal and abnormal ovaries. Females with normal ovarian development laid batches of 6 to 8 eggs each at regular intervals, while those with abnormal ovaries either laid no eggs at all or only laid batches of 2 to 3 eggs once or twice in their life. Ovaries from such females have a reduced number of ovarioles or none at all, compared with the 10 to 12 present in normal ovaries (plate III, fig. 1). The inhibition of ovary development in the hybrids seems to be similar to the corresponding arrest or slowing down of spermatogenesis in the male hybrids; in both cases we are probably dealing with an inability of gonial cells to enter on the growth phase and become spermatocytes or oocytes. No difference between the reciprocal F_1 hybrids in degree of ovarian development was observed.

In all those cases where female hybrids contained mature oocytes the first metaphases were almost completely normal, all the chromosomes forming bivalents (fig. 13). A very conspicuous cap of dark-staining material overlies the first metaphase spindle in eggs of the P52 group. Such a structure seems to occur in all species of morabine grasshoppers and has been previously illustrated in the eggs of the parthenogenetic species *Moraba virgo* (White, Cheney and Key, 1963); its exact nature and significance is not understood.

7. Discussion

The P52 group rather clearly consists of two pairs of fairly closely related species, P52*a*-P52*b* and P85-P53. There can be no reasonable

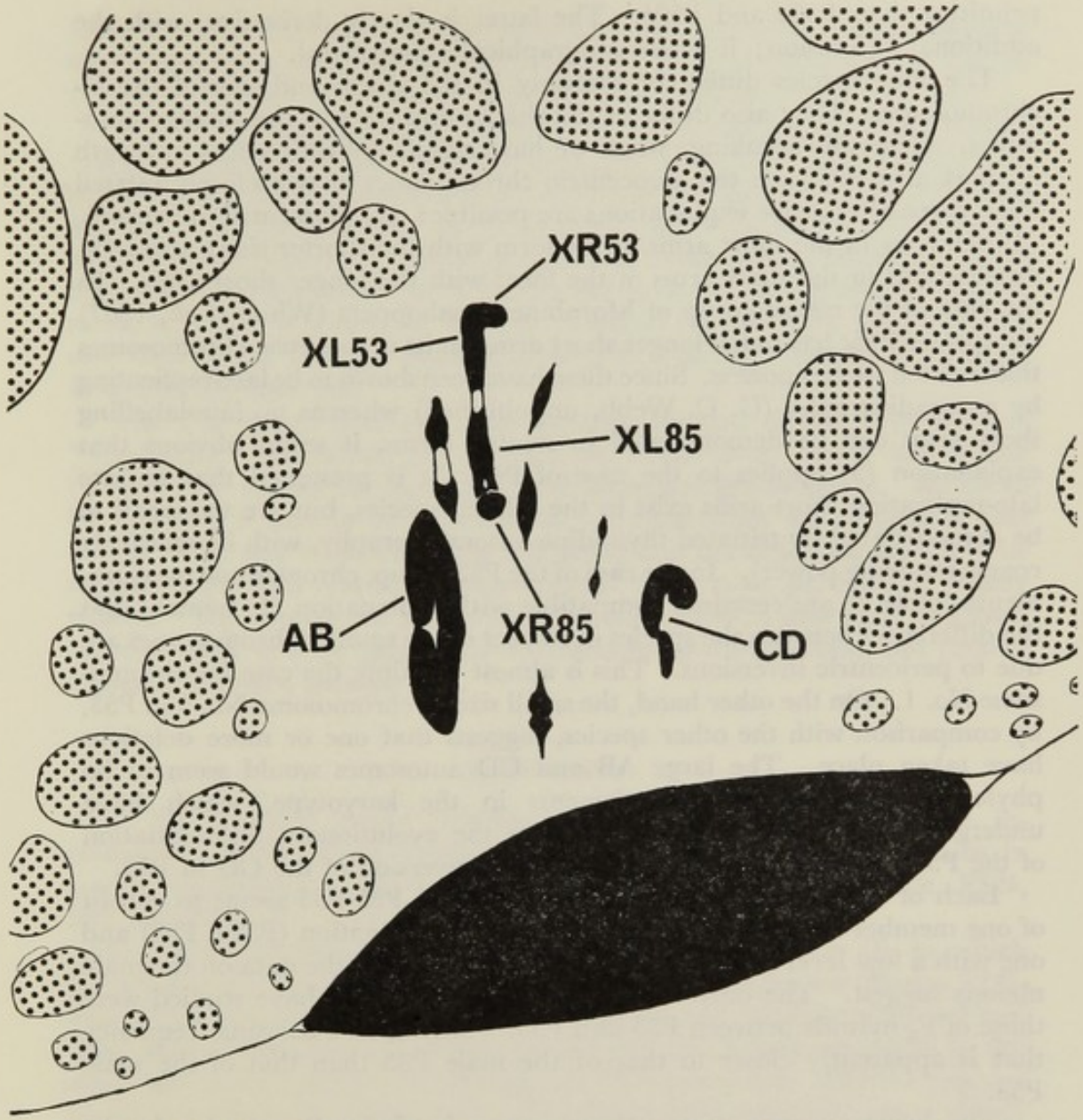


FIG. 13.—First metaphase in an oocyte of a female F_1 hybrid from the cross P85♂ × P53♀.

doubt that all these four taxa have reached the specific level. P52*a* and P52*b* have very similar internal male genitalia, but the numerous structural chromosomal differences between them as well as the difference in chiasma distribution indicate quite different genetic systems. Of the four species, only P52*a* shows a tendency to break up into local races, characterised by different types of Y-chromosomes.

The group as a whole is presumably descended from a species in which an XO sex chromosome mechanism was converted into a neo-XY one by a fusion (F14) between an acrocentric X and one of the six shorter chromosomes of the original $2n\,♂ = 17$ karyotype. The autosome in question was probably the longest or the second longest of the six smaller autosomes. We have no means of telling which of the four existing species is closest in karyotype to the common ancester of the group; the similarity in length between the Y and XR in P85 and especially in P53 suggests (without definitely proving) that those species are karyotypically more

primitive than P52*a* and P52*b*. The latter is clearly derivative, with the additional F15 fusion; it is also geographically peripheral.

The four species differ considerably in the shape and size of the Y-chromosome. They also differ in the shapes and sizes of the smaller autosomes. Generally speaking, when we have significant differences in length of short arms between the acrocentric chromosomes of two closely related taxa, three alternative explanations are possible: (1) pericentric inversions, (2) deletions in the short arms of the form with the shorter short arms, (3) duplications in the short arms of the form with the longer short arms. In the case of the *viatica* group of Morabine grasshoppers (White *et al.*, 1967) the species P45*c* has much longer short arms on its acrocentric chromosomes than related species possess. Since these have been shown to be late-replicating by autoradiography (G. C. Webb, unpublished) whereas no late-labelling short arms can be demonstrated in related forms, it seems obvious that explanation (3) applies to the case of P45*c* (it is presumed that minute late-replicating short arms exist in the related species, but are too small to be demonstrated by tritiated thymidine autoradiography, with its relatively coarse resolving power). In the case of the P52 group, chromosome measurements (table 2) are certainly compatible with explanation (1) namely that the differences between the species in respect of the smaller chromosomes are due to pericentric inversions. This is almost certainly the case for chromosome No. 1. On the other hand, the small size of chromosome No. 5 in P53, by comparison with the other species, suggests that one or more deletions have taken place. The large AB and CD autosomes would seem to be phylogenetically conservative elements in the karyotype, which have undergone few if any rearrangements in the evolutionary differentiation of the P52 group, except for the pericentric inversion of the CD in P53.

Each of the two species-pairs P52*a*-P52*b* and P85-P53 seems to consist of one member with a high level of genetic recombination (P52*a*, P53) and one with a low level (P52*b*, P85); at least that is what the data on the male meiosis suggest. The only female meiotic divisions we have studied were those of F_1 hybrids between P85 and P53, which have a chiasma frequency that is apparently closer to that of the male P85 than that of the male P53.

The heteropycnosis of sex chromosomes is obviously complex in the P52 group (quite apart from the very special behaviour of the Xs in tetraploid spermatogonia of P52*a*, reported by White, 1970*b*). Clearly, the neo-Y shows heteropycnosis under some circumstances, in most of the species. This may be an instance of heterochromatisation of this element since it became confined to the male line; homology with XR being now restricted to small terminal segments. It is not usual for the original X in XO morabines (or its derivative, XL in the neo-Y species) to be late-labelling in autoradiographs of female cells; *e.g.* it is not so in the parthenogenetic *Moraba virgo* (White and Webb, 1968) or in the *viatica* group of species (G. C. Webb, unpublished). But it clearly is so in P53. These differences illustrate the difficulty of attempting to establish any simple correlation between " heterochromatin " and late replication.

A number of naturally-occurring translocations have now been studied in Morabine grasshoppers. One of these, a 4-break translocation in *Keyacris scurra*, has already been described (White, 1963). The case dealt with in the present paper, in P52*a*, is somewhat less complex. Another instance of a

three-break translocation, but in that case with the breaks in three different non-homologous chromosomes, has been found in an individual of an undescribed member of the genus *Warramunga*, " PW11*b* ".

All these are rearrangements involving more than two breaks. Some 2-break rearrangements have also been encountered (White, 1956, 1961 and later unpublished work); these include pericentric inversions, deletions and 2-break translocations. It is quite clear, however, that rearrangements involving more than two breaks are far more common in Morabine grasshoppers than they would be if the breaks occurred independently and at random. One is forced to wonder whether the complex translocations owe their origin to some cause such as virus infection, leading to multiple breaks in the same cell.

The P52 group does not seem directly relevant to the hypothesis of " stasipatric " speciation, put forward by White *et al.* (1967) and White (1968) in the case of the *viatica* group of Morabine grasshoppers. We mean by this that it cannot be regarded as evidence either for or against that model. The only members of the P52 group that are definitely known to be sympatric are P85 and P52*a*, which do not seem to be particularly closely related. The two species pairs P52*a*-P52*b* and P85-P53 may each be shown by later work to have parapatric or overlapping distributions, but present evidence does not indicate this.

8. Summary

1. The " P52 " group (Orthoptera, Eumastacidae, Morabinae) includes four undescribed species of grasshoppers which live on shrubs in central Queensland. They are referred to provisionally as P52*a*, P52*b*, P85 and P53.

2. P52*a*, P85 and P53 are neo-XY species with $2n♂♀ = 16$, in which the original X has become fused to one of the small autosomes (Fusion F14). The XO ancester of these species is unknown, and is presumably extinct. They differ slightly in the dimensions of the neo-X and especially in the shape and size of the neo-Y; in addition P52*a* shows four different types of Ys, each apparently characteristic of a particular geographic area. There are also differences between the small autosomes of these species which indicate that numerous structural changes of these chromosomes must have occurred in their phylogeny.

3. One individual of P52*a* caught in the wild was heterozygous for a newly-arisen 3-break rearrangement involving a pericentric inversion and a mutual translocation.

4. P52*b* is an X_1X_2Y species with $2n♂ = 15$, $2n♀ = 16$, in which an additional neo-Y-autosome fusion (F15) has taken place.

5. The crosses P53♂ × P85♀ and P85♂ × P53♀ gave offspring of both sexes. The male hybrids had underdeveloped testes, some of which were inhibited in development to such an extent that no meiotic divisions occurred; others showed highly abnormal spermatogenesis with many univalents at first metaphase. Female hybrids had ovaries ranging from normal to abnormal with few oocytes; but those oocytes that had undergone development showed normal first meiotic divisions with complete synapsis of the chromosomes of the parental species.

9. References

CALLAN, H. G., AND JACOBS, P. A. 1957. The meiotic process in *Mantis religiosa* L. males. *J. Genet.*, *55*, 200-216.

HELWIG, E. R. 1955. Spermatogenesis in hybrids between *Circotettix verruculatus* and *Trimerotropis suffusa* (Orthoptera: Oedipodidae). *Univ. Colorado Study Ser. Biol.*, *10*, 49-64.

JOHN, B., AND HEWITT, G. M. 1966. Karyotype stability and DNA variability in the Acrididae. *Chromosoma*, *20*, 155-172.

JOHN, B., AND HEWITT, G. M. 1968. Patterns and pathways of chromosome evolution within the Orthoptera. *Chromosoma*, *25*, 40-74.

JOHN, B., AND LEWIS, K. R. 1965. Genetic speciation in the grasshopper *Eyprepocnemis plorans*. *Chromosoma*, *16*, 308-344.

KLINGSTEDT, H. 1939. Taxonomic and cytological studies on grasshopper hybrids. I. Morphology and spermatogenesis of *Chorthippus bicolor* Charp. × *Ch. biguttulus* L. *J. Genet.*, *37*, 389-420.

MESA, A. 1963. Mecanismo cromosómico de determinación sexual poco frecuente en *Scotussa daguerrei* Lieb. (Orthoptera-Acrididae). *Rev. Soc. Entomol. Argent.*, *26*, 119-124.

MESA, A., AND DE MESA, R. S. 1967. Complex sex determining mechanisms in three species of South American grasshoppers (Orthoptera, Acridoidea). *Chromosoma*, *21*, 163-180.

WHITE, M. J. D. 1941. The evolution of the sex chromosomes. I. The XO and X_1X_2Y mechanisms in praying mantids. *J. Genet.*, *42*, 143-172.

WHITE, M. J. D. 1956. Adaptive chromosomal polymorphism in an Australian grasshopper. *Evolution*, *10*, 298-313.

WHITE, M. J. D. 1957. Cytogenetics of the grasshopper *Moraba scurra*. I. Meiosis of interracial and interpopulation hybrids. *Austral. J. Zool.*, *5*, 285-304.

WHITE, M. J. D. 1958. Restrictions on recombination in grasshopper populations and species. *Cold Spr. Harb. Symp. Quant. Biol.*, *23*, 307-317.

WHITE, M. J. D. 1961. Cytogenetics of the grasshopper *Moraba scurra*. VI. A spontaneous pericentric inversion. *Austral. J. Zool.*, *9*, 784-790.

WHITE, M. J. D. 1963. Cytogenetics of the grasshopper *Moraba scurra*. VIII. A complex spontaneous translocation. *Chromosoma*, *14*, 140-145.

WHITE, M. J. D. 1965. Sex chromosomes and meiotic mechanisms in some African and Australian mantids. *Chromosoma*, *16*, 521-547.

WHITE, M. J. D. 1966*a*. Further studies on the cytology and distribution of the Australian parthenogenetic grasshopper *Moraba virgo*. *Rev. Suisse Zool.*, *73*, 383-398.

WHITE, M. J. D. 1966*b*. A case of spontaneous chromosome breakage at a specific locus occurring at meiosis. *Austral. J. Zool.*, *14*, 1027-1034.

WHITE, M. J. D. 1968. Models of speciation. *Science*, *159*, 1065-1070.

WHITE, M. J. D. 1969. Chromosomal rearrangements and speciation in animals. *Ann. Rev. Genet.*, *3*, 75-98.

WHITE, M. J. D. 1970*a*. Cytogenetics. Chapter 3 (pp. 72-82) in *Insects of Australia*. Melbourne Univ. Press.

WHITE, M. J. D. 1970*b*. Asymmetry of heteropycnosis in tetraploid cells of a grasshopper. *Chromosoma*, *30*, 51-61.

WHITE, M. J. D. 1970*c*. Cytogenetics of speciation. *J. Austral. Entomol. Soc.*, *9*, 1-6.

WHITE, M. J. D., BLACKITH, R. E., BLACKITH, R. M., AND CHENEY, J. 1967. Cytogenetics of the *viatica* group of morabine grasshoppers. I. The " coastal " species. *Austral. J. Zool.*, *15*, 263-302.

WHITE, M. J. D., CARSON, H. L., AND CHENEY, J. 1964. Chromosomal races in the Australian grasshopper *Moraba viatica* in a zone of geographic overlap. *Evolution*, *18*, 417-429.

WHITE, M. J. D., AND CHENEY, J. 1966. Cytogenetics of the *cultrata* group of morabine grasshoppers. I. A group of species with XY and X_1X_2Y sex chromosome mechanisms. *Austral. J. Zool.*, *14*, 821-834.

WHITE, M. J. D., CHENEY, J., AND KEY, K. H. L. 1963. A parthenogenetic species of grasshopper with complex structural heterozygosity. (Orthoptera : Acridoidea). *Austral. J. Zool.*, *11*, 1-19.

WHITE, M. J. D., KEY, K. H. L., ANDRÉ, M., AND CHENEY, J. 1969. Cytogenetics of the *viatica* group of morabine grasshoppers. II. Kangaroo Island populations. *Austral. J. Zool.*, *17*, 313-328.

WHITE, M. J. D., MESA, A., AND MESA, R. 1967. Neo-XY sex chromosome mechanisms in two species of Tettigonioidea (Orthoptera). *Cytologia*, *32*, 190-199.

WHITE, M. J. D., AND WEBB, G. C. 1968. Origin and evolution of parthenogenetic reproduction in the grasshopper *Morba virgo* (Eumastacidae : Morabinae). *Austral. J. Zool.*, *16*, 647-671.

THE CHROMOSOMES OF SOME CHRYSOMELID COLEOPTERA: DIABROTICITES *

STANLEY G. SMITH
Box 490, Sault Ste Marie, Ontario

1. Introduction

The 180 or so chrysomelid beetles investigated cytologically reveal the occurrence in the family of a wide spectrum of sex-determining systems and haploid chromosome numbers ranging from four to 30. The only Diabroticites so far recorded in the literature, six *Diabrotica* and one *Acalymma* species, all have *XO* males and *n* equalling either 10 (*Diabrotica*) or 11 (*Acalymma*), an unusual conservatism for Chrysomelidae (fig. 2).

Since it was first described by Dejean in 1837, the genus *Diabrotica* has undergone extensive revision, resulting in its subdivision into 14 genera, of which eight occur in the U.S.A. and Mexico.

In 1966, an invitation to participate, cytologically, in a wide survey of New World taxa, then being planned, was extended by Professor R. F. Smith, University of California, Berkeley. Despite their apparent karyotypic near-conformity, the offer was readily accepted as it promised an opportunity of gaining a broad insight into the general cytogenetic systems of Diabroticites by first-hand investigation of so many previously unstudied genera.

The following report, a first record of results, describes only the unexpected karyotypic plasticity revealed by the study.

2. Material and methods

Males, field collected and tentatively identified (by R. F. S.), were air mailed to Sault Ste Marie for cytological study, the field determinations being subsequently rechecked at Berkeley by examination of cadavers. These beetles are noxious pests and, to circumvent quarantine regulations, only males were imported into Canada.

During 1966 and 1967, accessions, many of them multiple, of 41 taxa in eight of the New World genera were received from southwestern U.S.A. and Mexico; two taxa failed to survive. Mortality in transit from Central America and Trinidad in 1968 was much higher, only nine of the 23 taxa arriving in satisfactory condition; they included eight species new to cytology. In 1967, collections were also received from several states east of Texas; they included three species not previously investigated. The 54 Diabroticites reported on by Ennis (1969) and herein, together with the three species studied earlier by Virkki (1964), are listed with locality data and chromosome numbers in table 1.

Gonads, fixed in modified Kahle's fluid, were squashed and then stained in basic fuchsin-methyl green (Smith and Takenouchi, 1969). When available in sufficient numbers, males were fed 0·2 per cent. colchicine in honey for 6-8 hours before dissection to increase the chances of obtaining

* Contribution No. 175, Insect Pathology Research Institute, Canada Department of Fisheries and Forestry, Sault Ste Marie, Ontario.

TABLE 1

Source and chromosome number of the Diabroticites investigated

Taxon	No. exam.	$2n =$	Source: U.S.A.	Source: Mexico	Source: Cent. Am.
Diabrotica					
adelpha Harold	—†	18+*X*			ES
balteata LeC.	14	18+*X*	5	B, D-F, L, M	ES
bioculata Bowd.	1	18+*X*		E	
capitata (F.)	2	18+*X*			Gu
circulata Harold	2	18+*X*			CR, ES
cortezi (undescr.)	8	18+*X*		I	
cristata (Harris)	12	18+*X*	8, 9	H	
dissimilis Jacoby	7	18+*X*		E	
flaviventris Jacoby	2	18+*X*		E	
graminea Baly	—†	18+*X*			ES
lemniscata LeC.	11	18+*X**	2, 3	C	
limitata 15-punctata (Germar)	1	18+*X*			Vz
longicornis longicornis (Say)	21	18+*X**	2-4		
longicornis barberi Smith & L.	23	18+*X**	6, 7, 10-12, 14		
longicornis nigricornis (undescr.)	6	18+*X**	10, 14, 16		
nummularis Harold	3	18+*X*		H	
ochreata (F.)	6	18+*X*			Gu
octoplagiata Jacoby	2	18+*X*		L	
porracea Harold	11	18+*X*		D, E, I, L, N	
scutellata Baly	4	18+*X**		E	
sexmaculata Baly	5	18+*X*		D, E	
tibialis Jacoby	4	18+*X**		E	
trifasciata (F.)	1	18+*X*			Tr
undecimpunctata 11-punctata Mann.	15	18+*X**	1		
undecimpunctata howardi Barber	67	18+*X**	2-6, 9-12, 17		
undecimpunctata tenella LeC.	14	18+*X**	2		
undecimpunctata 12-notata Harold	12	18+*X*	2, 3	A, C, D, M	
" *variabilis?* "	1	18+*X*			Vz
virgifera virgifera LeC.	27	18+*X*	2, 6, 7, 9, 10, 12	C, D	
virgifera zeae (undescr.)	16	18+*X*		D, E, G, M	
viridula (F.)	—†	18+*X*			ES
viridula rufibasis (undescr.)	9	18+*X*		E, M, N	
Cerotoma					
atrofasciata Jacoby	1	32+*X**		I	
ruficornis (Olivier)	2	32+*X*		I	
Amphelasma					
cavum cavum (Say)	2	16+*X*		E	
cavum vicinum (Jacoby)	5	16+*X**		A, B	
cavum n. subsp.	3	18+*X*		C	
Paranapiacaba					
connexa (LeC.)	1	18+*X*		E	
tricincta (Say)	17	22+*X*	2-4		
Paratriarius					
dorsata (Say)	2	18+*X*	13		
curtisii (Baly)	4	22+*X*		E	
Gynandrobrotica					
lepida (Say)	6	16+*X*		E, K	
variabilis (Jacoby)	3	16+*X*		E	
nigrofasciata (Jacoby) " Oaxaca "	3	16+*X**		M	
" Veracruz "	7	12+*X**		E	
Synbrotica					
multipunctata (Jacoby)	4	22+*X*		K	
tetraspilota (Baly)	6	36+*X*		E	
Acalymma					
blandulum (LeC.)	15	18+*X**	2-4		
gouldi Barber	9	18+*X*	13		
innubum (F.)	6	18+*X**		L, N	
vittatum (F.)	13	20+*X*	5, 13		
quadrivittatum (Latr.)	2	20+*X*			Tr
pallipe (Oliv.)	7	22+*X*			Tr
trivittatum (Mann.) " Chiapas "	39	20+*X**		A-F, K-M	
" Guerrero "	7	?+*XXY**		M, N	
thiemei (Baly)	18	22+*X*		E, L	Tr
fairmairei (Baly)	4	26+*X*		E	

* = excluding *B* chromosomes. † = Virkki (1964).

Locality code: 1, California; 2, Arizona; 3, New Mexico; 4, Texas; 5, Louisiana; 6, Kansas; 7, Nebraska; 8, South Dakota; 9, Iowa; 10, Minnesota, 11; Wisconsin; 12, Illinois; 13, Indiana; 14, Ohio; 15, North Carolina; 16, New York; 17, Connecticut.

A, Coahuila; B, Nuevo Leon; C, Durango; D, San Luis Potosi; E, Veracruz; F, Queretaro; G, Guanajuato; H, Jalisco; I, Morelos; J, Puebla; K, Mexico; L, Chiapas; M, Oaxaca; N, Guerrero. CR, Costa Rica; ES, El Salvador; Gu, Guadeloupe; Tr, Trinidad; Vz, Venezuela.

Plate I

FIG. 1.—*Cerotoma ruficornis*, MI = $16_{II}+X$.

FIG. 2.—*Amphelasma c. cavum*, diakinesis = $8_{II}+X$.

FIG. 3.—*Amphelasma c. vicinum*, MI = $8_{II}+X+1B$.

FIG. 4.—*Amphelasma c.* n. subsp., Spg = $18+X$ (phase contrast).

FIGS. 5 and 6.—*Paranapiacaba tricincta*. FIG. 5.—Spg = $22+X$. FIG. 6.—MI = $11_{II}+X$.

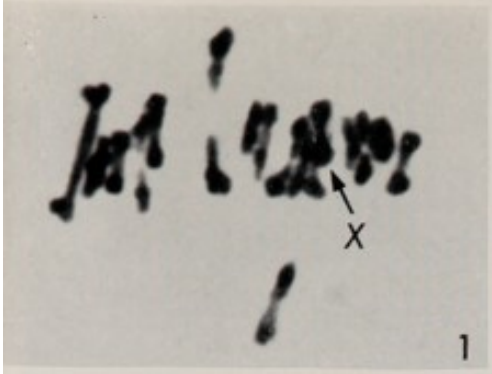
FIG. 7.—*Paratriarius dorsata*, Spg = $18+X$.

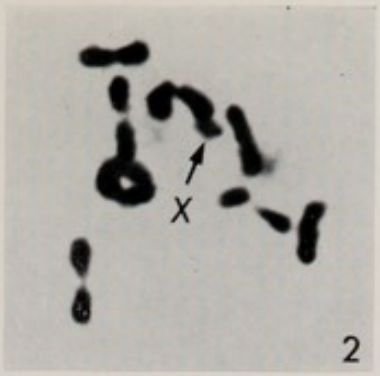
FIGS. 8 and 9.—*Paratriarius curtisii*. FIG. 8.—Spg = 23. FIG. 9. MI = $11_{II}+X$.

FIGS. 10 and 11.—*Synbrotica multipunctata*. FIG. 10.—Spg = 23. FIG. 11.—MI = $11_{II}+X$.

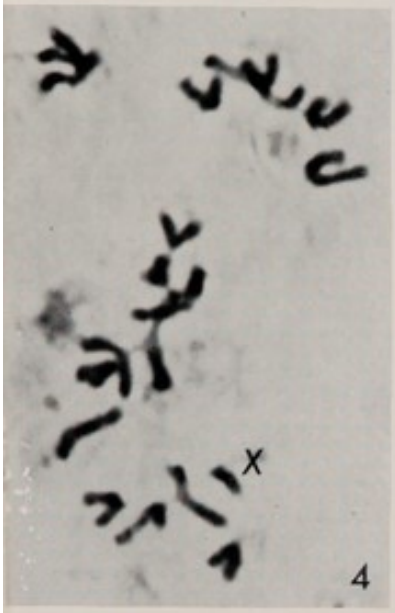
FIG. 12.—*Synbrotica tetraspilota*, MI = $18_{II}+X$.

FIGS. 13 and 14.—*Gynandrobrotica lepida*. FIG. 13.—Spg = 17. FIG. 14.—MI = $8_{II}+X$.

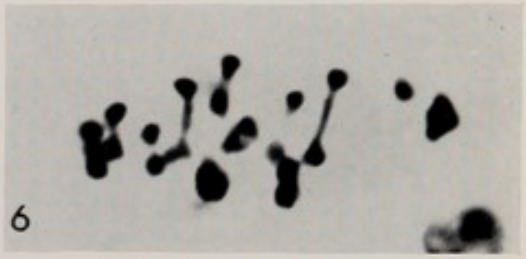

X
1

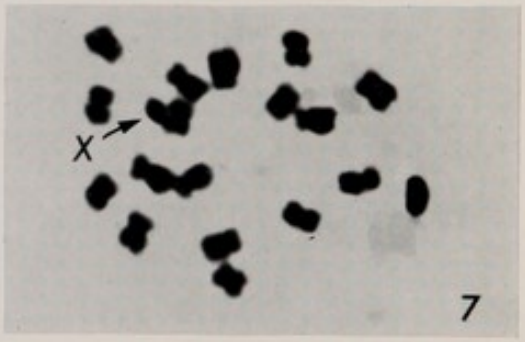

X
2

B
3

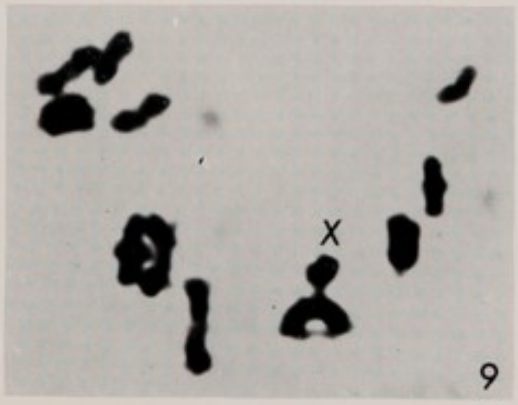

X
4

X
5

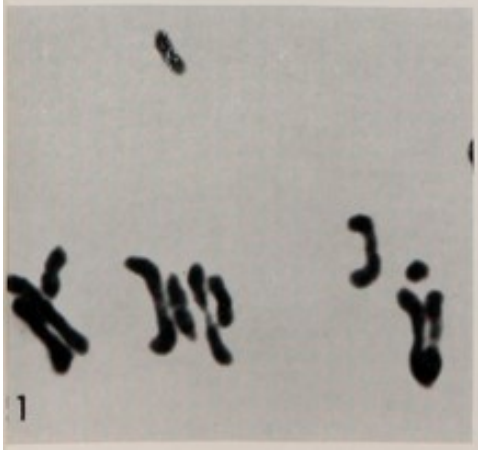

6

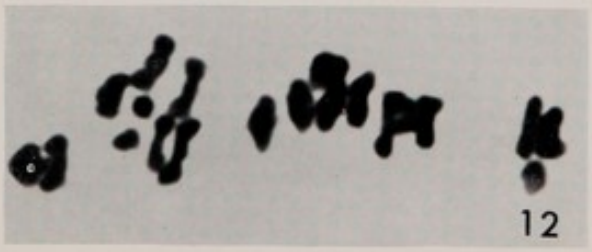

X
7

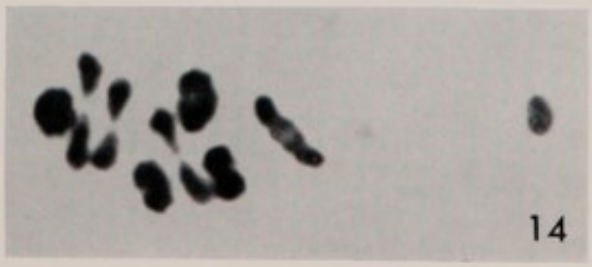
8

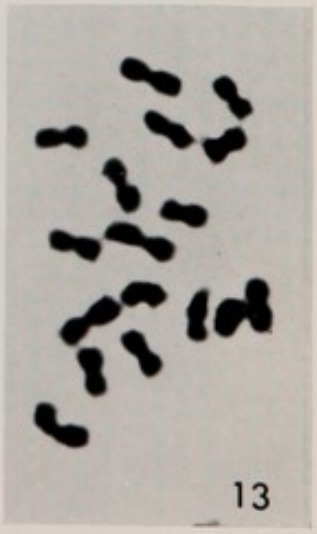

X
9

10


1


12


14


13

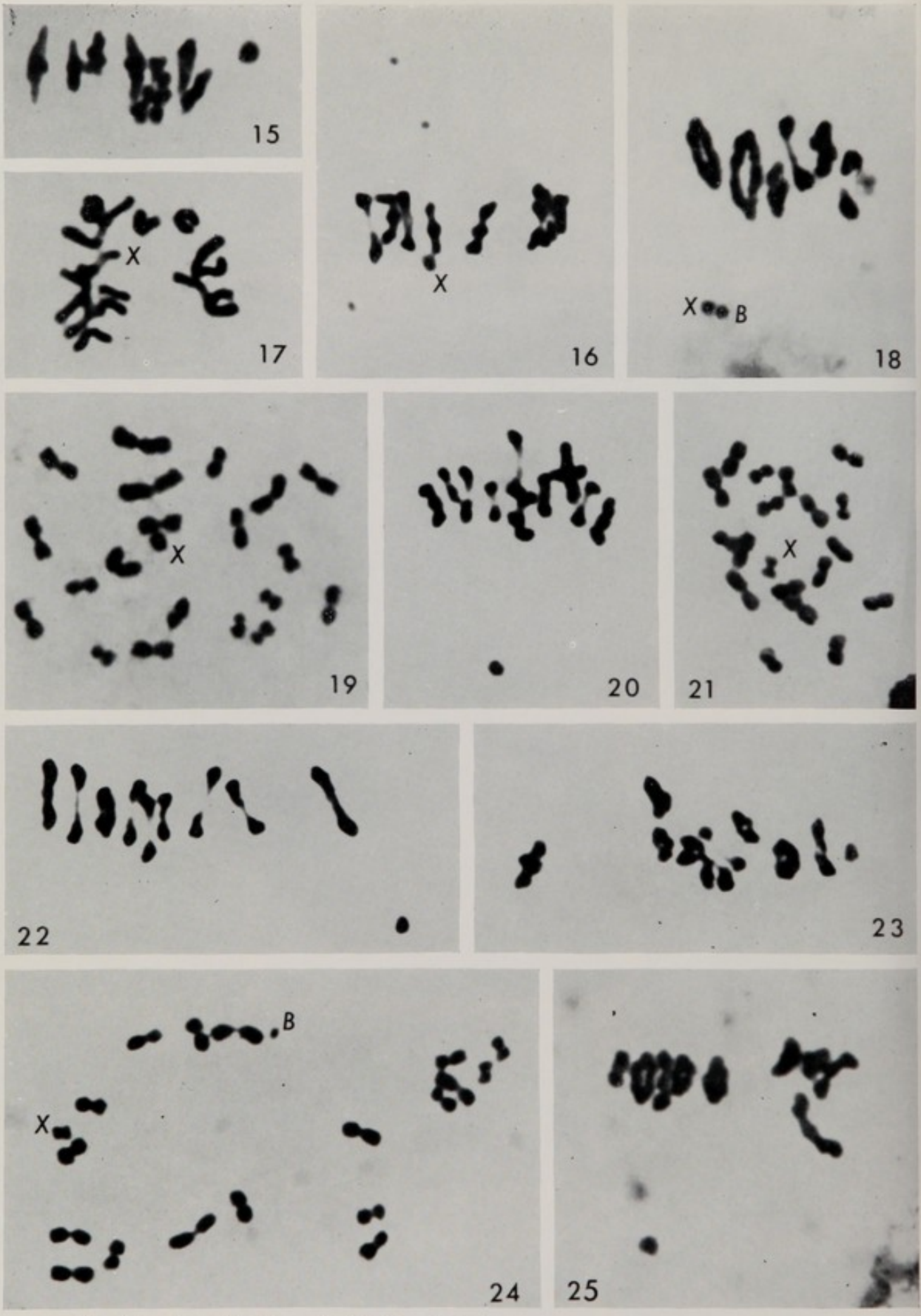
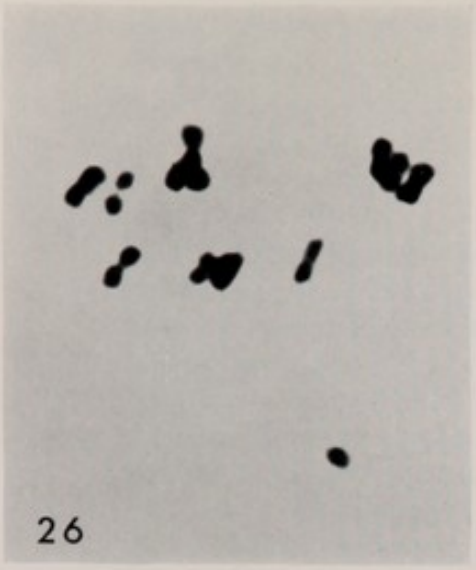


FIG. 15.—*Gynandrobrotica variabilis*, MI = $8_{II}+X$.
FIG. 16.—*Gynandrobrotica nigrofasciata* " O " phase, MI = $8_{II}+X+3B$.
FIGS. 17 and 18.—*Gynandrobrotica nigrofasciata* " V " phase. FIG. 17.—Spg = $12+X$. FIG. 18.—MI = $6_{II}+X+1B$.
FIGS. 19 and 20.—*Acalymma vittatum*. FIG. 19.—Spg = $20+X$. FIG. 20.—MI = $10_{II}+X$.
FIGS. 21 and 22.—*Acalymma blandulum*. FIG. 21.—Spg = $18+X$. FIG. 22.—MI = $9_{II}+X$.
FIG. 23.—*Acalymma gouldi*, MI = $9_{II}+X$.
FIGS. 24 and 25.—*Acalymma innubum*. FIG. 24.—Spg = $18+X+1B$. FIG. 25.—MI = $9_{II}+X$.



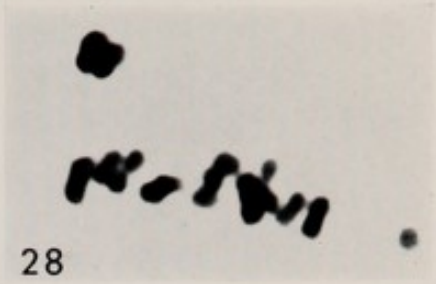


FIG. 26.—*Acalymma pallipe*, MI = $11_{II}+X$.
FIGS. 27 and 28.—*Acalymma thiemei*. FIG. 27.—Spg = $22+X$. FIG. 28.—MI = $11_{II}+X$.
FIG. 29.—*Acalymma fairmairei*, MI = $13_{II}+X$.
FIGS. 30 and 31.—*Acalymma trivittatum* " C " phase. FIG. 30.—Spg $20+X$. FIG. 31.—MI = $10_{II}+X$.
FIGS. 32-33*b*.—*Acalymma trivittatum* " G " phase. FIG. 32.—Spg *c*. $38+X+2B$. FIG. 33*a*.—MI = $18_{II}+XXY(?)+1B$. FIG. 33*b*.—MI = *c*. $18_{II}+XXY(?)$.

gonial metaphases. Prints, reproduced at *c.* 1900, have been retouched when necessary to eliminate artifacts that might be confused with *B* chromosomes.

3. Observations

A. *Diabrotica* Dejean, 1837

All 22 species and 7 subspecies representing this genus in recent collections (table 1) were studied by Ennis (1969), who recorded an invariable standard $2n = 19$ in males and the expected $2n = 20$ in females of the only two species where both sexes were obtained. For chrysomelids, and especially in relation to the other Diabroticites with which he was familiar, Ennis considered them to exhibit " . . . a surprising numerical and morphological similarity, apart from the presence of B chromosomes . . . " in nine taxa (table 1). The autosomes are graded in size and meta- or submeta-centric, the *X* being the smallest chromosome and metacentric. His description of the general course of spermatogenesis agrees with earlier published accounts. Briefly, the *X* and, where present, the *B* chromosome are positively heteropycnotic from early prophase until the beginning of diakinesis and precessional-prereductional at first anaphase; the *X* and *B*s commonly associate ectopically and then often remain attached as late as early anaphase. Chiasma interference is complete within arms and strong but variable across the centromere. A statistical indication of loss of a chiasma from ring bivalents during the course of terminalisation was attributed to variability in ring formation between cysts of an individual.

This outline so fittingly describes the meiotic process in most of the Diabroticites here reported that only atypical phenomena will be detailed here.

B. *Cerotoma* Chevrolat, 1843

The three available males, one *atrofasciata* and two *ruficornis*, were devoid of usable gonial mitoses, but cells at diakinesis and first metaphase established each as having basically $16_{II}+X$ (plate I, fig. 1), *i.e.* $2n = 33$. One or two ring bivalents were observed in an estimated 50 per cent. of the diakinesis cells and persisted until metaphase. One *B* chromosome occurred in the lone *atrofasciata*.

C. *Amphelasma* Barber, 1947

All accessions of this genus proved to be subspecies of *A. cavum* (Say), namely, *c. cavum*, *c. vicinum*, and *c.* n. subsp., and came from three different Mexican states (table 1). Spermatogonial metaphases of the first two showed $16+X$, all metacentric and *X* the smallest; of the last, $18+X$ (plate I, fig. 4). One of the *vicinum* males carried an isopycnotic *B* chromosome in gonial and first metaphase cells (plate I, fig. 3). Each subspecies had diakinesis and first metaphase stages with either $8_{II}+X$, in *cavum* (plate I, fig. 2) and *vicinum*, or $9_{II}+X$, in the n. subsp.

D. *Paranapiacaba* Bechyne, 1958

The single *connexa* male was not fed colchicine and showed only first metaphases ($9_{II}+X$) and anaphases. All stages were represented among

the 17 *tricincta* males; gonial metaphases with $2n = 23$ metacentrics, the smallest being unmatched and hence the X (plate I, fig. 5); diakineses with one or two rings; and first metaphases with 11_{II} and an isopycnotic X (plate I, fig. 6). In their compilation, Smith and Lawrence (1967) place the latter species in the " tricincta " group and the former in the " waterhousei " group.

E. *Paratriarius* Schaeffer, 1906

Both *dorsata* males, from Indiana, having been colchicined exhibited only gonial metaphases, with $2n = 18+X$ (plate I, fig. 7). The four *curtisii* males, all from Mexico, showed either gonial metaphases, with $2n = 22$ autosomes and a small, metacentric X (plate I, fig. 8), or first metaphases, with 11_{II} and an isopycnotic X (plate I, fig. 9). The former species belongs in the " dorsata " group and the latter in the " flavolimbata " group (Smith and Lawrence, 1967).

F. *Synbrotica* Bechyne, 1956

Two species of *Synbrotica* were provided, both from Mexico. In gonial metaphases, *S. multipunctata* had 23 chromosomes (plate I, fig. 10), including an X which, judging from first metaphases, is among the smallest and hence was isopycnotic in gonia. Up to three ring bivalents were present at first metaphase, and all chiasmata appeared to be terminal or nearly so (plate I, fig. 11). *S. tetraspilota* differs in having 37 spermatogonial chromosomes and diakineses and first metaphases with $18_{II}+X$ (plate I, fig. 12). As in *multipunctata*, rings are formed at meiosis in *tetraspilota* despite its gonial chromosomes being appreciably smaller and including about a dozen acrocentrics. According to Smith and Lawrence (1967) the two species are members of different groups, *multipunctata* being in the " mexicana " and *tetraspilota* in the " lugubris " group.

G. *Gynandrobrotica* Bechyne, 1955

Samples of this genus were received in 1966 and 1967 under three names, *lepida*, *variabilis*, and *nigrofasciata*. (The precise locality data for the collections are given in table 2.) The first two had spermatogonia with 17

TABLE 2

Locality data concerning Gynandrobrotica *accessions*

	G. lepida ($2n = 17$)	
Puebla	7·3 miles NE of Villa A. Camacho, Puebla	16.7.67
Veracruz	Huatusco	17.7.67
	3 miles N of Coscomatepec	17.7.67
	1 mile N of Fortin de las Flores	18.7.67
	G. variabilis ($2n = 17$)	
Oaxaca	27 miles S of Suchixtepec	26.10.66
	G. nigrofasciata	
	" Oaxaca " phase ($2n = 17$)	
Oaxaca	15·5 miles N of Pochutla	26.10.66
	27 miles S of Suchixtepec	26.10.66
	" Veracruz " phase ($2n = 13$)	
Veracruz	1 mile N of Fortin de las Flores	18.7.67
	1 mile S of San Andrés Tuxtla	18.7.67

near-metacentric chromosomes (plate I, fig. 13) and first metaphases with $8_{II}+X$ (plate II, fig. 15). At pachytene in *variabilis*, the bivalents and the *X* formed a bouquet in which the *X* was negatively heteropycnotic and associated with the nucleolus. In *lepida* the *X* was slightly understained at first metaphase (plate I, fig. 14).

Shipments of *nigrofasciata* differed in chromosome constitution depending on origin. Males from Oaxaca, the " O " phase, were not fed colchicine and consequently lacked spermatogonial metaphases of usable standard; however, they showed eight bivalents and a negatively heteropycnotic *X* at first metaphase. The three individuals had zero, one, and 1-3 *B* (plate II, fig. 16) chromosomes each. Males from Veracruz, the " V " phase, had a lower chromosome number, $2n = 13$ (plate II, fig. 17). The smallest member of the complement is a metacentric, which stains more lightly; presumably it is the *X*. One male had one and another two small, spherical *B* chromosomes at first metaphase (plate II, fig. 18). Diakineses showed, in addition to the, by then, isopycnotic *X*, up to the maximum of six ring bivalents possible; they persisted to metaphase.

The genus *Gynandrobrotica* is not subdivided into species complexes in Smith and Lawrence (1967), but the O and V phases of *nigrofasciata* clearly merit specific rank by conventional taxonomic criteria (R. F. S., personal communication.)

H. *Acalymma* Barber, 1947

Representatives of this genus were received under nine species names. Males of one, *vittatum* (= " *D. vittata* "), have long been known to possess $2n = 21$ and $10_{II}+X$ (see Smith, 1953, 1960*a*). This is confirmed (plate II, figs. 19, 20) and extended to include both *quadrivittatum* and the " Chiapas ", or C, phase of *trivittatum*.

Three species, *blandulum*, *gouldi*, and *innubum*, contained spermatogonia with 19 chromosomes (plate II, figs. 21, 24) and first metaphases with $9_{II}+X$ (plate II, figs. 22, 23, 25). Two of the 15 *blandulum* males carried one *B* and four of the six *innubum* males, either one (plate II, fig. 24) or two *B* chromosomes.

Two additional species, *pallipe* and *thiemei* (plate III, fig. 27), differed from the foregoing in having 23 spermatogonial chromosomes that were present at diakinesis and first metaphase as $11_{II}+X$ (plate III, figs. 26, 28). No *B* chromosomes were observed in either species.

Four *fairmairei* males were received from Veracruz, Mexico. The diploid number in gonia was 27, with all chromosomes metacentric and forming $13_{II}+X$ at first metaphase (plate III, fig. 29).

Purposeful collecting of *trivittatum* in 1967 was prompted by the discovery in 1966 that a multiple sex-determining mechanism operates in males from the State of Guerrero, the " G " phase, in contrast to the *XO* system established for C-phase males from the State of Chiapas. (These two states in southern Mexico lie respectively west and east of the State of Oaxaca (fig. 1)). The 1967 accessions, from eight other Mexican states, Arizona, and Texas (table 3), revealed the presence of the G phase only in Oaxaca; no material was collected from Guerrero in 1967.

C-phase males possess gonia with 21 chromosomes (table III, fig. 30) of which four are acrocentric and two are long metacentrics. The latter,

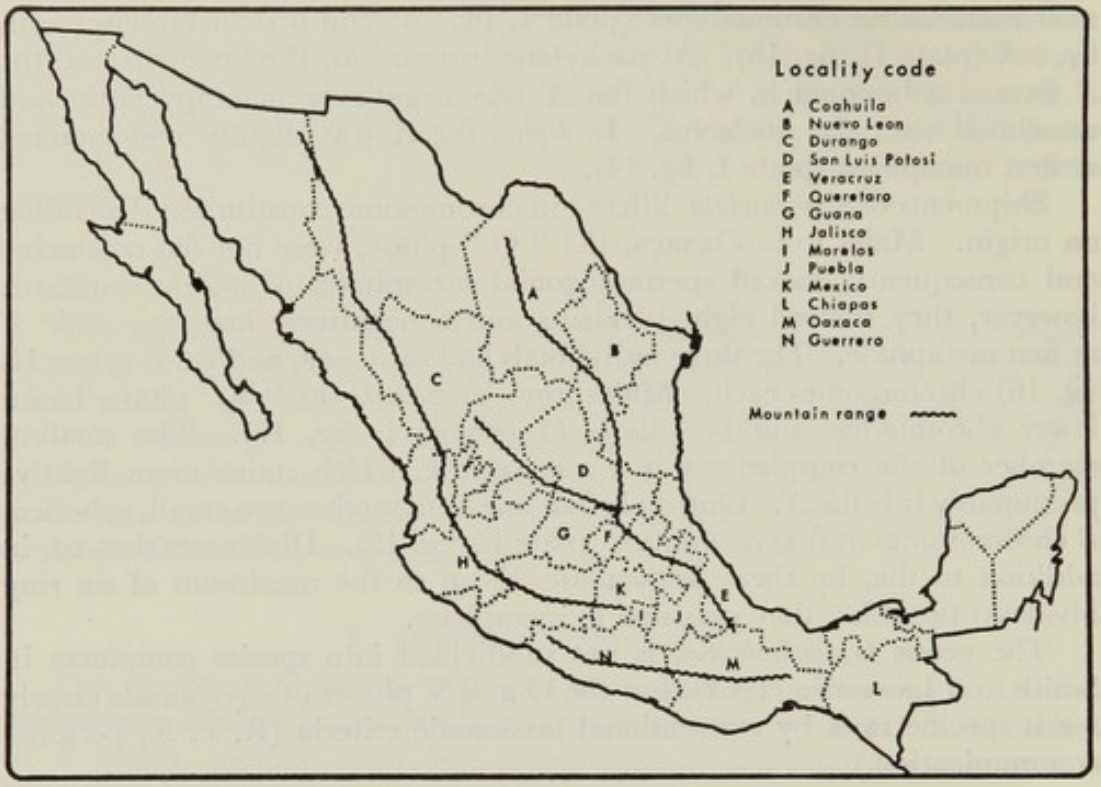


FIG. 1.—Map of Mexico showing location of states from which Diabroticites were studied cytologically.

TABLE 3

Locality data concerning Acalymma trivittatum *accessions*

	" Chiapas " phase ($2n = 20+X$)	
Chiapas	7·5 miles NW of Ocozocoautla	28.10.66
Arizona	7·5 miles NW of Sonoita	1.7.67
	Hereford	2.7.67
Texas	4 miles W of Cline	8.7.67
San Luis Potosi	0·4 miles W of Ciudad del Maiz	12.7.67
Mexico	1 mile S of Panuco	14.7.67
Veracruz	1 mile S of San Andrés Tuxtla	20.7.67
	4·3 miles N of Sayula	21.7.67
Oaxaca	7 miles S of El Tule	22.7.67
Queretaro	2 miles N of Queretaro	26.7.67
Nuevo Leon	3 miles N of Puerto Mexico	27.7.67
Durango	4 miles SW of Guadelupe Victoria	28.7.67
Coahuila	14 miles NW of Saltillo	28.7.67
	" Guerrero " phase ($2n = 36?+XXY?$)	
Guerrero	5 miles SW of Tierra Colorada	18.10.66
Oaxaca	5 miles E of Tequisistlan	21.7.67

occasionally along with four shorter ones, usually form a ring bivalent or else a rod bivalent in which the lone chiasma is intercalary at first metaphase (plate III, fig. 31). A single *B* chromosome was observed in two of the 39 males examined; both originated from El Tule, Oaxaca.

The two G-phase males obtained in October, 1966, were then too old to provide reliable counts, but they contained an undoubted trivalent at first metaphase in place of the single *X* otherwise universally present in known Diabroticites. The six males examined in 1967 had gonial (plate III, fig. 32) and/or first metaphases with various numbers of what I consider to be

B chromosomes (plate III, fig. 33*a* vs. 33*b*), paired or unpaired, and, again, a trivalent at metaphase. *B* chromosomes are so categorised because they commonly occurred as univalents—failure of standard *A* chromosomes to form bivalents is extremely rare (Ennis, 1969). The presence of *B*s, the high chromosome number, and the substandard quality of some preparations, taken together, render determination of the true karyotype of the G phase difficult. Moreover, importation of females being prohibited, the actual status of the sex-determining mechanism, *XXY* or *XYY*, remains questionable, though judging from other Coleoptera it is most probably *XXXX* : *XXY*. It can, however, be stated with some degree of confidence that most of the chromosomes, as seen in gonial metaphases, are metacentric or at least submetacentric, the few acro- or telo-centrics being presumed *B*s. The unmatched and particularly large metacentric is interpreted as the *X* chromosome.

4. Discussion

The family Chrysomelidae is conventionally grouped with the more primitive Cerambycidae and the more derivative Bruchidae to form the superfamily Chrysomeloidea (*e.g.* Crowson, 1960). All cerambycid males that have been examined possess an Xy_p bivalent, and 65 per cent. have nine pairs of autosomes; corresponding frequencies for bruchid males are 92 per cent. and 46 per cent. (fig. 2). Thus the large majority of species in these two families have the typical, modal coleopteran karyotype of $9_{II}+XY_p$. It marked contrast, the cytogenetic systems found among the chrysomelids are too diverse to exhibit any pronounced modal peak (fig. 2), and accordingly fail to reveal any significant evolutionary clues. Indeed, this is no more than one might expect of so heterogeneous a taxonomic assemblage, wherein the classical subfamilies are generally conceded to rate full familial status. But even when attention is focused on a small group, such as the Galerucinae of immediate interest, no clear-cut major cytogenetic pattern emerges: The diploid numbers of the 21 taxa so far recorded in the literature, or known to me personally, range between 18 and 60 (Smith, 1953, 1960*a*; Virkki, 1964, and elsewhere), and, moreover, embrace *XO*, Xy_p, neo-*XY*, and *XXY* male sex-determining mechanisms. It should therefore come as no surprise that the Diabroticites have evolved highly versatile breeding systems.

Since only 57 of the approximately 900 taxa that Smith and Lawrence (1967) list have been investigated (table 4), a detailed discussion of the possible mode of origin of such widely disparate karyotypes could be nothing but sheer speculation. However, in view of the conditions obtaining in the superfamily as a whole, it would seem both convenient and logical to reason from the premise that primitive Diabroticites had nine pairs of autosomes and an *XX* : *XO* sex system. This, then, is proposed as a credible working hypothesis when consideration of the structural basis of the chromosome rearrangements, and the speciation they entailed, becomes feasible.

Of the eight genera investigated, *Diabrotica* exhibits the greatest cytological stability, all 32 known taxa retaining the hypothetical formula (but see John and Lewis, 1965, on *Eyprepocnemis*, regarding possible pitfalls in equating karyotypic similarities). The interspecific polymorphisms here recorded in six of the seven allied genera (table 4) conform particularly

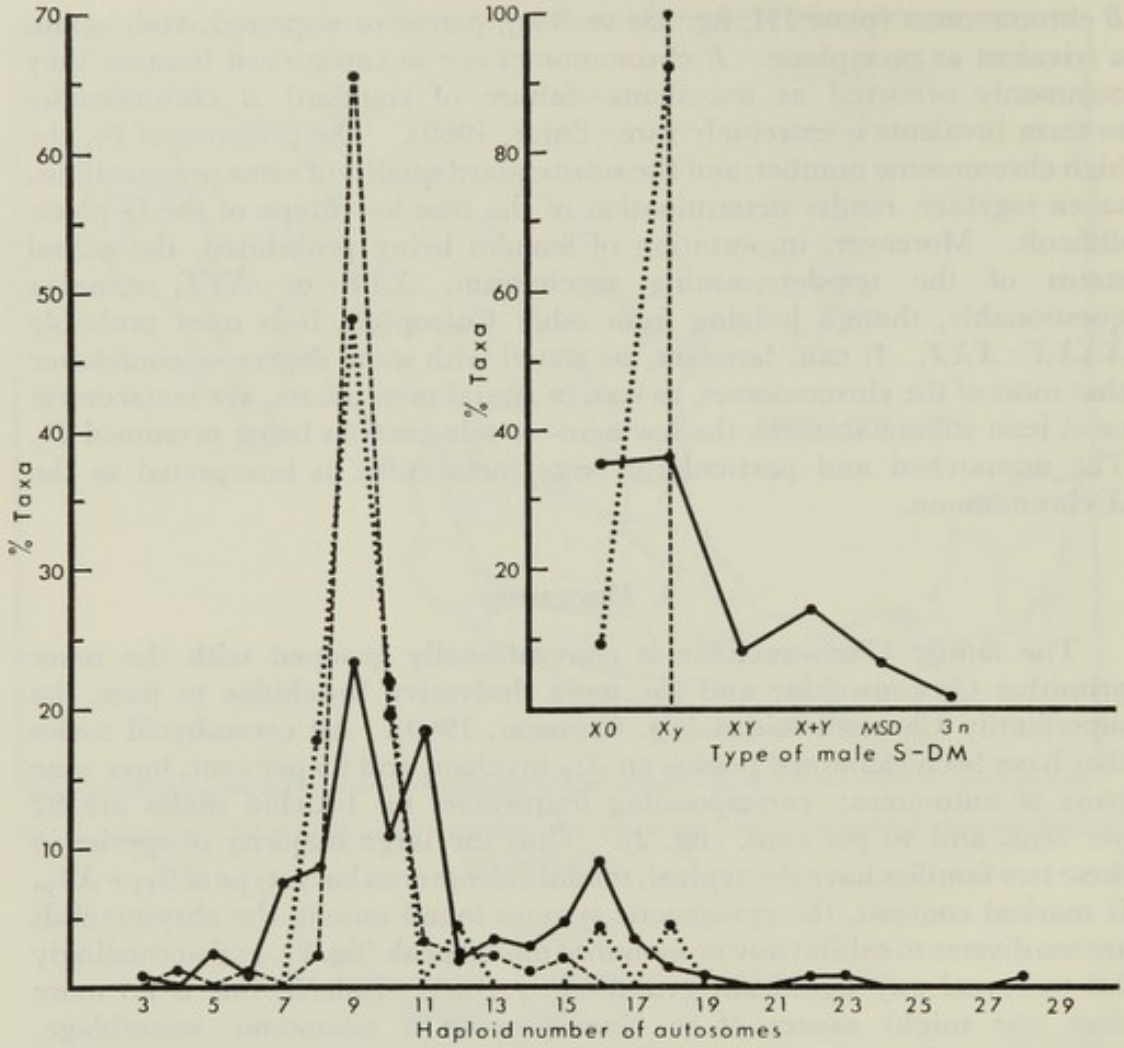


FIG. 2.—Graph showing the frequency distribution of taxa in the superfamily Chrysomeloidea by haploid number of autosomes and sex-determining mechanisms.——— = Chrysomelidae (N = 180); - - - - = Cerambycidae (N = 92); ..... = Bruchidae (N = 23).

TABLE 4

Cytotaxonomy of Diabroticites

Genus	Total recognised taxa*	No. of taxa studied with 2*n*								
		13	17	19	21	23	27	33	37	39?
Diabrotica	*c.* 420			32						
Paranapiacaba	*c.* 60			1		1				
Paratriarius	*c.* 60			1		1				
Acalymma	*c.* 75			3	3	2	1			1
Synbrotica	*c.* 200					1			1	
Cerotoma	?							2		
Amphelasma	13		2	1						
Gynandrobrotica	*c.* 46	1	3							
Total	*c.* 900	1	5	38	3	5	1	2	1	1

* According to Smith and Lawrence (1967).

well to White's (1964) principle of karyotypic orthoselection—note that no genus includes a species with more, and one with less, than 19 chromosomes. *Acalymma* and *Synbrotica*, each of which contains a species having an especially high chromosome number, about double the base number, pose the question

of whether polyploidy has played a role in numerical increase. Those cytologists who infer a casual relationship between polyploidy and multiple sex-determining mechanisms (*e.g.* Bauer, 1947; Goldschmidt, 1953; Ortiz, 1969) would almost certainly interpret the G phase of *A. trivittatum* as being a tetraploid form of the C phase. But it should be noted that (1) the finding of congeneric species with diploid numbers of 19, 21, 23, and 27 would more readily point to structural change of a reverse Robertsonian nature, were it not that acrocentric chromosomes seem to be absent from the standard *Diabrotica* karyotype; (2) no indication has been found of the presence in the basic complement of sizeable blocks of procentric heterochromatin, though donor chromosomes in the form of *B*s were seen in as many as 17 of the taxa (table 1); and (3) the unexpected failure to encounter neo-XY systems, of common occurrence elsewhere in the order; that these constitute a ready steppingstone to multiple formation has been established in *Chilocorus* (Smith, 1960*b*).

It is, however, by no means clear why, especially when the ancestors were unquestionably *XO*, a sex trivalent should be deemed indicative of, say, allotetraploidy: a simple *X-X* fusion would achieve an *XX* : *AA* balance in a single stroke, but it would call for a rare coincidence indeed, since the change is required to follow hybridisation with a minimum generation lag. Clearly, to arrive at a reasoned decision as to precisely how the G phase evolved is out of the question, hence this brief consideration is in no way intended to proselytise.

Although, in the present state of knowledge, it would be presumptuous to attempt a critical evaluation of the overall results of this study, there can be no denying that compelling substantiation is afforded the systematists' concept of a genus, at least regarding the seven of the 13 genera that have been erected since Dejean first described *Diabrotica* in 1837. What is more, trinomials, or subspecies, are obviously by no means trivial in the contemporary, non-Linnaean sense (*contra* John and Shaw, 1967). Without doubt, comparative cytology here again underlines Darlington's (1956) warning that until we know our subjects intimately, through purposeful research, we must use our names with diffidence.

5. Summary

1. Gonial chromosome numbers and/or MI karyotypes were determined for males of 54 New World diabroticite taxa representing 8 genera: 29 *Diabrotica* ($2n = 19$); 2 *Cerotoma* ($2n = 33$); 2 *Paranapiacaba* and 2 *Paratriarius* ($2n = 19$ and 23); 4 *Gynandrobrotica* ($2n = 13$ and 17); 2 *Synbrotica* ($2n = 23$ and 37); and 10 *Acalymma* ($2n = 19$, 21, 23, 27, and *c.* 39).

2. *B*-chromosome carriers were observed in 9 *Diabrotica*, 1 *Cerotoma*, 1 *Amphelasma*, 2 *Gynandrobrotica*, and 4 *Acalymma* taxa.

3. Colchicine-treated standard gonial chromosomes only rarely appear to be other than meta- or submeta-centric; *B*s, acro- or telo-centric.

4. Males are *XO* in 53 of the taxa, the *X* like the *B*s being precessional and prereductional.

5. *Acalymma trivittatum* comprises two distinct cytotypes, the widespread " Chiapas " phase, with $10_{II}+X$, and the restricted " Guerrero " phase, with *c.* $18_{II}+1_{III}$. Because females were not available, the status of the " G " phase sex system, *XXY* vs. *XYY*, is unknown.

6. The concurrence of a high diploid number and a sex multiple may imply tetraploidy, but reverse Robertsonian structural change as the cause is equally tenable.

7. Since only 57 of the near-900 Diabroticites listed by systematists have been investigated cytologically, speculation regarding the structural basis of their extensive karyotypic versatility would obviously be premature, but $9_{II}+X$ is proposed as the working hypothesis for the future.

Acknowledgments.—Thanks are extended to the following university personnel for provision of material: Dr J. W. Apple, Wisconsin; Dr W. V. Campbell, North Carolina State; Dr H. C. Chiang, Minnesota; Dr G. E. Guyer, Michigan State; Dr R. E. Hill, Nebraska; Dr H. C. Knutson, Kansas State; Dr F. L. McEwen, Cornell; Dr L. D. Newsom, Louisiana State; Dr J. D. Paschke, Purdue; Dr D. C. Peters, Iowa State; Dr J. B. Polivka, Ohio State; and also to Dr J. B. Kring, The Connecticut Agricultural Experiment Station, New Haven; Dr B. W. George, United States Department of Agriculture, Brookings, D.C.; and Dr R. E. Sechriest, Illinois Natural History Survey, Urbana. Above all, I am deeply indebted to Dr R. F. Smith for the invitation to participate in this project, for provision of materials, and for soliciting the eastern accessions. T. J. Ennis, M.Sc., shared in the preparation of slides and, with J. A. MacDonald, B.A., who prepared figs. 1 and 2, was responsible for the photography.

6. References

BAUER, H. 1947. Karyologische Notizen. I. Über generative Polyploidie. *Z. Naturf.*, 2*b*, 63-66.

CROWSON, R. A. 1960. The phylogeny of Coleoptera. *Ann. Rev. Entom.*, *5*, 111-134.

DARLINGTON, C. D. 1956. *Chromosome Botany*. Allen and Unwin, London.

ENNIS, T. J. 1969. Meiosis in *Diabrotica* (Chrysomelidae, Coleoptera): Chiasma frequency and variation. M.Sc. thesis, University of Toronto.

GOLDSCHMIDT, E. 1953. Multiple sex-chromosome mechanisms and polyploidy in animals. *J. Genet.*, *51*, 434-440.

JOHN, B., AND LEWIS, K. R. 1965. Genetic speciation in the grasshopper *Eyprepocnemis plorans*. *Chromosoma*, *16*, 308-344.

JOHN, B., AND SHAW, D. D. 1967. Karyotype variation in dermestid beetles. *Chromosoma*, *20*, 371-385.

ORTIZ, E. 1969. Chromosomes and meiosis in Dermaptera. *Chromosomes Today* (ed. C. D. Darlington and K. R. Lewis), *2*, 33-40.

SMITH, R. F., AND LAWRENCE, J. F. 1967. Clarification of the status of type specimens of Diabroticites (Coleoptera, Chrysomelidae, Galerucinae). *University of California Publ. Entom.*, *45*, 145-154.

SMITH, S. G. 1953. Chromosome numbers of Coleoptera. *Heredity*, *7*, 31-48.

SMITH, S. G. 1960*a*. Chromosome numbers of Coleoptera. II. *Canad. J. Genet. Cytol.*, *2*, 67-88.

SMITH, S. G. 1960*b*. Cytogenetics of insects. *Ann. Rev. Entom.*, *5*, 69-84.

SMITH, S. G., AND TAKENOUCHI, Y. 1969. Chromosomal polymorphism in *Pissodes* weevils: Further on incompatibility in *P. terminalis*. *Canad. J. Genet. Cytol.*, *11*, 761-782.

VIRKKI, N. 1964. On the cytology of some neotropical chrysomelids (Coleoptera). *Ann. Acad. Sci. Fenn. IV.*, *75*, 1-30.

WHITE, M. J. D. 1964. Principles of karyotype evolution in animals. *Proc. XIth Int. Cong. Genet.*, 391-397. Pergamon Press, London.

7. Addendum

The following is a précis of notes on the phases of *A. trivittatum* and *G. nigrofasciata* that were kindly provided by Douglas Munroe, Department of Entomology and Parasitology, University of California, Berkeley.

A. trivittatum. The Chiapas phase occurs throughout Mexico and south to Panama. The Guerrero phase is common in western drainage systems

of the Sierra Madre Occidental from Sonora south to Costa Rica and in the eastern lowlands of Veracruz. Throughout its range, it appears to be sympatric with the Chiapas phase, although there is some evidence of ecological (microgeographic) isolation. The genitalia of the two phases are distinct.

G. nigrofasciata. The Veracruz phase is common in Chiapas, Oaxaca, Veracruz, and San Luis Potosi, and also occurs in Panama. In addition to the three " cytological cadavers ", the Oaxaca phase is represented in the Berkeley reference collection by only two specimens, from Michoacan, Mexico. The two phases show slight but distinct genitalic differences.

THE STRUCTURE AND ROLE OF B-CHROMOSOMES IN THE MOTTLED GRASSHOPPER

GODFREY HEWITT
School of Biological Sciences, University of East Anglia, Norwich, G.B.

1. Introduction

There have been many reports of B-chromosomes. For example, White (1954) in a comprehensive survey of 250 species of grasshoppers found Bs in 29 of them. This is a low estimate since it is necessary to examine many populations to adequately describe the chromosomal variation of a species. Despite this abundance, in only a few cases has their significance been pursued further. Where the B-chromosome systems have been studied closely a number of interesting features have been described. For example the B-chromosomes of maize possess an accumulation mechanism in which both the centromere and distal heterochromatin are involved in producing non-disjunction at the second pollen mitosis (Carlson, 1969). Several other organisms tend to accumulate supernumeraries by a variety of mechanisms (Nur, 1969*a*). Secondly, whilst in rye, for example, the Bs appear to have no genes with major effects, they do cause reductions in fertility, germination and flowering; at the cell level they effect the frequency and distribution of chiasmata as well as the composition of interphase nuclei (Jones and Rees, 1969). Thirdly, in coccids—and several other organisms—there is evidence that B chromosomes are harmful leading to a lower fitness in their carriers (Nur, 1969*b*).

However, while there are some similarities between supernumerary systems the most impressive feature is their extreme variety, in size, shape, number, stability, meiotic behaviour, phenotypic effects and inheritance from generation to generation. It is not surprising therefore that different authors have given very different interpretations of the role of B-chromosomes. Each individual system requires a study in depth of its functioning at the population and biochemical levels as well as of its cytogenetics.

2. The B-system of *Myrmeleotettix*

Certain populations of the mottled grasshopper, *Myrmeleotettix maculatus* (Thunb.), in Britain possess a large B chromosome which is usually a mediocentric isochromosome (B^m). Some southern populations also have a submetacentric supernumerary (B^{sm}) which is a pericentric derivative of the B^m chromosome and both form chiasmata between their two sister arms as well as between each other (John and Hewitt, 1965*a*).

Individuals may contain from zero to three to these B-chromosomes, however 2B individuals are infrequent and a 3B individual is a rarity. They are mitotically quite stable in the germ line of an individual. Other tissues such as midgut diverticula and ovariole wall, as well as whole embryos, show this stability and this indicates that all cells in an individual possess the same number of supernumeraries (John and Hewitt, 1965*b*).

The B-chromosome behaves randomly through meiosis in the male,

and this coupled with the mitotic stability denies any possibility of an accumulation mechanism in the production of the male gametes. The fact that the males and females in a population have the same B-chromosome frequency, and that 2B individuals are infrequent suggest that no accumulation of supernumeraries is occurring in the female gametes either; but this requires direct testing despite its technical difficulty. It is a very different situation from the accumulation systems which have been nicely demonstrated in two grasshoppers, *Camnula pellucida* and *Locusta migratoria*, where unstable Bs undergo non-disjunction in the sperm line mitoses to increase the frequency of Bs in the male gametes by 37 per cent. (Nur, 1969*a*).

The chiasma frequency and its variance in individuals containing B-chromosomes is significantly higher than in the non B members of the same population. This has been found in all the populations examined and represents an increase in the recombination potential of the individual and of the population (Hewitt and John, 1967). Similar effects have been reported for maize (Ayonoadu and Rees, 1968), rye (Zecevic and Paunovic, 1969) and recently in another grasshopper *Melanoplus differentialis* (Abdel-Hameed *et al.*, 1970). However, such effects are not a property of all B-chromosomes in grasshoppers.

So far no exophenotypic effects of the B-chromosomes have been observed in *Myrmeleotettix* (Hewitt and John, 1970). This does not mean that there are no such effects, but it does suggest that the function of the B-chromosomes is physiologic and endophenotypic, affecting cellular functions such as chiasma formation.

3. Distribution of B-chromosomes in Britain

The strategies of marginal and central populations for the release of variability are quite different and the favoured genotypes in the two situations will also differ.

The South of England provides many environments favourable for this grasshopper, having a relatively warm and dry climate; while the North of Britain is at the margins of the species distribution and populations are only found in places less cold and wet than the rest of the area. Because of their effect on variability it is important to define the distribution of supernumeraries in populations *Myrmeleotettix*.

The general situation to date (fig. 1) is striking in that B-chromosomes are present in the southern half of Great Britain only, more northerly and thus more marginal populations being devoid of supernumeraries. There are three southern areas, however, in which some populations do not possess supernumeraries; these are in Wales, East Anglia and the South West peninsula.

The area inland from Aberystwyth varies from a warm, dry coastal plain, eastward through the cold, wet mountains to the warmer and drier Marches, providing a wide range of conditions in a small area. The B-chromosome frequency mirrors this climatic variation, decreasing from around 40 per cent. of individuals with Bs near the coast as one progresses into the mountains and conditions marginal for the grasshopper, until the B-chromosome vanishes, to reappear on the East side. Indeed the species disappears in the higher and wetter mountains. Variations in the B-frequencies in some 16 populations have been insignificant since 1965, and the

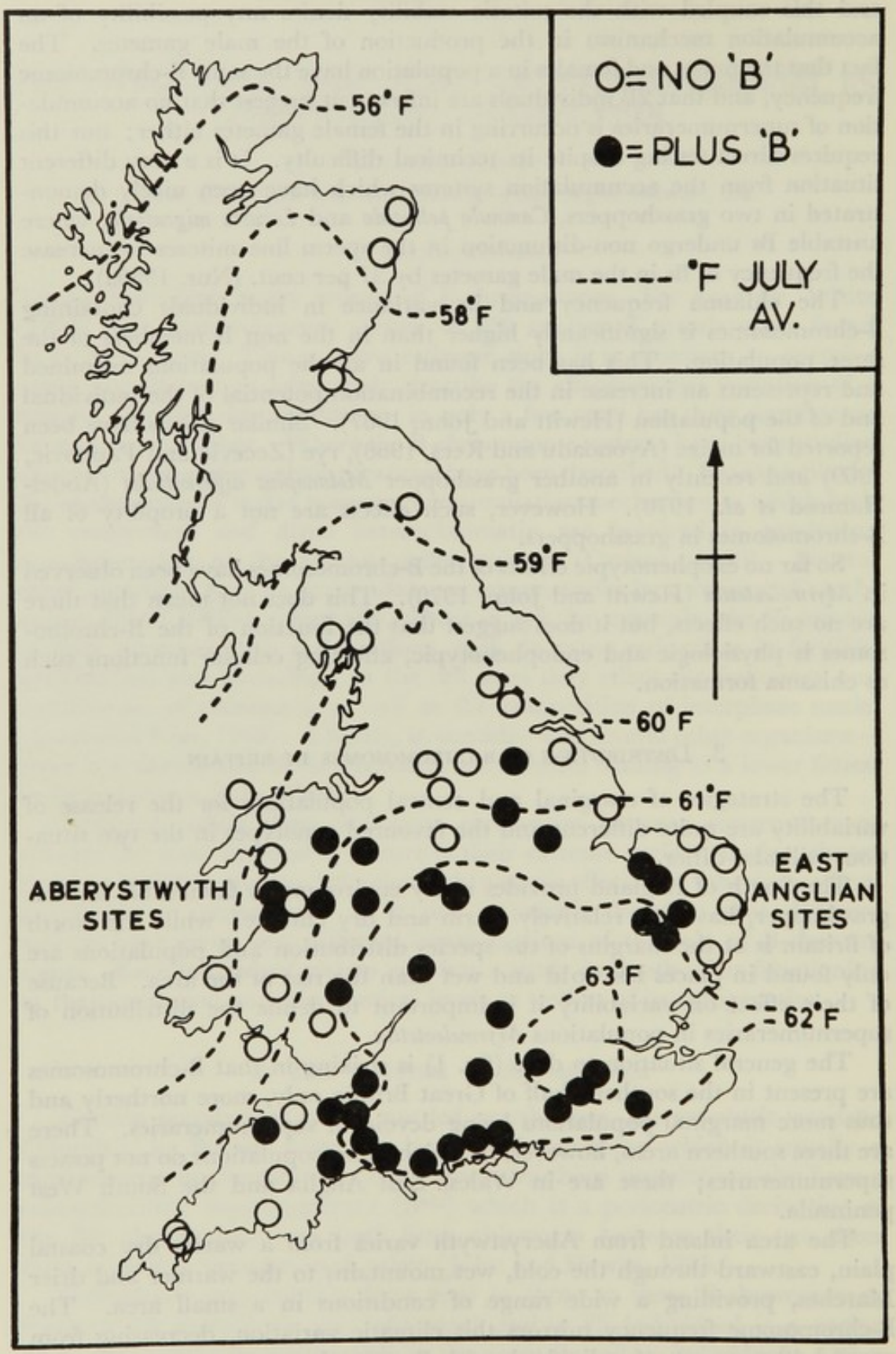


FIG. 1.—The distribution of B-chromosomes in populations of *Myrmeleotettix maculatus* in Great Britain.

frequencies themselves correlate well with the gross climate (*e.g.* rainfall) of the location (Hewitt and John, 1970).

The climate in East Anglia is quite different, being more continental; rainfall is low and even over the whole region (22″-27″ p.a.) and so is the altitude! The B-chromosomes are absent from populations in the North and East of the region, but present in the west of the region which adjoins the Midland Plain. This distribution follows closely the summer temperatures in the region, since the north and east of East Anglia are subject to a cool maritime influence (Hewitt and Brown, 1970).

Both the Aberystwyth and East Anglian sites demonstrate the close relationship between the B-frequency and the favourability of the environment to the species, in one area rainfall is the main factor in the other it is summer temperatures. It is noteworthy that the supernumeraries of some plant species, *Festuca pratensis*, *Secale cereale*, *Centaurea scabiosa* and *Phelum phleoides*, are prevalent in areas favourable to each species (*e.g.* Bösemark, 1967). Therefore, any increase in variability which the Bs bring with them is for the central populations in favourable locations, and is not available in the more marginal populations under more stringent conditions.

Another interesting feature emerges from the East Anglian investigation, and that is the very short distance over which the B-chromosome frequency changes from zero per cent. to the 40 per cent. which occurs in most populations in southern Britain. On the Breckland it is as little as five miles. There are a number of possibilities which may explain this relatively steep cline, including, (1) a sharp undetected microclimate gradient, (2) stabilising selection for 1B individuals above a certain threshold of environmental favourability, (3) limited B-chromosome flow, because of low insect dispersal or incompatibility of the B-containing migrant and the resident OB individuals.

4. The Goginan population

Most interestingly, further examination of the Welsh sites has revealed a similar sharp cline five miles inland from Aberystwyth at the head of the Melindwr valley in the area of Goginan (fig. 2). The spoil from nineteenth century mining of a heavy metal seam provides suitable environments for *Myrmeleotettix* from the valley bottom at 300 ft. up to more bleak conditions near 1000 ft. The main Goginan spoil heap runs from A to B on fig. 2 and is shown in cross section in fig. 3; this has *Myrmeleotettix* all over it with many local concentrations of grasshoppers. A location at the bottom (Goginan Isaf) and one near the top (Goginan Uchaf) of this mine had been sampled earlier in 1965, 1966 and 1967, along with one (Bwlch site D, fig. 2) higher up the seam in 1966 and 1967. These three populations show that there is a sharp drop in B-frequency up the Goginan mine, particularly from Goginan Isaf (27·2 per cent. of individuals have Bs) to Goginan Uchaf (13·1 per cent.). The populations in the mountains above Goginan, including Bwlch, have an average 5·7 per cent. of their individuals with Bs, those around and below Goginan in the valley and coastal plain have between 30 and 60 per cent. Bs, on average 41·3 per cent. Consequently this mine was sampled further in six areas in 1968 and 1969 (see fig. 3 for locations) along with the Bwlch population.

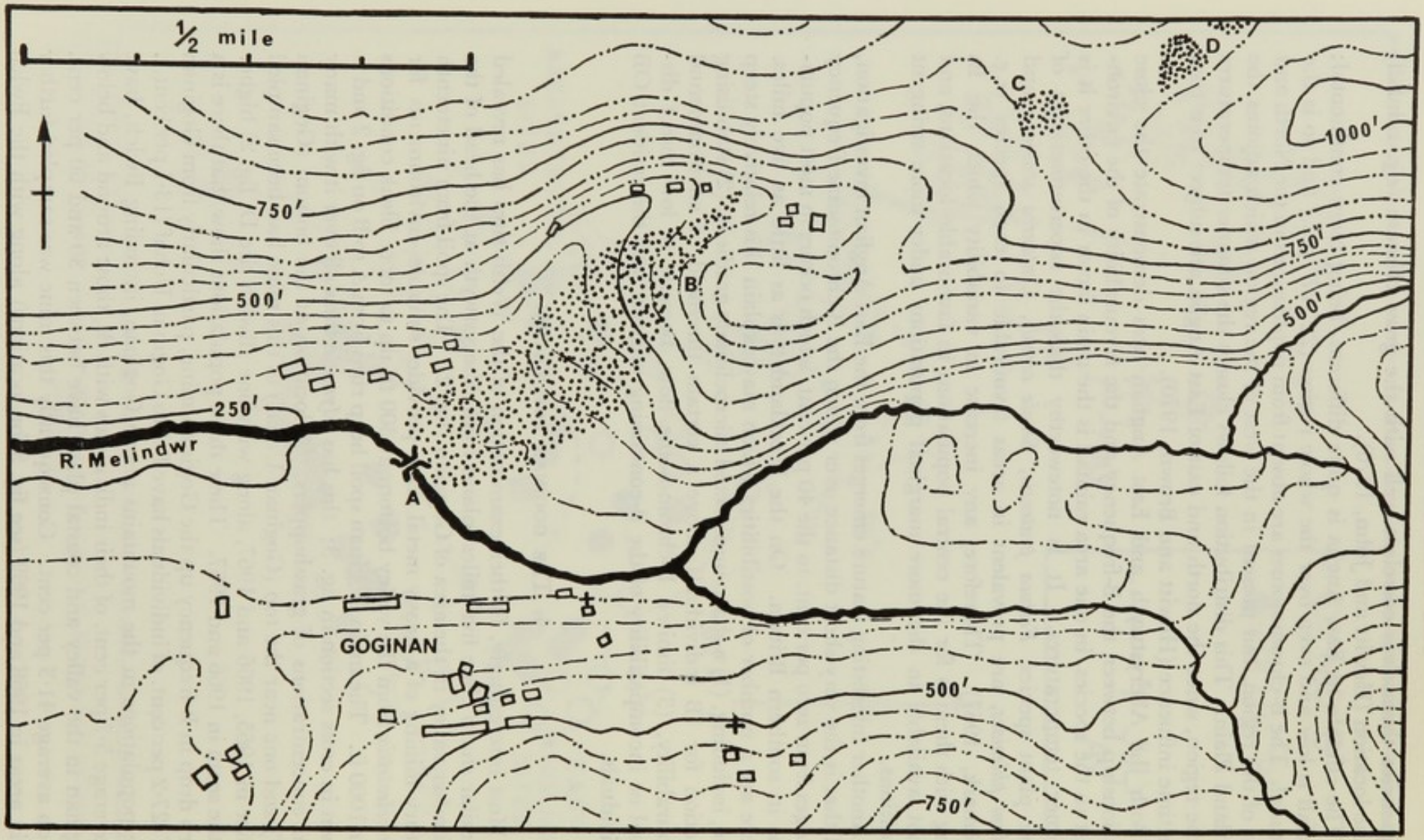


Fig. 2.—A map of the Goginan District and Melindwr Valley showing the topography and extent of the mine area (stippled). A to B indicates the line of the transect shown in Fig. 3, C is the Twyll mawr site, and D is the Bwlch site.

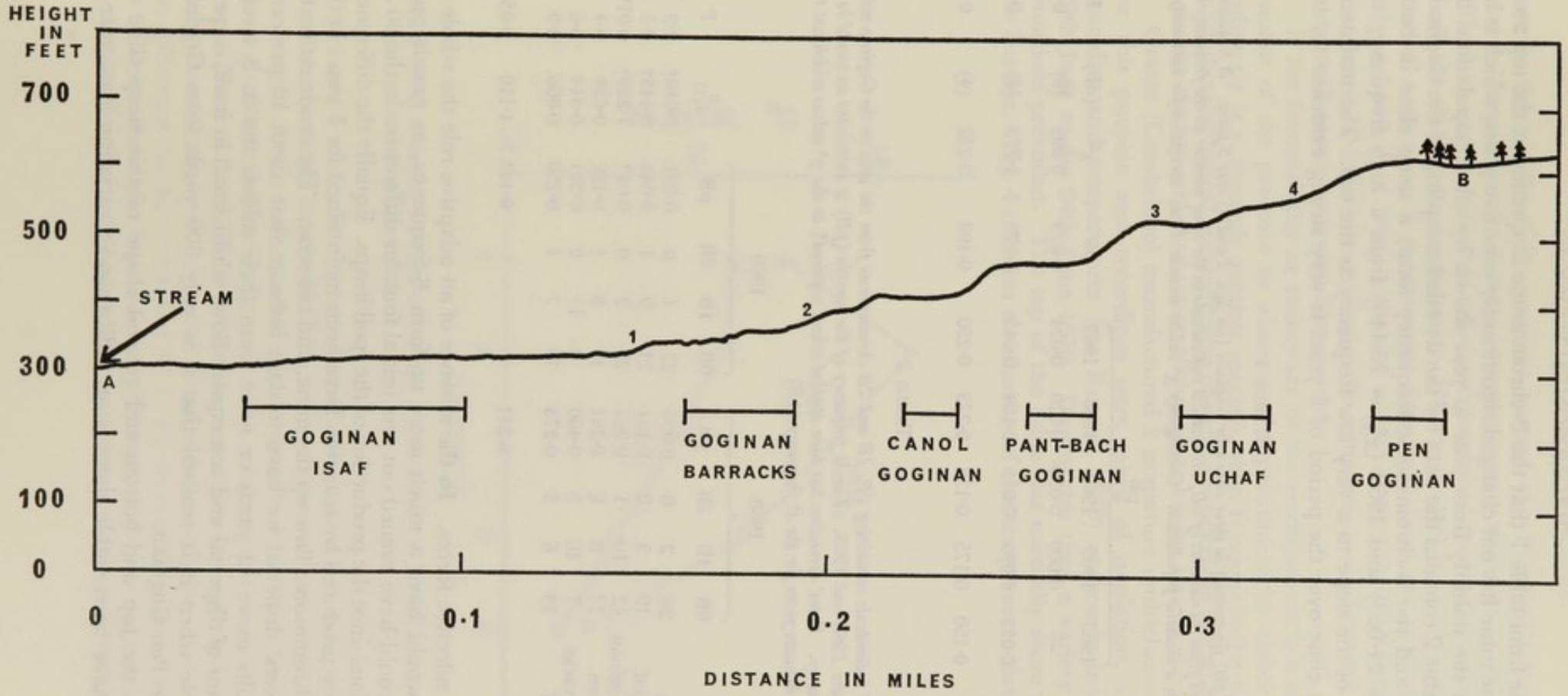


FIG. 3.—A section of the Goginan mine from A to B on fig. 2 with both axes to same scale and showing the locations of the sampling sites on the mine. Figs. 1, 2, 3 and 4 refer to the positioning of the four sets of micrometeorological recording equipment.

It is clear from table 1 that the B-chromosome frequency at the top and bottom of the mine has not changed significantly over five years, which is in accord with the stability from year to year shown by other populations in Britain. Table 2 contains the data for the detailed sampling of the Goginan mine itself, and the B-chromosome frequency forms a steep cline in both 1968 ($\chi^2_{(5)} = 21{\cdot}003$) and 1969 ($\chi^2_{(5)} = 26{\cdot}446$) from a high frequency at the bottom of the slope to a very low frequency at the top. The maintenance of this cline over the period of 5 years is very strong evidence for its

TABLE 1

B-chromosome (pB) frequencies in three sites near Goginan (see figs. 2 and 3) over 5 years. B frequency is calculated from the numbers of 0B, 1B and 2B individuals in the same manner as the frequency of an allele in a diallelic population. Contingency χ^2 values include Yates' correction for continuity

	pB								
Site	1965	1966	1967	1968	1969	pB	χ^2	(dF)	P
Bwlch	—	0·000	0·050	0·020	0·024	0·023	0·899	(3)	0·8
Goginan Uchaf	0·025	0·095	0·075	0·104	0·045	0·070	1·278	(4)	0·9
Goginan Isaf	0·150	0·175	0·132	0·273	0·250	0·198	5·232	(4)	0·3

TABLE 2

The number of individuals containing 0B, 1B and 2B chromosomes from six sites on the Goginan mine for the years 1968 and 1969. The B frequency of the sample (pB) is calculated as would be a gene frequency. Yates' correction has been applied where required† to the χ^2 values which test the difference between years for the B frequency (pB)

	1968				1969					
Site	0B	1B	2B	pB	0B	1B	2B	pB	$\chi^2_{(1)}$	P
Pen Goginan	26	2	0	0·036	23	1	0	0·021	0·018†	0·9
Goginan Uchaf	19	5	0	0·101	21	0	1	0·045	0·445†	0·5
Pant-bach Goginan	12	10	1	0·261	22	2	0	0·042	7·259†	0·01**
Canol Goginan	13	8	2	0·261	17	8	1	0·192	0·659	0·4
Goginan Barracks	7	10	3	0·400	9	11	2	0·341	0·314	0·6
Goginan Isaf	13	6	3	0·273	10	7	1	0·250	0·006	0·9
Total				0·211				0·145	4·110	0·05*

control by selective forces. In the absence of an adaptive role the whole of the mine would have a much more uniform B-frequency, as grasshopper dispersal would have evened out any initial founder differences in the 90 or so generations since the production of the spoil heaps. Equally the differences in frequency produced by selection have been maintained for 5 years in the face of B-chromosome flow up the mine, and *vice-versa*. The crude estimates of this insects' dispersal we have to date indicate that about 10 per cent. of the adults move 12 yards or more from their release site in 2 weeks. This amount of dispersal and consequent flow, whilst small in itself, is quite considerable when it is realised that it is only 300 yards from Goginan Barracks to Pen Goginan.

Whilst the top and bottom and general shape of this steep cline on Goginan have been stable there is an indication of variation from year to

year in the middle of the gradient around the Pant-bach locality. This may be just chance or it may be a response to different selective forces in different years, the effects of which are magnified on this steep cline. Indeed, if all the Goginan material from 1968 and 1969 is considered there is a hint of a drop in B chromosome frequency from one year to the next ($\chi^2_{(1)} = 4{\cdot}110$, P = 0·05) over the whole mine. It is obviously vital to continue such detailed sampling of this mine area to confirm and understand such small season to season fluctuations.

If the favourability or stringency of the environment is a major determinant of the presence or absence of supernumeraries either directly or indirectly (*e.g.* through another species), then it ought to be possible with micrometeorological techniques to observe considerable climatic differences between the top and the bottom of the mine at some time in the year.

Grants (Cambridge) manufactured 2 miniature multichannel recorders for this purpose and recordings were taken of insolation, wind speed, humidity and temperatures (see Ruscoe, 1970) at hourly intervals from 20th June to the 24th June 1970. During this time a variety of weather situations prevailed. The top of the mine was normally more windy than the bottom. Fig. 4 contains a summary of the temperature data from sites

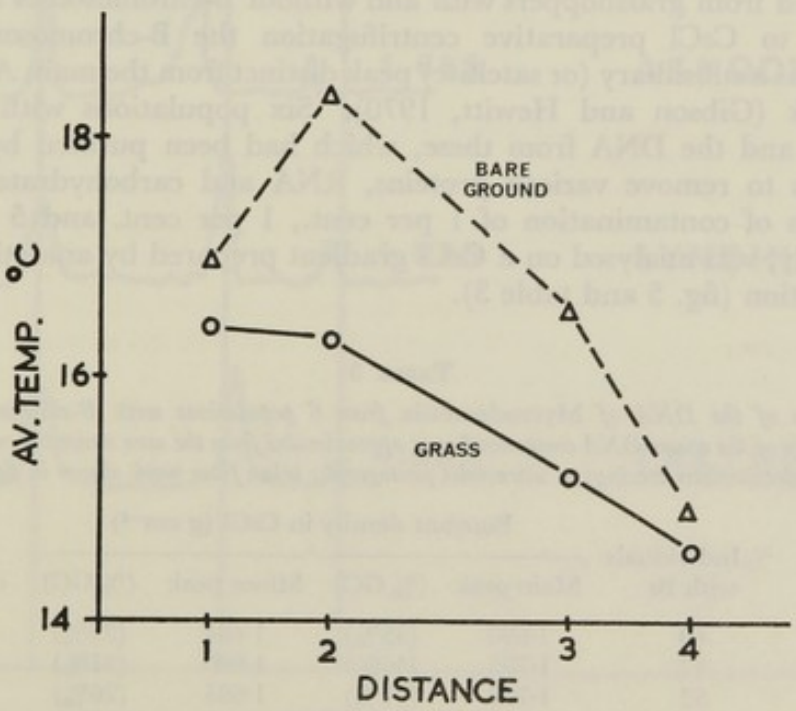


FIG. 4.—The difference between sites 1, 2, 3 and 4 (see fig. 3) for the average temperature from 20th to 24th June 1970.

1, 2, 3 and 4 on the bare ground and in the grass, expressed as an average of all the readings obtained in the 4 days of recording. It is obvious that the bottom of the slope near Goginan Barracks is warmer than the top of the slope towards Pen Goginan in both microenvironments. This higher temperature at the bottom of the slope is observed under all the weather conditions encountered and at all times of day and night. On a sunny day the difference can be as much as 10° C. for short periods and averages about 2° C. throughout the day. During the night a 2 to 3 degree difference is maintained between top and bottom and also through a rainy or cloudy

day. Consequently during the summer months, when the grasshoppers are hatching mating and laying next year's eggs, there will be an accumulative effect of higher temperatures at the bottom of the mine which will promote grasshopper development in the Barracks locality ahead of that at Goginan Uchaf. Such a gradient of local climate favours B-containing individuals at the bottom and eliminates them at the top. It may do this directly by differentiating between the physiology and/or behaviour of the 0B and B containing individuals. It may possibly be more indirect acting primarily on the distribution of food or shelter plants or on the behaviour of a predator; in either case the 0B and B individuals must be selectively distinguished. The climatic gradient could allow a longer growing season at the bottom and also greater population growth, and the variability produced by the Bs may be of value in such conditions.

5. The DNA of the B-chromosome

With this information at the chromosome and population levels an investigation of the structure and function of the B-chromosome at a molecular level will lead to a more complete understanding of its role. DNA was isolated from grasshoppers with and without B-chromosomes and when subjected to CsCl preparative centrifugation the B-chromosome DNA appeared as a subsidiary (or satellite) peak distinct from the main A-chromosome peak (Gibson and Hewitt, 1970). Six populations with Bs were collected, and the DNA from these, which had been purified by enzyme treatments to remove various proteins, RNA and carbohydrates to give final levels of contamination of 1 per cent., 1 per cent. and 5 per cent. respectively, was analysed on a CsCl gradient prepared by analytical ultracentrifugation (fig. 5 and table 3).

Table 3

Characteristics of the DNA of Myrmeleotettix *from 6 populations with B-chromosomes. The percentage of the minor DNA component* was approximated from the area under the curves obtained by microdensitometer tracings of ultraviolet photographs taken from work shown in fig. 4*

Source of DNA	% Individuals with Bs	Buoyant density in CsCl (g cm^{-3})				% Minor component*
		Main peak	(% GC)	Minor peak	(% GC)	
Talybont	69	1·698	(39%)	1·684	(25%)	5
Foxhole	47	1·705	(46%)	1·690	(31%)	6
Lyndhurst	52	1·700	(41%)	1·685	(26%)	5
Ashurst	40	1·700	(41%)	1·694	(35%)	4
Midford	32	1·700	(41%)	1·692	(33%)	4
Ashdowne	38	1·708	(49%)	1·688	(29%)	5

There is a major and minor peak in each corresponding with A and B chromosomes respectively. It can be seen from table 3 that there is variation between the main A chromosome peaks; three are very similar to each other and the other three are somewhat different from these and from each other. All six of the B-chromosome DNA samples appear to be different from each other. Of particular interest is the difference in base composition of B-chromosome DNA from different populations calculated from these gradients, they are of the same order of magnitude as those between pro-

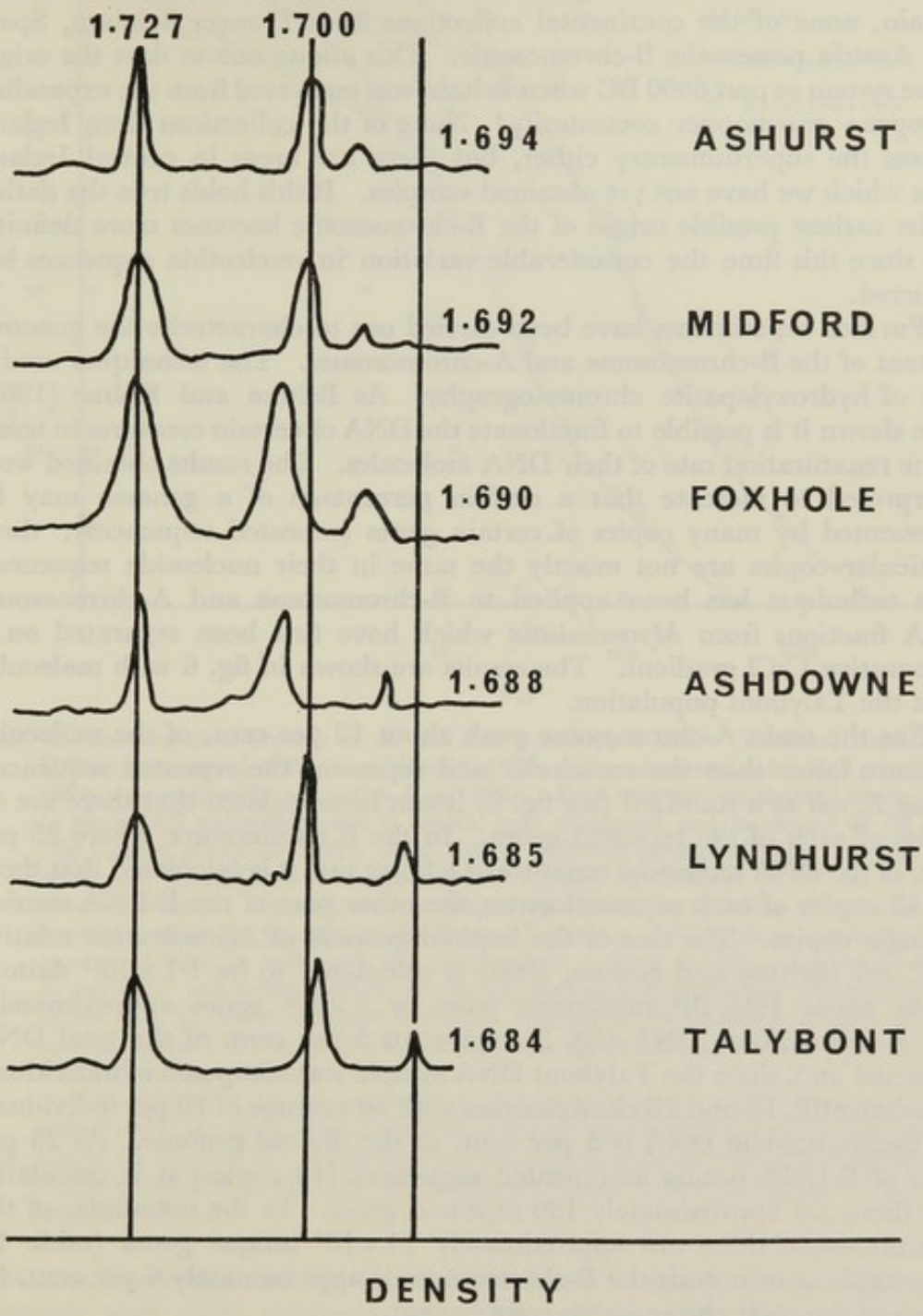


FIG. 5.—2 μg of the DNA samples and 1 μg of *M. luteus* marker DNA (density 1·727 g/Cm³) were centrifuged to equilibrium in a CsCl gradient at 42,040 r.p.m. at 25° C. for 20 hours in a Model E ultracentrifuge. The buoyant density and per cent. GC of the DNA was determined by the method described by Schildkraut, Marmur and Doty.

tozoons, for example, and greater than that within the Arthropods or Chordates (McCarthy, 1969).

Different species of *Apodemus* which have diverged in the recent evolution of this genus (5×10^7 years?) have satellite DNAs which vary from 31 to 35 per cent. G-C (Hennig and Walker, 1970). The considerable differences in B chromosome G-C contents (25 to 35 per cent.) take on added significance

when it is realised that the supernumerary has been found only in Southern Britain, none of the continental collections from France, Belgium, Spain and Austria possess the B-chromosome. This allows one to date the origin of the system as post 6000 BC when Britain was separated from the expanding European grasshopper community! None of the collections from Ireland possess the supernumerary either, but there are areas in central Ireland from which we have not yet obtained samples. If this holds true the dating of the earliest possible origin of the B-chromosome becomes more definite, and since this time the considerable variation in nucleotide sequences has occurred.

Further experiments have been carried out to characterise the genomic content of the B-chromosome and A-chromosomes. The techniques used is that of hydroxylapatite chromatography. As Britten and Kohne (1968) have shown it is possible to fractionate the DNA of certain creatures in terms of the renaturation rate of their DNA molecules. The results obtained were interpreted to indicate that a certain percentage of a genome may be represented by many copies of certain genes (repeated sequences): these particular copies are not exactly the same in their nucleotide sequences. This technique has been applied to B-chromosome and A-chromosome DNA fractions from *Myrmeleotettix* which have first been separated on a preparative CsCl gradient. The results are shown in fig. 6 with molecules from the Talybont population.

For the main A-chromosome peak about 10 per cent. of the molecules renature faster than the remainder and represent the repeated sequences. Using *E. coli* as a standard (see fig. 6) it can be calculated that there are 43 copies of each of the repeated genes. In the B chromosome where 25 per cent. of the DNA molecules renature at a faster rate it is calculated that there are 43 copies of each repeated genes, the other part of the B-DNA consists of single copies. The size of the haploid genome of *Myrmeleotettix* relative to *E. coli* (Britten and Kohne, 1968) is calculated to be $1{\cdot}1 \times 10^{11}$ daltons which equal $1{\cdot}8 \times 10^{8}$ nucleotide pairs or 2×10^{5} genes approximately. The B-chromosome DNA (fig. 5) represents 5 per cent. of the total DNA extracted and, since the Talybont DNA sample was composed of individuals containing 0B, 1B and 2B chromosomes with an average of 1B per individual, the B-chromosome DNA is 5 per cent. of the diploid genome. As 25 per cent. of B-DNA occurs as repeated sequences (43 copies) it is calculated that there are approximately 130 repeated genes. In the remainder of the B-chromosome there are approximately 14×10^{3} unique genes (table 4). At metaphase of mitosis the B-chromosome is approximately 6 per cent. by length of the total chromosome complement.

These results indicate that there are both repeated and unique sequences of DNA in the B-chromosome of *Myrmeleotettix maculatus*. The functions of repeated sequences are at present speculative, and those of the guinea-pig α-satellite, mouse satellite and crab A-T satellite do not appear capable of coding for active protein (Southern, 1970). On the other hand it has been concluded from *in vitro* DNA/RNA hybridisation experiments that repeated sequences are capable of synthesising RNA (Britten and Kohne, 1968; Robertson *et al.*, 1969). It has been suggested that these sequences may be important in chromosome pairing, centromeric function and gene regulation in eukaryotic organisms (*e.g.* Walker *et al.*, 1969; Britten and Davidson, 1969). In the case of *Myrmeleotettix maculatus* they may effect

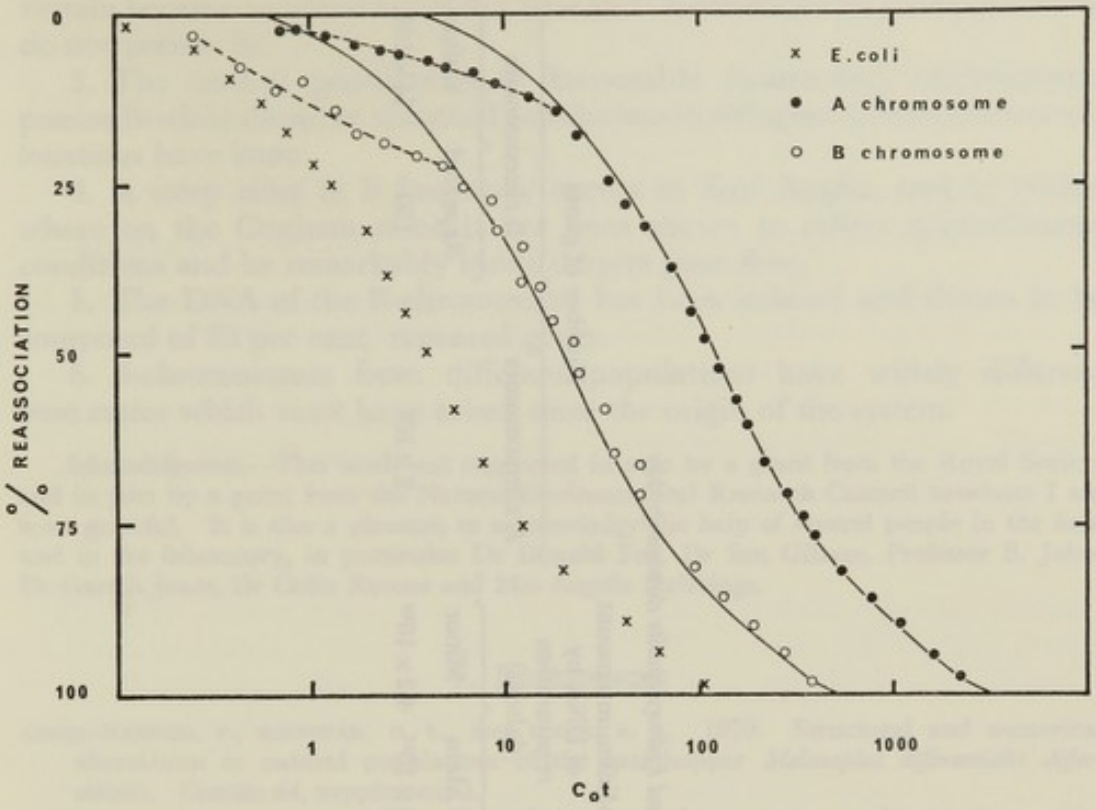


FIG. 6.—Renaturation kinetics of *E. coli* and grasshopper DNA (Talybont population). DNA was prepared (Gibson and Hewitt, 1970) and a mixture of unlabelled grasshopper DNA was made with radioactive *E. coli* DNA. Each mixture was sheared by sonication to pieces 500 nucleotide pairs in length, heat denatured and incubated at 60 °C. in 12M sodium phosphate buffer pH = 6·8 for different periods of time. Samples were taken and adsorbed to a hydroxylapatite column equilibrated at 60° C. in 0·01 M phosphate buffer. The column was washed with 3 volumes of 0·01 M phosphate buffer, 6 volumes of 0·14 M phosphate 3 buffer and 5 volumes of 0·5 M phosphate buffer. The amount of DNA in each wash was estimated by reading at OD_{262} and the amount of *E. coli* DNA was determined by measuring radioactivity following precipitation with 10 per cent. TCA and collection on filters. Denatured DNA elutes in the 0·14 M washes and renatured DNA in the 0·5 M washes. The fraction of each sample that renatures is plotted on the ordinate.

The solid lines on the grasshopper plots represents the renaturation curve for a second-order reaction. The dashed lines are drawn through fast renaturing samples. Cot is an acronym for the product of the concentration of original denatured DNA molecules (Molarity of nucleotides) and length of time in seconds during which reaction has taken place.

chiasma frequency and have a cellular function which is advantageous in warmer and drier environments. Interestingly, the B-chromosome is heterochromatic, and the satellite DNAs of mouse and guinea-pig have recently been shown to reside in the heterochromatin fraction of the genome (Yunis and Yasmineh, 1970, see also p. 47).

6. SUMMARY

1. The large B-chromosome of *Myrmeleotettix maculatus* is stable and apparently has no accumulation mechanism. Its presence is associated with an increase in chiasma frequency, the only effect that has been observed to date.

2. It is probable that the B-chromosome arose after 6000 BC since

TABLE 4

Analysis of the A and B DNA components by renaturation and hydroxylapatite chromatography

	Cot $\frac{1}{2}$				Multiplicity		Minimum amount of DNA by renaturation (daltons)		Number of genes		
	Main		Minor		Average number repeated sequences				A chromosomes	B chromosomes	
Source	Repeated	Unique	Repeated	Unique	Main	Minor	Main	Minor		Repeated	Unique
Talybont	4	170	1·5	65	43	43	$1{\cdot}1 \times 10^{11}$	$4{\cdot}2 \times 10^{10}$	2×10^{5}	130	14×10^{3}

Britain became an island about this time and Continental and Irish population do not possess Bs.

3. The central populations in favourable (warm-dry) environments possess Bs while the more marginal populations in stringent (colder and wetter) locations have none.

4. A steep cline in B frequency occurs in East Anglia, and in Wales, where on the Goginan mine it has been shown to reflect microclimatic conditions and be remarkably stable despite gene flow.

5. The DNA of the B-chromosome has been isolated and shown to be composed of 25 per cent. repeated genes.

6. B-chromosomes from different populations have widely different base ratios which must have arisen since the origin of the system.

Acknowledgments.—This work was supported in part by a grant from the Royal Society and in part by a grant from the Natural Environmental Research Council to whom I am most grateful. It is also a pleasure to acknowledge the help of several people in the field and in the laboratory, in particular Dr Donald Fox, Dr Ian Gibson, Professor B. John, Dr Gareth Jones, Dr Colin Ruscoe and Mrs Angela Stebbings.

7. References

ABDEL-HAMEED, F., ROOTHAM, D. L., AND FLINN, R. R. 1970. Structural and numerical aberrations in natural populations of the grasshopper *Melanoplus differentialis differentialis*. *Genetics 64*, supplement 1.

AYONOADU, U., AND REES, H. 1968. The influence of B-chromosomes on chiasma frequencies in Black Mexican Sweet Corn. *Genetica*, *39*, 75-81.

BÖSEMARK, N. O. 1967. Edaphic factors and the geographical distribution of accessory chromosomes in *Phleum phleoides*. *Hereditas*, *57*, 239-262.

BRITTEN, R. J., AND DAVIDSON, E. H. 1969. Gene regulation for higher cells: a theory. *Science*, *165*, 349-357.

BRITTEN, R. J., AND KOHNE, D. E. 1968. Repeated sequences in DNA. *Science*, *161*, 529-540.

CARLSON, W. R. 1969. A test of homology between the B-chromosome of Maize and Abnormal Chromosome 10, involving the control of non-disjunction in Bs. *Molec. Gen. Genetics*, *104*, 59-65.

GIBSON, I., AND HEWITT, G. M. 1970. Isolation of DNA from B-chromosomes in Grasshoppers. *Nature*, *225*, 67-68.

HENNIG, W., AND WALKER, P. M. B. 1970. Variations in the DNA from two rodent families (Cricetidae and Muridae). *Nature*, *225*, 915-919.

HEWITT, G. M., AND BROWN, F. M. 1970. The B-chromosome system of *Myrmeleotettix maculatus*. V. A steep cline in East Anglia. *Heredity*, *25*, 363-371.

HEWITT, G. M., AND JOHN, B. 1967. The B-chromosome system of *Myrmeleotettix maculatus*. III. The statistics. *Chromosoma*, *21*, 140-162.

HEWITT, G. M., AND JOHN, B. 1970. The B-chromosome system of *Myrmeleotettix maculatus*. IV. The dynamics. Evolution, *24*, 169-180.

JOHN, B., AND HEWITT, G. M. 1965*a*. The B-chromosome system of *Myrmeleotettix maculatus*. I. The mechanics. *Chromosoma*, *16*, 548-578.

JOHN, B., AND HEWITT, G. M. 1965*b*. The B-chromosome system of *Myrmeleotettix maculatus*. II. The statics. *Chromosoma*, *17*, 121-128.

JONES, R. N., AND REES, H. 1969. An anomalous variation due to B-chromosomes in rye. *Heredity*, *24*, 265-271.

MCCARTHY, B. J. 1969. The evolution of base sequences in nucleic acids. Handbook of Molecular Cytology (ed. Lima-de Faria), North Holland Research Monographs. *Frontiers of Biology*, *15*, 4-20.

NUR, U. 1969*a*. Mitotic instability leading to an accumulation of B-chromosomes in Grasshoppers. *Chromosoma*, *27*, 1-19.

NUR, U. 1969*b*. Harmful B-chromosomes in a mealy bug: additional evidence. *Chromosoma*, *28*, 280-297.

ROBERTSON, F. W., CHIPCHASE, M., AND MAN, N. T. 1969. The comparison of differences in reiterated sequences by RNA-DNA hybridisation. *Genetics*, *63*, 369-385.

RUSCOE, C. N. E. 1970. Ph.D. Thesis. University of East Anglia.

SOUTHERN, E. M. 1970. Base sequence and evolution of guinea-pig α-satellite DNA. *Nature, 227*, 794-798.

WALKER, P. M. B., FLAMM, W. G., AND MCLAREN, A. 1969. Highly repetitive DNA in rodents. Handbook of Molecular Cytology (edi Lima-de Faria), North Holland Research Monographic. *Frontiers of Biology, 15*, 52-66.

WHITE, M. J. D. 1954. *Animal Cytology and Evolution.* 2nd edition. University Press, Cambridge.

YUNIS, J. J., AND YASMINEH, W. G. 1970. Satellite DNA in constitutive heterochromatin of the guinea-pig. *Science, 168*, 263-265.

ZECEVIC, L. J., AND PAUNOVIC, D. 1969. The effect of B-chromosomes on Chiasma frequency in wild populations of rye. *Chromosoma, 27*, 198-200.

POPULATION CYTOGENETICS OF THE MALAYAN HOUSE RAT, *RATTUS RATTUS DIARDII*

YONG HOI-SEN

Division of Genetics, School of Biological Sciences, University of Malaya, Kuala Lumpur, Malaysia

THE Malayan house rat is currently recognised as a form of *Rattus rattus*, viz. *R. r. diardii* (Chasen, 1940; Medway, 1969). Heteromorphism of the longest autosome pair and numerical polymorphism were identified in samples of this rat trapped at two localities. Additionally, a single animal at Kuala Lumpur was heterozygous for a Robertsonian translocation (Yong, 1971*a*).

2. MATERIAL

The rats were trapped live within or close to domestic and public buildings in Kuala Lumpur (November, 1968 to July, 1970) and Batu Pahat (November, 1969). Seventy animals were obtained from Kuala Lumpur and 20 from Batu Pahat.

3. HETEROMORPHISM OF THE LONGEST AUTOSOME

The longest autosome pair was found to be represented by alternative acrocentric and subacrocentric forms, presumably related by pericentric inversion (Yong and Dhaliwal, 1970). Three karyotypic classes were observed (plate, figs. 1-3).

Heteromorphism of the longest autosome has also been reported for the Japanese and Korean taxa (Yosida *et al.*, 1965, 1967) and for the Vietnamese taxon (T. C. Hsu, personal communication). All these taxa have $2n = 42$. In contrast several widely separated populations (from South America, Europe, Africa and Oceania) with $2n = 38$ are homomorphic for the subacrocentric member of this autosomal pair (Bianchi *et al.*, 1969; Capanna *et al.*, 1970, 1971 and in press; Yosida *et al.*, 1969). Additionally, three distinct populations ($2n = 38$, $2n = 42$ and $2n = \pm 54$) within the same locality in Egypt also apparently exhibit homomorphism (Badr and Badr, 1970).

In a random sample of 70 rats collected in Kuala Lumpur, 6 were homozygous acrocentric, 25 homozygous subacrocentric and 39 heterozygous (table 1). The frequencies of homozygous and heterozygous individuals,

TABLE 1

Frequency of homomorphic and heteromorphic pairs of the longest autosome in Rattus rattus diardii *collected in Kuala Lumpur*

	Homomorphic pair		Heteromorphic pair	
	A/A*	S/S	A/S	Total
Males	3	12	16	31
Females	3	13	23	39

* A = acrocentric S = subacrocentric

however, differ from sample to sample. In another Malayan sample (Batu Pahat), there seems to be a relatively higher number of homozygous acrocentric individuals—25 per cent. as compared to 8·6 per cent. in the Kuala Lumpur sample. The Batu Pahat sample, however, is rather small and therefore may not reflect the actual frequency of each karyotypic class. The difference is most striking between the Malayan (Kuala Lumpur) and Japanese populations. In the former, heterozygous individuals constitute the majority (55·7 per cent.) while homozygous acrocentric individuals are the least frequent (8·6 per cent.). In the latter 69·6 per cent. of the Misima sample are homozygous acrocentric and 30·4 per cent. heterozygous but without homozygous subacrocentric. Further, the Sapporo (Japan) sample is homomorphic with only homozygous acrocentric animals. The Korean population also shows a different frequency for these three karyotypic classes (table 2).

TABLE 2

Frequency of homomorphic and heteromorphic Rattus rattus diardii *compared with those of* Rattus rattus *with similar heteromorphism occurring in Japan and Korea (Yosida, Moriguchi, Kang and Shimakura, 1967)*

	Kuala Lumpur (Malaya)	Batu Pahat (Malaya)	Misima (Japan)	Seoul (Korea)
A/A	6 (8·6%)	5 (25·0%)	39 (69·6%)	9 (34·6%)
A/S	39 (55·7%)	7 (35·0%)	17 (30·4%)	13 (50·0%)
S/S	25 (35·7%)	8 (40·0%)	0 (0%)	4 (15·4%)
Total number of animals	70	20	56	26

The difference in the frequency of the 3 karyotypic classes (A/A, A/S, S/S) is also reflected in the frequencies of subacrocentric and acrocentric members of the longest autosomal pair (table 3). The Malayan population

TABLE 3

Frequencies of subacrocentric and acrocentric chromosomes of the longest autosome pair calculated from data in table 2

Longest autosome	Kuala Lumpur	Batu Pahat	Misima	Korea
Subacrocentric	0·64	0·575	0·15	0·40
Acrocentric	0·36	0·425	0·85	0·60

seems to be the direct reverse of the Korean population while the Japanese population has an extremely low frequency of the subacrocentric. These populations do not differ significantly from the Hardy-Weinberg distribution (Batu Pahat $X_1^2 = 1{\cdot}62$, $P > 0{\cdot}2$; Misima $X_1^2 = 1{\cdot}84$, $P > 0{\cdot}1$; Korea $X_1^2 = 0{\cdot}04$, $P > 0{\cdot}8$). The frequencies of the subacrocentric and acrocentric members do not differ significantly in the two Malayan populations ($X_1^2 = 0{\cdot}49$, $P \sim 0{\cdot}50$). However, the Kuala Lumpur population is distinct from that of Misima ($X_1^2 = 53{\cdot}95$, $P < 0{\cdot}001$) and Korea ($X_1^2 = 8{\cdot}33$; $P < 0{\cdot}01$). Similarly, the Japanese population differs from the Korean ($X_1^2 = 12{\cdot}67$, $P < 0{\cdot}001$).

In the Kuala Lumpur population, although the three karyotypic classes do not differ significantly from those expected from the Hardy-Weinberg

distribution ($X_1^2 = 2{\cdot}92$, $P > 0{\cdot}05$), there is a small excess of heterozygous individuals (table 4). The numbers are small but the result is consistent

TABLE 4

Comparisons of the three karyotypic classes of Rattus rattus diardii *collected in Kuala Lumpur with those expected from the Hardy-Weinberg distribution*

	A/A	A/S	S/S
Observed number	6	39	25
Expected number	9·07	26·67	32·26

$X_1^2 = 2{\cdot}92$, $P > 0{\cdot}05$

with the assumption of random breeding and a system of balanced polymorphism, possibly involving a small selective advantage of the heterozygotes.

4. Numerical variation

In addition to heteromorphism of the longest autosome, a high proportion of *Rattus rattus diardii* possesses chromosome numbers other than the diploid number of 42. Out of the 70 rats collected in Kuala Lumpur, 17 (24 per cent.) had variant numbers ranging from 41 to 45 (table 5). In the Batu

TABLE 5

Variation in chromosome number in homomorphic and heteromorphic individuals of Rattus rattus diardii *collected in Kuala Lumpur*

	Chromosome number					Percent numerical variants
	41	42	43	44	45	
A/A males		3				0
A/A females		3				0
S/A males		12	2	1	1	33·3
S/A females	2	16	5			30·0
S/S males		10	2			16·7
S/S females	1	9	2	1		30·8
Total	3	53	11	2	1	24·3

Pahat sample 3 out of 20 rats were numerical variants—2 had 43 chromosomes and one had 44 chromosomes. The additional chromosomes all appeared to be small metacentrics, indistinguishable from the small metacentrics of the normal complement. Counts of 41 chromosomes were surprising but they were made repeatedly and confidently in the three animals recorded in table 5. The interpretation of this observation will be considered elsewhere (Yong, in press).

Numerical variation was not found in a relatively much larger sample from a Japanese population of *Rattus rattus* (Yosida *et al.*, 1967).

5. Significance of chromosome polymorphism

There are two groups of *Rattus rattus* viz. Asian populations with $2n = 42$ and European, African, American and Oceanian populations with $2n = 38$. The Asian populations are characterised by heteromorphism of the longest

autosome due to presumptive pericentric inversion while other populations are characterised by Robertsonian-type translocations in which 2 pairs of large biarmed chromosomes have been established in the complement. The probability that " centric fusion has occurred between the same pairs of autosomes independently " (Capanna *et al.*, 1970) in these widely separated populations is rather remote. It is more probable that these $2n = 38$ " populations are interconnected by the same system that is responsible for the ubiquitous diffusion of the species, which is that associated with the black rat's condition as man's commensal " (Capanna *et al.*, 1970).

In addition, an extraordinary situation has been reported from Cairo. Three distinct populations of *Rattus rattus* with $2n = 38$, $2n = 42$ and $2n = \pm 54$, apparently coexist in the same locality, without interbreeding (Badr and Badr, 1970). If it could be confirmed that the $2n = 38$ and $2n = 42$ individuals do not hybridise in nature and the F_1 hybrids produced under laboratory conditions (*cf.* Yosida *et al.*, 1969) are markedly infertile, it would throw more light on the systematic status of these animals.

Further, only homozygous individuals have been reported for the $2n = 38$ populations. It would be noteworthy if intrapopulation polymorphism is found, such as that in the Malayan house shrew, *Suncus murinus*, in which Robertsonian-type translocation is responsible for the existence of $2n = 38$, 39 and 40 individuals within the same population (Yong, 1971*b*).

6. Summary

1. The longest pair of autosome in the Malayan house rat, *Rattus rattus diardii*, is heteromorphic and in three karyotypic classes of individuals—homozygous acrocentric (A/A), homozygous subacrocentric (S/S) and heterozygous (A/S) are found in Hardy-Weinberg proportions.

2. The frequencies of both the three classes and the two chromosomes differ in different populations.

3. The heteromorphism is attributed to pericentric inversion.

4. Variation in chromosome number has been observed only in homozygous subacrocentric and heterozygous individuals.

5. A numerical variant ($2n = 41$) apparently due to Robertsonian translocation has also been found.

6. The results are compared with those for other forms of *Rattus rattus* from both $2n = 42$ and $2n = 38$ populations.

Acknowledgments.—I wish to thank Dr Charles E. Ford for his valuable suggestions and discussions. Thanks are also due to Mr Teh Kok Leng for technical assistance and Miss Kuan Lai Wah for clerical assistance.

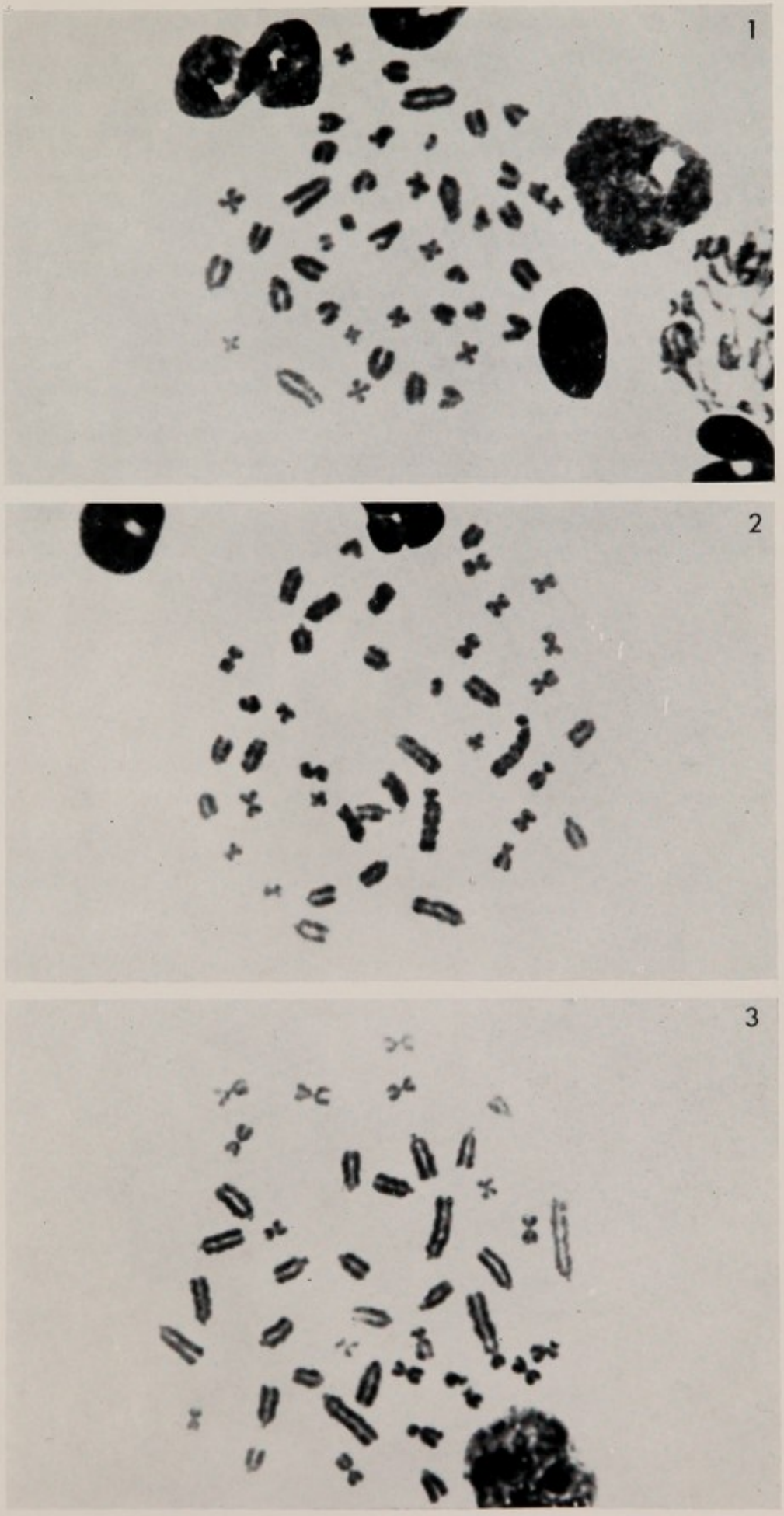
7. References

BADR, F. M., AND BADR, R. S. 1970. The somatic chromosomes of a wild population of rats: numerical polymorphism. *Chromosoma, Berl.*, 465-475.

BIANCHI, O. N., PAULETTE-VANRELL, J., AND DE VIDAL TIOJA, L. A. 1969. Complement with 38 chromosomes in two South American populations of *Rattus rattus*. *Experientia, 25*, 1111-1112.

CAPANNA, E., CIVITELLI, M. V., AND NEZER, R. 1970. The karotype of the Black rat (*Rattus rattus* L.). Another population with a 38-chromosome complement. *Experientia, 26*, 422-525.

CAPANNA, E., AND CIVITELLI, M. V. 1971. On the chromosomic polymorphism of *Rattus rattus* L. A study on West-European populations. *Experientia, 27*, 583-584.



The three karyotypes observed with regard to heteromorphism in the longest autosome.
1 = AA homozygote
2 = SS homozygote
3 = AS heterozygote.

CAPANNA, E., AND CIVITELLI, M. V. Karyological analysis of four African populations of *Rattus rattus* L. A statement of the problem of chromosomal polymorphism in the black rat. *Bolletino di Zoologia, 38* (in press).

CHASEN, F. N. 1940. A handlist of Malaysian mammals. *Bull. Raffles Mus.*, No. 15.

MEDWAY, LORD. 1969. *The Wild Animals of Malaya and offshore Islands including Singapore, Kuala Lumpur*. Oxford University Press.

YONG, H. S. 1969. Karyotypes of Malayan rats (Rodentia-Muridae, genux *Rattus* Fischer). *Chromosoma, 27*, 245-267.

YONG, H. S. 1971*a*. Centric fusion in the Malayan house rat, *Rattus rattus diardii* (Rodentia-Muridae). *Experientia, 27*, 467-468.

YONG, H. S. 1971*b*. Chromosome polymorphism in the Malayan house shrew, *Suncus murinus* (Insectivora, Soricidae). *Experientia, 27*, 589-591.

YONG, H. S. Presumptive *X* monosomy in Black rats from Malaya. *Nature* (in press).

YONG, H. S., AND DHALIWAL, S. S. 1970. Chromosomal polymorphism in the Malayan House rat, *Rattus rattus diardii* (Jentinck). *Mamm. Chrom. Newsletter, 11*, 30-32.

YOSIDA, T. H., NAKAMURA, A., AND FUKAYA, T. 1965. Chromosomal polymorphism in *Rattus rattus* L. collected in Kusudomari and Misima. *Chromosoma, Berl., 16*, 70-78.

YOSIDA, T. H., MORIGUCHI, Y., KANG, Y. S., AND SHIMAKURA, K. 1967. Population survey of No. 1 chromosome polymorphism of Black rats (*Rattus rattus*) collected in Japan and Korea. In Annual Report No. 17, 1966, National Institute of Genetics, Japan.

YOSIDA, T. H., TSUCHIYA, K., IMAI, H., AND MORIWAKI, K. 1969. New chromosome types of the Black rat, *Rattus rattus*, collected in Oceania and F_1 hybrids between Japanese and Australian rats. *Japan. J. Genetics, 44*, 89-91.

HYBRIDISATION IN NATURE BETWEEN TWO CHROMOSOME FORMS OF SPINY MICE

JACOB WAHRMAN and RUTH GOITEIN
Laboratory of Genetics, The Hebrew University, Jerusalem, Israel

1. Introduction

Different species of mammals are distributed in Israel and Sinai either in the northern relatively humid parts of the country, or in the southern semi-arid and arid regions. Related species often show complementary distributions, thus occupying together the entire country, with little or no overlapping (Wahrman, 1970).

Among the rodents there are two exceptions to this distribution pattern: *Gerbillus dasyurus* Wagner, 1842 and *Acomys cahirinus* Desmarest, 1819, that have an almost country-wide distribution. Both species thrive in all three climate regions, namely the Mediterranean, steppe and desert (according to Köppen's classification). The mean annual rainfall in these regions ranges from more than 1,100 mm. to less than 50 mm., and the mean annual ambient temperatures range from below 15° C. to above 25° C. The genetic basis of this remarkable adaptibility to an extremely wide range of environmental conditions is unknown. We report on the chromosomal variability of the common spiny mouse, *A. cahirinus*, from different parts of the country.

For several years we have been familiar with the uniform $2n = 38$ karyotype of *A. cahirinus* from Israel (Wahrman and Zahavi, 1953). Recently we found that animals from the southern parts of the Sinai Peninsula were morphologically indistinguishable from Israeli specimens, but had two chromosomes less. A distributional gap in Central Sinai (Haim, 1969) separates the two chromosome forms from each other, except for a narrow stretch of land in the north-eastern corner of the peninsula connecting the Sinai population with that of Israel. We directed our efforts to this area since we assumed that the two forms might meet there. Indeed, late in 1969 we found the anticipated hybrid animals.

2. Material and methods

The material (table 1) consisted of 73 specimens, 30 females and 43 males, collected by live-trapping. Five animals were studied in 1952-53 (Wahrman and Zahavi, 1953), 5 in both 1959-62 and 1966-67, and 58 in 1969-70.

Spiny mice were among the first mammals whose chromosomes were examined after hypotonic pre-treatment (Wahrman and Zahavi, 1953). We studied mitotic chromosome complements in bone marrow and spleen cells, leukocytes and spermatogonia. The examination of colcemid-treated bone marrow was our basic method. The marrow of both femora was introduced into 8-10 ml. of tissue culture medium 199, including one drop of colcemid (CIBA) 0·05 per cent. for each millilitre of medium. After mechanically dispersing the marrow, it was incubated at 37° C. for 90 minutes. Hypotonic treatment, fixation and air-drying were performed

TABLE 1

The chromosome constitution of 73 individuals of Acomys cahirinus according to their geographic origin. The localities' numbers refer to figs. 1 and 2. The Israeli co-ordinate grid appears on maps of the Survey of Israel

Number of locality	Israel grid notation	Name of locality	Number of individuals		
			$2n = 36$	$2n = 37$	$2n = 38$
—	—	Israel, origin unknown			2
1	211/295	Dan			1
2	167/272	Nahal Keziv			2
3	168/131	Jerusalem			3
4	161/133	ʿEn Hemed			1
5	162/129	Sea ʿdim			6
6	187/097	ʿEn Gedi			4
7	187/054	Sedom			1
8	096/006	Qadesh Barnea			2
9	068/018	Gebel Halal			1
10	060/005	Gebel Halal, Bir el-Khadira			2
11	147/908	Timnaʿ			1
12	144/884	Elat			1
13	137/889	Ras en-Naqb			1
14	109/899	35 km. NWW of Ras en-Naqb			1
15	090/900	Wadi Abu Buteima			1
16	134/872	Mersa Morakh			1
17	130/868	Wadi el-Hamra			2
18	128/863	Wadi Umm Yehia			1
19	122/850	Wadi Suweir	4	6	2
20	124/848	Ras el-Burqaʿ		1	
21	123/845	Wadi Himeira	2		
22	120/844	—	1	1	
23	118/834	—		2	
24	113/825	Wadi Watir	3		
25	105/831	ʿEin el-Furtaga	1		
26	092/830	ʿEin Umm Ahmed	1		
27	115/820	Nuweiba el-Emzeina	1		
28	112/818	—	2		
29	098/799	Wadi el-Gha ʿib	1		
30	076/797	Wadi Saʿal	1		
31	049/775	St Catherine's Monastery	1		
32	047/773	El-Arbaʿin	1		
33	039/741	Wadi Isla	2		
34	972/860	Wadi Wata	1		
35	977/878	ʿEin Shallala	2		
36	974/898	Wadi Sumar	1		
37	970/942	Mitla Pass	1		
38	007/974	Gebel Yiʿallaq	1		
		Total	27	10	36

by the methods of human leukocytes. Acetic orcein was the standard staining method for all tissues. Good results were also obtained with Giemsa stain. In fact, these two staining methods were often complementary in revealing cytological detail.

Mitoses were induced in leukocytes by phytohaemagglutinin. Whole blood, in 1 ml. aliquots, was introduced into tissue culture medium 199 (7·5 ml.), to which foetal-calf serum (2·5 ml.) and phytohaemagglutinin Wellcome (0·5 ml.) were added. The best results were obtained when the cells were harvested after 96 hours. Colcemid 0·05 per cent. (1 ml. per

100 gm. body weight) was injected intraperitoneally to the animals, about 90 minutes before the blood was drawn. Successful cultures can possibly be obtained without colcemid injections.

Meiotic preparations were made from males only. Testicular tubules were either directly treated hypotonically and then squashed, or cell suspensions were incubated for 90 minutes prior to the hypotonic treatment, in tissue culture medium with colcemid. In the latter case the bone marrow procedure was followed after the incubation with colcemid. Both methods yielded good results. Meiotic slides often include clear second meiotic metaphases which may be difficult to obtain in some other mammals. These are useful for checking fine morphological detail.

3. Results

(i) *The geographic distribution of the chromosome types*

The distribution of *Acomys cahirinus* in Israel, Sinai and some neighbouring areas is shown in fig. 1. The chromosome complements of 73 individuals from 38 localities were determined. They fall into three classes: Twenty-seven animals had $2n = 36$ chromosomes, 10 had $2n = 37$ chromosomes and 36 had $2n = 38$ chromosomes (table 1). In correlating the cytological findings with the geographic distribution, the following three phenomena called for special attention.

1. The range of *A. cahirinus* is divided into two sub-regions separated by a large, nearly complete distribution gap in Central Sinai. These two regions, the northern and the southern, are inhabited by the $2n = 38$ and $2n = 36$ chromosome forms, respectively. We designate the northern race the " Israeli Form " and the southern race the " Sinaitic Form ", despite some minor penetrations of the Israeli Form into areas which, strictly speaking, belong to Sinai.

2. The Israeli and Sinaitic areas of distribution are continuous through a 15 km. wide stretch of land along the coast of the Gulf of Elat, in north-eastern Sinai (figs. 1 and 2). Here the two forms are in contact and they hybridise. We shall designate this contact area as the " Hybrid Zone ". The hybrid, $2n = 37$ animals (5 males and 5 females) were found in four localities all of which are within 3 km. from the coast. The two extreme localities (numbers 19 and 23) are 16 km. apart. The Hybrid Zone is probably somewhat more extensive, but sampling at localities about 10 km. to the north and south has yielded, so far, only homozygous animals (fig. 2). It remains to be established whether the hybrid zone extends all across the 15 km. wide distribution strip.

3. There are three isolated mountain ranges in north-western Sinai that are known to harbour the common spiny mouse. These mountains are surrounded by sandy biotopes which are not suitable for this species. Gebel Halal is close to the northern, Israeli region. The two specimens trapped in the Gebel Halal localities (numbers 9 and 10) had $2n = 38$ chromosomes. The other two mountain ranges, namely Gebel Yia'llaq (locality number 38) and Gebel Maghara, are near the southern, Sinaitic region. A single animal from Gebel Yia'llaq had $2n = 36$ chromosomes, while no specimens from Gebel Maghara have yet been examined. This limited information does not preclude the possibility of chromosome polymorphism in the isolated populations.

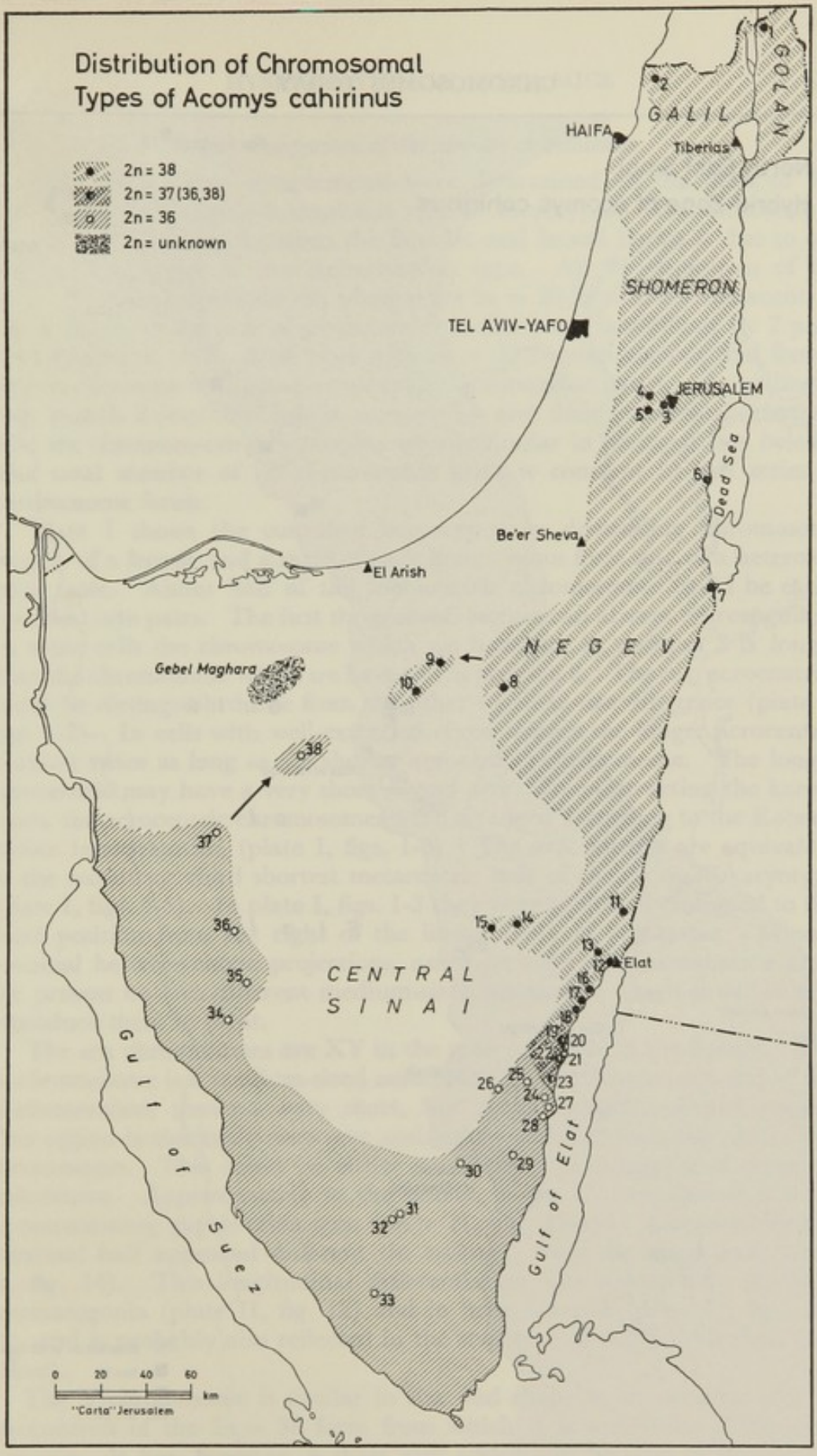


FIG. 1.—The distribution of chromosomal types of *Acomys cahirinus* in Israel and Sinai. The numbers refer to localities from which cytological information was obtained (compare with table 1). The general distribution is based on Haim (1969 and unpublished results), Shkolnik (1966) and Wahrman (1970). Chromosomal data for Lebanon, Jordan, Saudi-Arabia and Egypt are not available. For details of the Hybrid Zone see fig. 2.

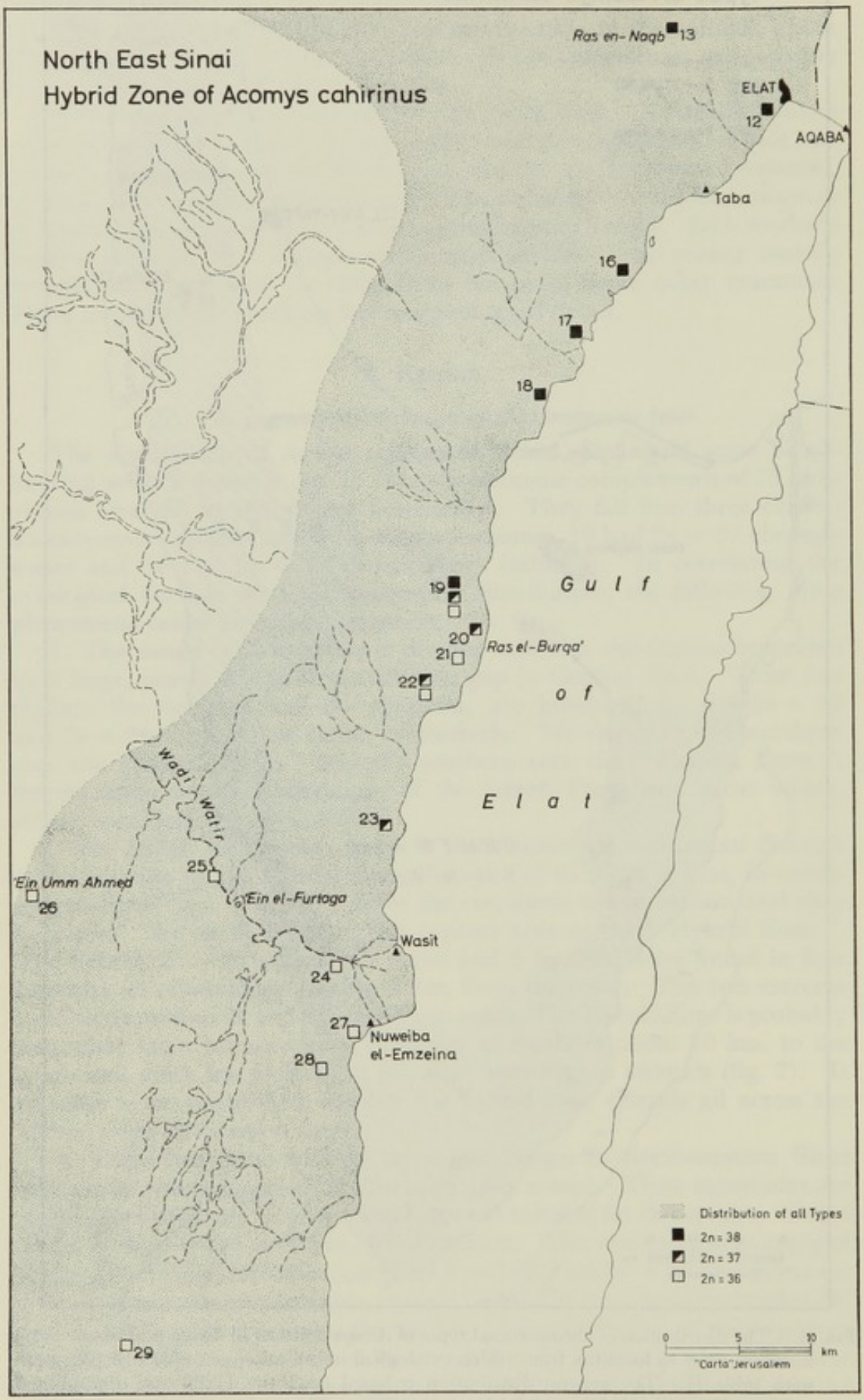


FIG. 2.—The Hybrid Zone of *Acomys cahirinus* in north-eastern Sinai. The figures refer to the corresponding localities in table 1.

(ii) *Comparison of the somatic chromosomes*

The chromosome complements were determined on the basis of 387 photographs of mitotic metaphases and 48 karyotypes. The difference in chromosome number between the Sinaitic and Israeli Forms is due to one whole-arm change of the Robertsonian type. All 34 autosomes of the $2n = 36$ form are metacentric, whereas the $2n = 38$ form has 32 metacentrics only. The missing pair of metacentrics in the latter is replaced by 2 pairs of acrocentrics. The third class with $2n = 37$ consists of male and female hybrids between these two structurally homozygous types. Accordingly, they possess 2 non-homologous acrocentrics and their metacentric partner. The sex chromosomes are morphologically similar in all types (see below). The total number of 70 chromosome arms is constant in this series of chromosome forms.

Plate I shows the complete karyotypes, in descending chromosome length, of a female and a male of each homozygous form and of a heterozygous male. About half of the metacentric chromosomes could be easily matched into pairs. The first three chromosomes can always be recognised. In some cells the chromosome which we have put in position 3 is longer than the chromosome which we have put in position 2. The two acrocentrics could be distinguished one from the other by their size difference (plate I, figs. 1-3). In cells with well-extended chromosomes the longer acrocentric is about twice as long as the shorter acrocentric chromosome. The longer acrocentric may have a very short second arm. In constructing the karyotypes, the acrocentric chromosomes were arranged according to the Robertsonian interpretation (plate I, figs. 1-3). The acrocentrics are equivalent to the second or third shortest metacentric pair of the $2n = 36$ karyotype (plate I, figs. 4-5). In plate I, figs. 1-3 they were arbitrarily assigned to the third position from the right of the linear array of autosomes. Minute terminal heteropycnotic projections, narrower than the chromosome arm, are present on two different medium-sized autosomes, but it is difficult to reproduce them in print.

The sex chromosomes are XY in the male and XX in the female. The X-chromosome is a medium-sized acrocentric. The centromeric end of the X-chromosome shows a very short, but clear triangular-shaped region. This region is markedly narrower and lighter staining than the rest of the chromosome. This could be either a minute second arm or a terminal centromere. Approximately in the middle of the X-chromosome a bend or non-staining gap is often seen (plate II, figs. 12-14). Occasionally the proximal half appeared different (in coiling?) from the distal half (plate II, fig. 14). This longitudinal differentiation was observed in dividing spermatogonia (plate II, fig. 12) and in bone marrow (plate II, figs. 13-14), and is probably also reflected in the structure of the XY bivalent (see below).

The Y-chromosome is similar in size and shape to the smaller pair of acrocentrics of the $2n = 38$ form from which it is sometimes difficult to separate. It has, however, a short arm which can be seen in favourable cells.

Polyploid cells, usually tetraploid, were occasionally observed in the soma and germ line. In the bone marrow of a $2n = 38$ individual a polyploid mass consisting of about 290 chromosomes was found.

(iii) *Meiosis*

The meiotic behaviour in the diplotene-metaphase II interval has been studied in 22 males belonging to all three chromosome types (plate II, figs. 6-8). Several of the bivalents can be individually recognized, including bivalent 1 which is the longest, usually displaying three chiasmata, and the two small acrocentric bivalents in the $2n = 38$ form (plate II, fig. 7). The small bivalents tend to separate before the larger bivalents, and could sometimes be seen separated already in diakinesis. In mid-anaphase their diads advance to the poles in front of the other chromosomes. The separation of the XY bivalent is sometimes precocious as well.

No systematic counts of chiasma frequency were attempted. In the $2n = 38$ form the following distribution was frequent: 1 bivalent with 3 chiasmata, 11 with 2 chiasmata and 6 with 1 chiasma which totals 31 autosomal chiasmata.

In the males with $2n = 37$ chromosomes, 18 bodies were counted in prophase and metaphase I. These consisted of 16 autosomal bivalents, one autosomal trivalent and the sex bivalent (plate II, fig. 8). The trivalent is a three-segment chain, with 2 chiasmata, one between each of the acrocentric lateral elements and the metacentric central chromosome (plate II, figs. 8-10). One of the lateral chromosomes is shorter than the other and tends to separate precociously. A few first spermatocytes were thus found which included a minute univalent. The pairing relationships between the chromosomes in the hybrid thus demonstrated the structural homology between 16 pairs of autosomes and convincingly supported the Robertsonian interpretation of the chromosome difference between the $2n = 36$ and $2n = 38$ karyotypes.

The sex bivalent has a striking appearance at all stages due to its characteristic shape and heteropycnosis. Late in the first meiotic division it appears like an exclamation mark (plate II, fig. 7). This is a common form of the sex bivalent in murine rodents that possess a long acrocentric X and a short Y. However, in the earlier stages, before contraction sets in, the elongated X is bent in the shape of a walking stick (plate II, fig. 6). The bend appears at a constant position and may be related to the previously described achromatic gap seen in some mitotic metaphases. A segmentation of the X may still be visible at metaphase I. The XY bivalent is negatively heteropycnotic throughout.

Second meiotic metaphases are of two kinds in the homozygous forms, and of four kinds in the hybrids. The X chromosome, when present, is positively heteropycnotic. Its chromatids are parallel and close to each other, in contrast to the autosomal chromatids which are widely separated (plate II, fig. 11).

4. Discussion

The *Acomys cahirinus* complex (in the sense of Ellerman and Morrison-Scott, 1951) includes a considerable number of Palaearctic and African forms that have originally been named as either subspecies or closely related species. We have chosen, for the time being, to use the name *A. cahirinus* for all these forms in preference to *A. dimidiatus* (Setzer, 1959) or to separate specific names. The distribution of the whole complex includes

Plate I

FIGS. 1-5.—The somatic chromosomes arranged in descending length. The sex chromosomes at right. Bone marrow, acetic orcein. × 2,500.

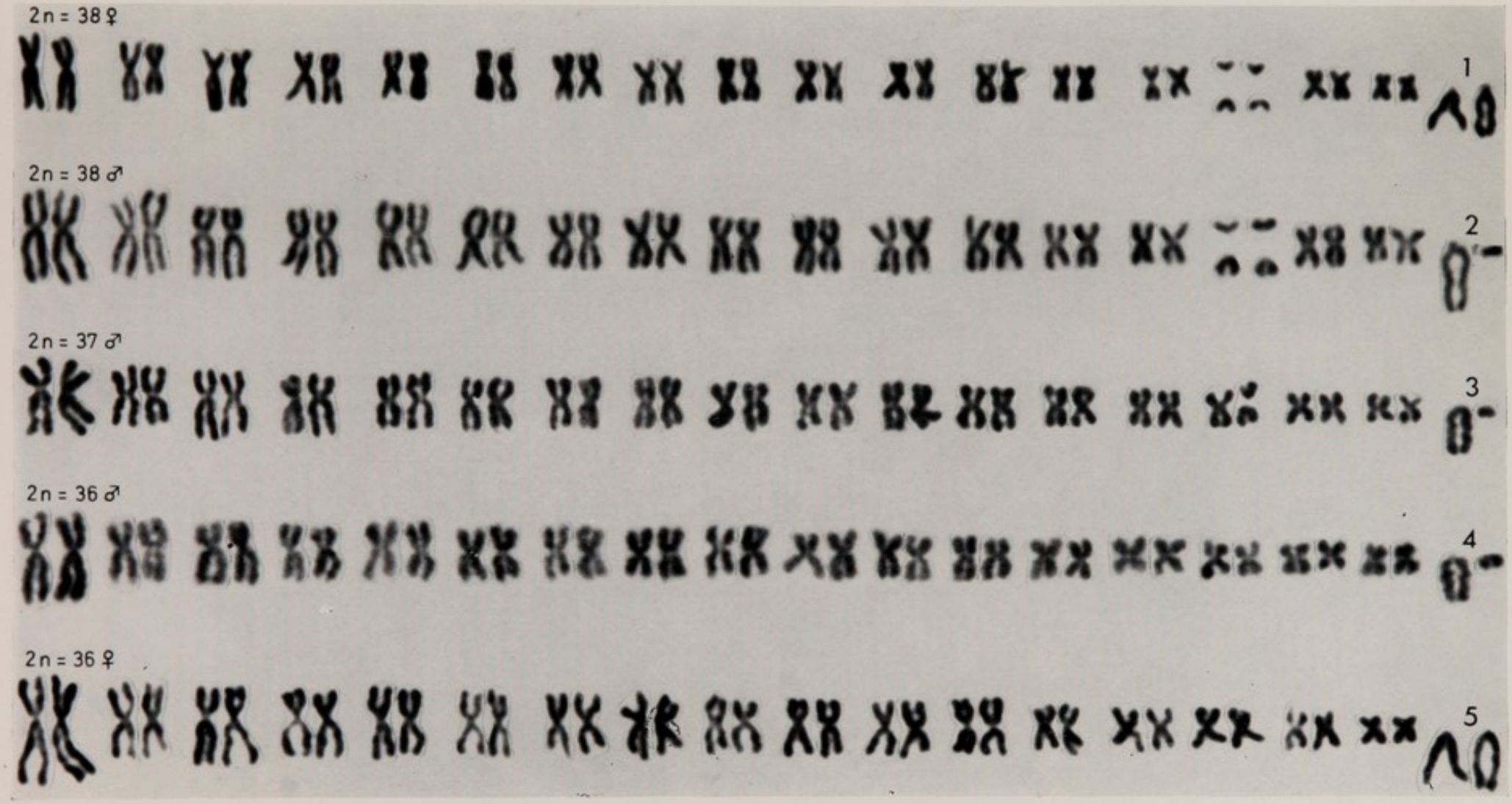
FIG. 1.—Female from Ras en-Naqb. $2n = 38$.

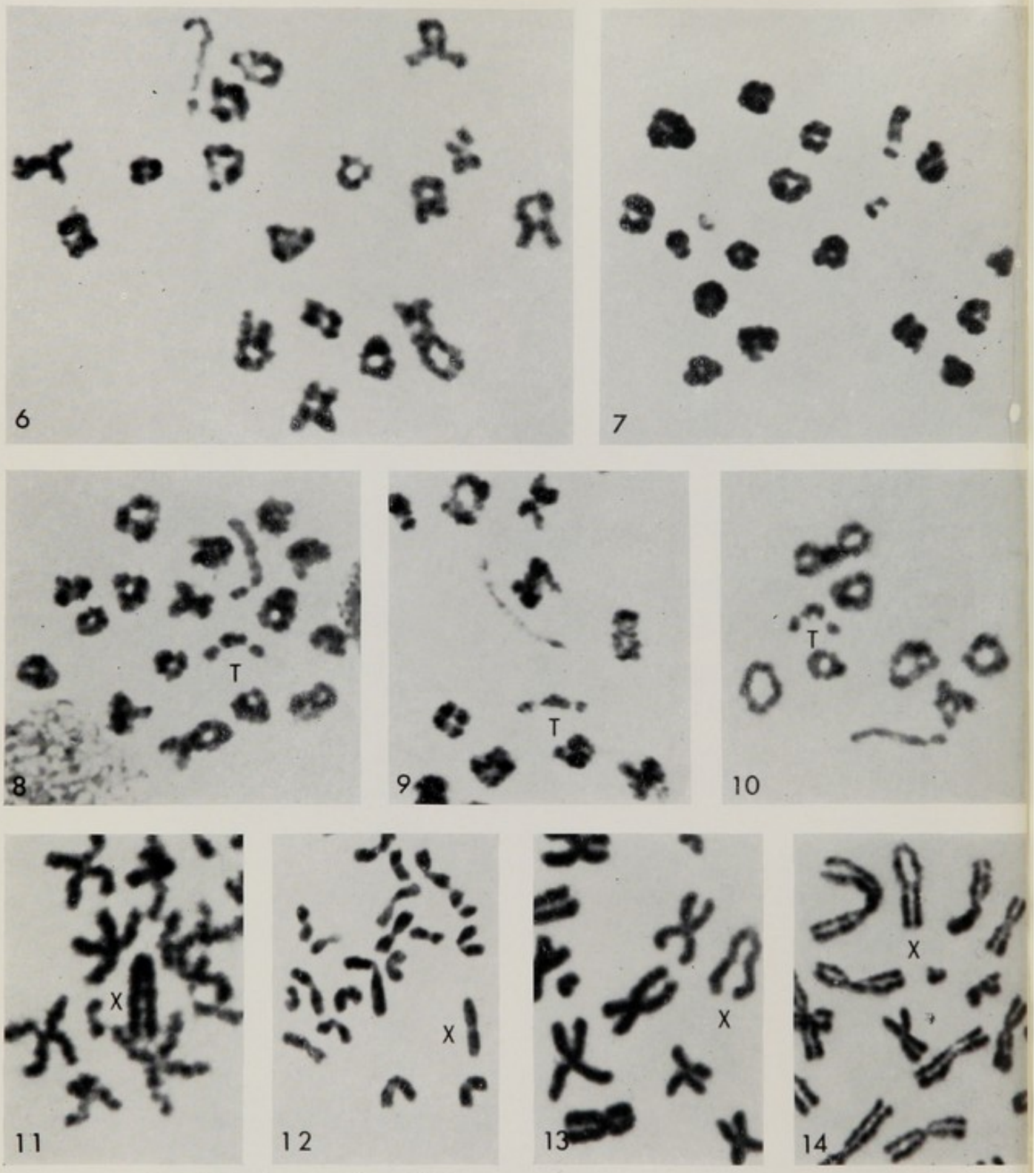
FIG. 2.—Male from 35 km. NWW of Ras en-Naqb. $2n = 38$.

FIG. 3.—Male from Wadi Suweir. $2n = 37$. The two acrocentric autosomes are shown associated as they were found in the cell.

FIG. 4.—Male from 'Ein Shallala. $2n = 36$.

FIG. 5.—Female from St. Catherine's Monastery. $2n = 36$.


2n = 38 ♀
1
2n = 38 ♂
2
2n = 37 ♂
3
2n = 36 ♂
4
2n = 36 ♀
5



Figs. 6-14.—Acetic orcein. × 2,500.

Fig. 6.—Diakinesis in male from Wadi Watir. $n = 18$. The XY bivalent has a walking-stick-like shape.

Fig. 7.—First metaphase in male from Gebel Halal. $n = 19$. The XY bivalent resembles an exclamation mark.

Fig. 8.—Diakinesis from a hybrid male ($2n = 37$) from Wadi Umm Yehia. The XY bivalent resembles an exclamation mark. T = autosomal trivalent.

Figs. 9-10.—Parts of cells in diakinesis from the same animal as in fig. 8. The XY bivalents are of the exclamation mark type. T = autosomal trivalent.

Fig. 11.—Part of second meiotic metaphase including the heteropycnotic X-chromosome. $2n = 38$.

Fig. 12.—Part of a spermatogonial metaphase showing the achromatic gap in the X-chromosome. $2n = 36$.

Fig. 13.—Part of a bone marrow metaphase from a male showing a bend in the X-chromosome. $2n = 38$.

Fig. 14.—Part of a bone marrow metaphase from a hybrid male showing the longitudinal differentiation of the X-chromosome. $2n = 37$.

north-western India, southern Iran (Lay, 1967) the Arabian Peninsula (Atallah and Harrison, 1967), the western coast of the Red Sea, Sudan (Setzer, 1956) and it extends into North Africa, north West Africa and East Africa. Cyprus and Crete are the only Mediterranean islands inhabited by forms of this group. Israel lies on a northern extension from the centre of origin which is presumably in the Ethiopian Region. From Israel northwards spiny mice taper off. There are only isolated records from Lebanon (Lewis, Lewis and Atallah, 1967) and southern Turkey (von Lehmann, 1966). Historically, it seems that this species is a newcomer to the eastern more humid coastal area of the Mediterranean. It appeared in Israel for the first time during the upper Palaeolithic, that is, some 20,000-30,000 years ago (Tchernov, 1968). The Israeli Form may therefore be a relatively recent offshoot of the *A. cahirinus* stock. The chromosomal relationships within the whole complex and their cyto-taxonomic implications will be discussed separately.

The Israeli and Sinaitic populations of the common spiny mouse are almost completely isolated one from the other by an uninhabited area in Central Sinai. Animals of the two populations are morphologically indistinguishable, but each population is characterised by a different chromosome constitution. Similar situations are common and have been described, for example, in the genera *Thomomys* (Patton and Dingman, 1968), *Gerbillus* (Wahrman and Zahavi, 1955, 1958) and *Spalax* (Soldatović, Živković, Savić and Milošević, 1967; Wahrman, Goitein, and Nevo, 1969).

The present distributions of the Israeli and Sinaitic Forms do not correspond to climatic regions. Moreover, the Israeli Form tolerates the whole spectrum of climatic conditions that can be encountered in the country. Although it is still possible that the chromosome forms are related to some micro-factors, it seems more likely that little correlation exists between the chromosome forms and the present environmental conditions of their respective areas of distribution.

Chromosomal differences distinguishing taxonomically similar or identical populations are of different kinds. In the present case the two Forms differ only by a single whole-arm change, a Robertsonian transformation, which is the most common change among related forms of mammals. The selective value of a Robertsonian change has not yet been elucidated experimentally and thus remains largely unknown. The adaptive significance of symmetric versus asymmetric karyotypes, centromere position and the number of linkage groups may be important in this connection.

The heterozygous animals are confined to one narrow zone where secondary intergradation occurs. At this place hybridisation became possible presumably because complete reproductive isolation has not been reached. In addition, the Robertsonian type of chromosome change, in contrast to inversions, does not interfere with the linearity of meiotic synapsis. A loss of gametes due to errors in segregation is also relatively small, especially if, as in this case, only one trivalent is involved.

In considering the origin of the Israeli and Sinaitic Forms, it should be borne in mind that the karyotypes of the forms belonging to the *Acomys cahirinus* complex were derived by stepwise Robertsonian evolution from ancestral karyotypes that were mostly or totally acrocentric. It has been assumed, for example, that *different* combinations of acrocentrics have produced the metacentrics of Israel, Cyprus and Crete (Matthey, 1963;

Wahrman and Zahavi, unpublished results, 1959). The structural homology that exists between 32 metacentric autosomes of the two Forms indicates that they are the penultimate and ultimate members in a series of chromosomal transformations. On this basis, two alternative explanations should be considered for the origin of the Israeli and Sinaitic Forms.

1. The Sinaitic, completely metacentric karyotype may be the last member of the series. In this case the Israeli, $2n = 38$ form is the ancestor of the two.

2. On the other hand, the Israeli Form might have been derived from the Sinaitic Form through the fission of one metacentric chromosome.

The present knowledge of the history and distribution of the *Acomys cahirinus* complex, as summarised earlier, tends to support the second hypothesis. However, the first hypothesis requires fewer, cytologically less complicated, steps, and is therefore simpler.

The Israeli and Sinaitic Forms are structurally homozygous throughout most of their respective ranges. We have not found any special factors that would seem to encourage the establishment of structural homozygosity in this line of chromosomal evolution. In other species Robertsonian chromosomal polymorphism has been shown to exist *within* populations. It appears that in certain cases there are selective advantages in the achievement of homozygosis for such changes, whereas in others the establishment of polymorphic systems is advantageous. However, Robertsonian polymorphism may, in fact, be only a transitional stage in the attainment of structural homozygosis.

5. Summary

1. Two populations of *Acomys cahirinus* in Israel and Sinai are almost completely isolated but taxonomically indistinguishable.

2. The Israeli and Sinaitic populations are continuous at only a narrow stretch along the coast of the Gulf of Elat. This meeting is, apparently, a secondary intergradation.

3. The Israeli specimens, obtained from all climatic regions, possess $2n = 38$ chromosomes, while the animals from Sinai have $2n = 36$ chromosomes. The two populations are completely homozygous except for the inter-gradation zone where hybrids with $2n = 37$ are found as well.

4. A comparison of the three karyotypes and a study of the synaptic relationships at meiosis, demonstrate that the chromosome difference is due to a single whole-arm (Robertsonian) change.

5. The Sinaitic and Israeli populations are presumably the penultimate and ultimate members in one particular line of stepwise chromosomal evolution in the *A. cahirinus* complex. Which of the two Forms is ancestral has not been decided.

Acknowledgments.—We thank A. Borut, Y. Cohen, M. Katznelson, E. Nevo and A. Shkolnik, who took part in the field trips. The recent renewal of our interest in this problem was mainly due to the intensive distribution studies in Sinai by Haim, to whom we are especially grateful. M. Shaham took part in karyotype analysis.

6. References

ATALLAH, S. I., AND HARRISON, D. L. 1967. New records of rodents, bats and insectivores from the Arabian Peninsula. *J. Zool., Lond., 154*, 311-319.

ELLERMAN, J. R., AND MORRISON-SCOTT, T. C. S. 1951. *Checklist of Palaearctic and Indian Mammals 1758 to 1946.* British Museum, London.

HAIM, A. 1969. *The Distribution of Rodents in the Sinai Peninsula.* M.Sc. Thesis, Hebrew University, Jerusalem.

LAY, D. M. 1967. A study of the mammals of Iran, resulting from the Street Expedition of 1962-63. *Fieldiana, Zool., 54,* 1-282.

LEWIS, R. E., LEWIS, J. H., AND ATALLAH, S. I. 1967. A review of Lebanese mammals. Lagomorpha and Rodentia. *J. Zool., Lond,. 153,* 45-70.

MATTHEY, R. 1963. Polymorphisme chromosomique intraspecifique et intraindividuel chez *Acomys minous* Bate (Mammalia-Rodentia-Muridae). *Chromosoma, 14,* 468-497.

PATTON, J. L., AND DINGMAN, R. E. 1968. Chromosome studies of pocket gophers, genus Thomomys. 1. The specific status of *Thomomys umbrinus* (Richardson) in Arizona. *J. of Mammal., 49,* 1-13.

SETZER, H. W. 1956. Mammals of the Anglo-Egyptian Sudan. *Proc. U.S. Nat. Mus., 106,* 447-587.

SETZER, H. W. 1959. The spiny mice (Acomys) of Egypt. *J. Egypt. Publ. Health Assoc., 34,* 93-101.

SHKOLNIK, A. 1966. *Studies in the Comparative Biology of Israel's two Species of Spiny Mice.* Ph.D. Thesis, Hebrew University, Jerusalem.

SOLDATOVIĆ, B., ŽIVKOVIĆ, S., SAVIĆ, I., AND MILOŠEVIĆ, M. 1967. Vergleichende Analyse der Morphologie und der Anzahl der Chromosomen zwischen Populationen von *Spalax leucodon* Nordmann, 1840. *Z.f. Säugetierkunde, 32,* 238-245.

TCHERNOV, E. 1968. *Succession of Rodent Faunas during the Upper Pleistocene of Israel.* Paul Parey, Hamburg and Berlin.

VON LEHMANN, E. 1966. Ein Nachweis der Stachelmans (*Acomys cahirinus*) in der Türkei. *Zool. Beitr., 12,* 465-467.

WAHRMAN, J. 1970. Distribution of land Vertebrates. *Atlas of Israel,* Sheet VII/1. Survey of Israel, Jerusalem and Elsevier, Amsterdam.

WAHRMAN, J., GOITEIN, R., AND NEVO, E. 1969. Geographic variation of chromosome forms in Spalax, a subterranean mammal of restricted mobility. In *Comparative Mammalian Cytogenetics* (ed. by K. Benirschke). Springer-Verlag, New York.

WAHRMAN, J., AND ZAHAVI, A. 1953. Intra-generic difference in chromosome numbers of spiny mice (Rodentia: Murinae). *Bull. Res. Counc. Israel, 3,* 265.

WAHRMAN, J., AND ZAHAVI, A. 1955. Cytological contributions to the phylogeny and classification of the rodent genus Gerbillus. *Nature, 175,* 600-602.

WAHRMAN, J., AND ZAHAVI, A. 1958. Cytogenetic analysis of mammalian sibling species by means of hybridization. *Proc. X Int. Congr. Genet., 2,* 304-305.

CYTOLOGY OF SOME INDIAN ASCLEPIADACEAE

MIRA JASH and ARCHANA SHARMA
Department of Botany, University of Calcutta, Calcutta 19, India

1. Introduction

The family Asclepiadaceae has a wide distribution throughout India and is represented by a large number of species under fifty-six genera, occurring mainly in the wild condition, in ecological habitats ranging from tropical forests to temperate regions. The specialised nature of the flowers makes the family a homogeneous complex. Several of its members have important medicinal properties. Different taxonomists have assigned different positions to this family under their systems of classification, for example under the Loganiales (Bentham and Hooker, 1883) together with the Oleaceae, Loganiaceae and Apocynaceae; under the Contortae (Engler, 1887) and under the Apocynales with Apocynaceae (Hutchinson, 1959).

However, little cytological data is available on the representatives in India, except for sporadic reports of chromosome numbers. A detailed study of the chromosomes of the Indian members of this family, including karyotype analysis and meiotic studies, has therefore been undertaken to work out the phylogeny and the lines of evolution of the genera concerned.

2. Materials and methods

Seventeen species under fourteen genera of the family have been studied.

The plants were collected mostly from the Indian Botanic Gardens at Shibpur, Howrah, different nurseries near Calcutta, from rural areas of Midnapur and different regions of West Bengal.

For somatic study temporary acetic orcein squash preparations were made. Sections were also cut from paraffin blocks of root tips and stained in crystal violet but were found to be not suitable for the analysis of karyotypes. For scattering and clarification of chromosome morphology several pre-treatment chemicals were tried. These included a mixture of saturated solutions of paradichlorobenzene (Sharma and Mookerjea, 1955) and aesculin (Sharma and Sarkar, 1955) in the proportion of 1 : 1 at 10° – 12° C. for 2-3 hours which was found to be the most suitable. For all the species studied here, 2-3 hours fixation in propionic acid: ethyl alcohol (1 : 2) following pre-treatment was found to be essential. After fixation roots were heated in a mixture of 2 per cent. propionic-orcein solution and normal hydrochloric acid (9 : 1) for 4-5 seconds and were kept in the mixture overnight. Then they were squashed in a drop of 45 per cent. propionic acid.

Meiotic study presented considerable difficulties owing to the heavy cell contents. To remove cell inclusions, the flower buds in most cases were fixed and kept in a propionic acid : ethyl alcohol mixture (1 : 2) for four days, then transferred to 45 per cent. propionic acid and the pollinia were squashed in 1 per cent. propionic carmine solution; the slide was warmed to intensify the stain, sealed and then observed.

For paraffin block sections, the buds were fixed in a mixture of propionic

acid and ethyl alcohol (1 : 2) for two to four days and then transferred to absolute alcohol for one day. Following the usual schedule, blocks were made and sections were cut at a thickness of 14μ. Sections were stained by crystal violet staining procedure. Temporary squash preparations of both root tips and flower buds were made permanent by inverting the slide in tertiary butyl alcohol and subsequently mounting in euparal.

Figures were drawn at a table magnification of approximately × 3000 and chromosomes with secondary constrictions are shown in outline.

3. Observations

Seventeen species under fourteen genera of this family have been investigated. They form cytologically a homogenous group, with chromosome numbers of $2n = 22$ and 44. The chromosomes are, in general, very short. Slight size differences are present, forming a graded series. On critical analysis, the following types of chromosomes can be distinguished:

Type A—Long chromosome with two constrictions, primary and secondary, one submedian and the other nearly subterminal in the long arm (4·8-2·3μ).

Type B—Long chromosome with two constrictions, primary and secondary, one nearly median and the other submedian (4-2·3μ).

Type C—Long chromosome with a nearly median primary constriction and a satellite, at the distal end of one of the arms (3·7-2·2μ).

Type C′—Long chromosome with a submedian primary constriction and a satellite located at the distal end of the shorter arm (3·6-2·2μ).

Type C″—Long chromosome with a subterminal primary constriction and a satellite located at the distal end of the longer arm (2·7-1·9μ).

Type D—Long chromosome with two submedian constrictions dividing the chromosome into three equal segments (2·7-1·7μ).

Type E—Long chromosome with median to submedian primary constriction (3-2·5μ).

Type F—Medium sized chromosome with subterminal to median primary constriction (2·3-2μ).

Type G—Very short chromosome with submedian to median primary constriction (1·7-1·2μ).

Genus: *Asclepias*

A. curassavica Linn

$2n = 22 = 2A+2C''+2E+10F+6G$; 2·7-2·0$\mu$ (fig. 1);
$n = 11$ bivalents in diakinesis and metaphase I (fig. 2).

Genus: *Calotropis*

C. gigantia Ait. (i) *Variety with violet flowers*

$2n = 22 = 2B+2C+2E+12F+4G$; 2·3-1·0$\mu$ (fig. 3);
$n = 11$ bivalents in diakinesis and metaphase I (fig. 4).

C. gigantia Ait. (ii) *Variety with white flowers*

$2n = 22 = 4A+2E+8F+8G$; 3·3-1·3μ (fig. 5);
$n = 11$ bivalents in diakinesis and metaphase I (fig. 6).

FIGS. 1 and 2.—*Asclepias curassavica*, somatic and meiotic metaphases.
FIGS. 3 and 4.—*Calotropis gigantia* var. I, somatic and meiotic metaphases.
FIGS. 5 and 6.—*C. gigantia* var. II, somatic and meiotic metaphases.
FIGS. 7, 8 and 9.—Somatic metaphase plates of *Caralluma fimbriata*, *Ceropegia woodii* and *Cryptolepis buchanani* respectively.
FIGS. 10 and 11.—*Cryptostegia grandiflora*, somatic and meiotic metaphases.
FIGS. 12, 13 and 14.—*Daemia extensa*, somatic, meiotic and pollen grain metaphases.
FIGS. 15 and 16.—*Dregea volubilis*, somatic and meiotic metaphases.
FIGS. 17, 18, 19, 20, 21, 22, 23.—Somatic metaphase plates of *Hemidesmus indicus*, *Hoya sikkimensis*, *H. parasitica*, *Huernia macrocarpa*, *Stapelia grandiflora*, *S. reflexa*, and *Stephanotis floribunda* respectively.
GS. 24, 25.—*Tylophora asthmatica*, somatic and meiotic metaphases.

14
15
17
16
25
18
19
23
22
24
21

Irregularities in meiotic behaviour like lagging chromosomes and early separation of chromosomes are very common.

Genus = *Caralluma* R. Br.

C. fimbriata Wall.

$2n = 44 = 2A+2C'+8E+26F+6G$; 2·6-1·1$\mu$ (fig. 7).

Genus: *Ceropegia* Linn.

C. woodii Schlechter

$2n = 44 = 2C+2C'+2C''+4E+26F+8G$; 2·7-1·0$\mu$ (fig. 8).

Genus: *Cryptolepis* R. Br.

C. buchanani Roem and Schult

$2n = 22 = 2B+2C+6E+8F+4G$; 2·8-1·0μ (fig. 9).

Genus: *Cryptostegia* R. Br.

C. grandiflora R. Br.

$2n = 22 = 4A+2E+10F+6G$; 3·3-1·7μ (fig.10).

Meiotic study reveals regular eleven bivalent formation in diakinesis and metaphase I (fig. 11). The bivalents form secondary associations which persist in some cases up to metaphase II. The basic number, as determined from the maximum association, is $x = 4$.

Genus: *Daemia* R. Br.

D. extensa R. Br.

$2n = 22 = 2A+2C+6E+10F+2G$; 4·3-1·3$\mu$ (fig. 12).

Meiosis in this species shows eleven bivalents in diakinesis and metaphase I (fig. 13). Pollen grain mitosis was also studied which supports the number and types of the chromosome observed in the somatic complement (fig. 14).

Genus: *Dregea* E. Mey.

D. volubilis Benth.

$2n = 22 = 2A+6E+8F+6G$; 3·3-1·6μ (fig. 15);

$n = 11$ bivalents in meiotic metaphase I (fig. 16).

Genus: *Hemidesmus* R. Br.

H. indicus H. Br.

$2n = 22 = 2C''+4E+6F+10G$; 2·3-1·1μ (fig. 17).

Genus: *Hoya* R. Br.

H. sikkimensis Hook. f.

$2n = 22 = 4A+2E+14F+2G$; 3·8-1·5μ (fig. 18).

H. parasitica Wall.

$2n = 22 = 2B+2C'+6E+10F+2G$; 3·8-1·3$\mu$ (fig. 19).

Genus: *Huernia* R. Br.

H. macrocarpa Schweinf.

$2n = 44 = 2C+2D+8E+26F+6G$; 3·2-1·1$\mu$ (fig. 20).

Genus: *Stapelia* Linn.

S. grandiflora Mass.

$2n = 22 = 2B+2C''+2D+4E+10F+2G$; 2·6-1·3$\mu$ (fig. 21).

S. reflexa Haw.

$2n = 44 = 2C'+2C+6E+18F+16G$; 2·8-1·1$\mu$ (fig. 22).

Genus: *Stephanotis* Thou.

S. floribunda Brongn.

$2n = 22 = 4B+2C+8E+6F+2G$; 8·6-1·6μ (fig. 23).

Genus: *Tylophora* R. Br.

T. asthmatica Wight and Arn.

$2n = 22 = 2D+2E+12F+6G$; 1·6-1·0μ (fig. 24);

$n = 11$ bivalents in meiotic metaphase I (fig. 25).

4. Discussion

The Asclepiadaceae is primarily tropical with only a few genera in the temperate regions of the northern and southern hemispheres. Both the taxonomic status of the family and the number of genera included in it have been subjects of dispute amongst taxonomists. It has been included with Oleaceae, Loganiaceae, Gentianaceae (Contorteae of Engler) based on the aestivation of the perianth, structure of calyx and the number of carpels. The Asclepiadaceae and Apocynaceae are the most closely related with regard to structure of flowers, seeds, fruits, adherence of the anthers to the stigma and the semi-free carpels. The Asclepiadaceae differs from the Apocynaceae in the modification of the stamens associated with the presence of translators and the gynostegium. Hutchinson (1959) however, split up the order Loganiales into three separate orders Loganiales, Apocynales and Gentianales. Within the Apocynales again he formed four families mainly on the character of the pollen. The families Plocospermaceae, Apocynaceae and Periplocaceae have granular pollen and they are therefore supposed to be closely related. Asclepiadaceae with pollen in waxy sub-pallucid pollinia is regarded as an independent group.

Hallier (1905) included the Asclepiadaceae with the Apocynaceae and derived them from the Linaceae. Bessey (1951) and his contemporary taxonomists regarded the two families as distinct and included both in Gentianales, an order he derived from the Geraniaceous stock. Hutchinson considered the Apocynales to be derived from a stock ancestral to the Loganiaceae. The genera *Cryptolepis*, *Cryptostegia*, *Hemidesmus*, etc. have been transferred by Hutchinson (1959) from the Asclepiadaceae to the family Periplocaceae on the basis of pollen character.

Under the system of classification adopted by Hooker (1883) the genera *Hemidesmus*, *Cryptolepis*, *Cryptostegia* and *Periploca* in addition to others have been included under the tribe Periploceae; *Secamone* under Secamoneae; *Asclepias*, *Calotropis*, *Pentatropis*, *Daemia*, *Cynanchum* under Cynancheae; *Tylophora*, *Dregea*, *Hoya*, *Stephanotis*, *Dischidia*, *Pycnorachis*, *Pergularia*, etc., in tribe Marsdenieae and *Leptadenia*, *Ceropegia*, *Frerea*, *Caralluma* under Ceropegieae. Of these, the first tribe is included under a separate sub-order Periploceae while the remaining ones have been placed under another sub-order Eusclepiadeae.

In the system of classification given by Mitra (1964) there are two sub-families Periplocoideae corresponding to the sub-order Periploceae of Hooker and Cynanchoideae corresponding to a second sub-order, Eusclepiadeae. The distribution of different genera under the different tribes is slightly different from that of Hooker but the positions of *Hemidesmus*, *Cryptolepis*, *Cryptostegia* and *Periploca* together in a group separate from the other genera remain constant.

Cytological data from present and previous reports show the predominance of the basic number of $n = 11$ chromosomes in almost all genera of Asclepiadacea e.g. *Asclepias*, *Calotropis*, *Caralluma*, *Ceropegia*, *Cryptolepis*, *Cryptostegia*, *Cynanchum*, *Dischidia*, *Daemia*, *Dregea*, *Frerea*, *Gomphocarpus*, *Hemidesmus*, *Hoya*, *Hoodia*, *Huernia*, *Leptadenia*, *Mangenotia*, *Metaplexia*, *Pachycarpus*, *Pergularia*, *Periploca*, *Pentatropis*, *Pycnostelma*, *Sarcostemma*, *Secamone*, *Stapelia*, *Tylophora* and *Vincetoxicum* (Bezbaruah, 1963; Biswas, 1955, 1957; Böcher and Larsen, 1955; Bowden, 1940; Chopde, 1965; Diers, 1961;

Harvey, 1966; Huynh, 1965; Jaworska, 1964; Lewis, 1961; Lopane, 1951; Löve and Löve, 1961, 1964; Mangenot and Mangenot, 1958, 1962; Miege, 1960; Moore, 1946, 1959; Moyer, 1936; Mulay *et al.*, 1965; Pardi, 1934; Raghavan and Arora, 1958; Riley and Van't-Hoff, 1961; Sax and Husted, 1936; Sokolovskaya, 1966).

Other basic numbers like $n = 10$ and 12 have been observed in *Araugia* and *Metaplexia* (Schnack and Covas, 1947; Sokolovskoya, 1966). Two basic sets $n = 11$ and 12 have been reported in *Periploca*, *Cryptostegia* and *Daemia* (Bowden, 1940; Lopane, 1951; Nirula, 1945; Pardi, 1934; Sax and Husted, 1936) and $n = 11$ and 11 simultaneously in different species of *Philibertia* (Covas and Schnack, 1946, 1947; Diers, 1961). These numbers suggest that $n = 11$ is deep seated for this family but $n = 10$ and 12 indicate the role of aneuploidy in evolution within the genera and a relationship between the two sets.

The family Apocynaceae shows several basic sets, *i.e.* $n = 8$, 9, 10, 11 and 12, $n = 11$ being most common. This similarity in basic numbers provides further support to the taxonomists' contention that the Asclepiadaceae and Apocynaceae are closely related and justify their inclusion within the same group Apocynales by Hutchinson as distinct from Gentianales and Loganiales.

A study of the karyotypes shows that the members of the family Asclepiadaceae from a very homogeneous complex. Not only is the basic number the same, being $n = 11$ in almost all of them, but the chromosome types are also very similar. In all, seven general chromosome types which form a graded series have been distinguished amongst the members of the family studied here. The karyotypes of different species belonging to the same genus and also different genera differ from each other with respect to the combinations of these different types.

Based on chromosome morphology, the genera *Cryptolepis*, *Cryptostegia* and *Hemidesmus* appear to show as much similarity with the other genera of the family Asclepiadaceae as amongst themselves and so their inclusion under a separate family Periplocaceae as suggested by Hutchinson does not appear justified. As mentioned before, data from meiotic behaviour and karyotype analysis show the close affinity of these genera with the rest of the Asclepiadaceae and possibly structural changes have been responsible for the diverging morphological characters which encouraged taxonomists to place them in a distinct group.

The records of chromosome number include cases of both $2n = 22$ and 44 chromosomes in the same species as, for example, in *Caralluma fimbriata* Wall, *Cynanchum vincetoxicum* (L) Pers., and species of *Stapelia* and *Vincetoxicum* (Böcher and Larsen, 1955; Chopde, 1965; Löve and Löve, 1961; Pardi, 1934). $2n = 44$ chromosomes are present in *Ceropegia woodii debilis*, *Huernia nacrocarpa*, *Stapelia reflexa*, *S. variegata*, *Frerea indica*, etc. (Chopde, 1965; Pardi, 1934). These observations indicate the presence of polyploid series both at intra- and inter-specific levels.

Since the family is rather successful with a wide distribution over the tropics and a highly specialised floral structure, the cytological data so far obtained suggest that the structural alterations of chromosomes have been the major factor in the evolution of the different members of the family aided to some extent by aneuploidy.

The report of polysomaty in the roots of *Tylophora asthmatica* by Biswas

(1955) is interesting but its significance in evolution is yet to be established.

Both varieties of *Calotropis gigantia* have $2n = 22$. They differ principally in the number of satellited chromosomes, one in the variety with violet flowers and two in the variety with white flowers. Of the two species of *Hoya*, *H. parasitica* contains one pair of satellited chromosomes and one pair of chromosomes with secondary constrictions whereas *H. sikkimensis* has two pairs of chromosomes with secondary constrictions. These are interesting examples where only structural changes in the satellited chromosomes are associated with evolution of species.

Regarding the interrelationships of the genera included within different tribes, a gross similarity of the chromosome morphology and the presence of the same basic number in all of them show that these genera are closely related forming a homogenous complex. Therefore, within this family evolution from the intervarietal stage to interspecific and even intergeneric levels has evidently proceeded through a series of structural changes both at the submicroscopic genic level and at the level of the structure of the chromosomes.

It is a remarkable fact that both in vegetative and floral characters, the family represents a wide range of variation resembling xerophytic cacti on the one hand and even certain epiphytes on the other. Intermediate forms are also abundant. In spite of this diversity, homogeneity is specially manifested in certain fundamental floral and vegetative characters.

Concomitant with this diversity against a background of basic homogeneity there has been very little change in the basic set of chromosomes which is mostly eleven with occasional aneuploids as pointed out above. Polyploidy however is not uncommon specially in xerophytic forms indicating their adaptation to extreme climatic conditions (Löve and Löve, 1949; Cain, 1944). It is clear, therefore, that detectable chromosomal diversity has not been as profuse as the phenotypic diversities exhibited by the taxa. This may be taken to imply that the importance of undetectable gene mutation has also been an important factor in the evolution of different taxa.

5. Summary

1. Cytological studies have been carried out on seventeen species under fourteen genera of the family Asclepiadaceae. All the members of the family studied here show a uniform basic number of $n = 11$ chromosomes. On the basis of detailed chromosome analysis, changes at the genic level and minor chromosomal alterations have been shown to be the principal factors in their evolution. Evidence presented indicate that these genera form a homogenous assemblage.

2. The impropriety of the inclusion of *Cryptolepis*, *Cryptostegia* and *Hemidesmus* under a separate group as claimed by certain taxonomists has been pointed out on the basis of the observations presented here.

3. The position of this family under the order Apocynales, having affinity with the Apocynaceae, is justified on cytological grounds.

6. References

BENTHAM, G., AND HOOKER, J. D. 1883. Genera Plantarum, London.

BESSEY, C. E. 1915. Phylogenetic taxonomy of flowering plants. *Ann. Mo. Bot. Gard.*, *2*, 1-155.

BEZBARUAH, H. P. 1963. Induced intraploidy in *Asclepias curassavica* L. *Curr. Sci., 32,* 376.

BISWAS, I. 1955. Cytological and embryological investigations in the Asclepiadaceae. *Curr. Sci., 24,* 204-205.

BISWAS, I. 1957. Chromosome numbers of some Asclepiadaceae. *Curr. Sci., 26,* 218-219.

BÖCHER, T. W., AND LARSEN, K. 1955. Chromosome studies on some European flowering plants. *Bot. Tidsskr., 52,* 125-131.

BOWDEN, W. M. 1940. Diploidy, polyploidy and winter hardiness relationships in the flowering plants. *Amer. J. Bot., 27,* 357.

CAIN, S. A. 1944. *Foundations of Plant Geography.* Harper and Bros., New York and London.

CHOPDE, V. P. 1965. Chromosome numbers in some flowering plants. *Sci. Cult., 31,* 30.

*COVAS, G., AND SCHNACK, B. 1946. *Rev. argent. Agron., 13,* 153.

*COVAS, G., AND SCHNACK, B. 1947. *Rev. argent. Agron., 14,* 224.

DIERS, L. 1961. Der Anteil an polyploiden in den vegetations-gurteln der West Koradillera perus. *Zeits. Bot., 49,* 437-488.

ENGLER, A. 1887. *Further durch den koniglichen botanischen Garten der Universitat zu Breslau.* Breslau Pfanzenreich, Leipzig.

HALLIER, H. 1905. Phylogenetic studies of flowering plants. *New Phytologist, 5,* 151-162.

HARVEY, M. J. 1966. In IOPB chromosome number reports. VIII. *Taxon, 15,* 155-163.

HOOKER, J. D. 1883. *The Flora of British India,* IV, 1-78. L. Reeve and Co. Ltd., Kent, U.K.

HUTCHINSON, J. 1959. The Families of Flowering Plants. Vol. I. Macmillan and Co., Ltd., N.Y.

HUYNH, K. L. 1965. Contribution a' l'etude caryologique et embryologique des phanerogames du Pe'rou. *Deutschr. Schweiz. Nat. Ger., 85,* 1-178.

JAWORSKA, H. 1964. Kariological study of *Vincetoxicum officinale. Stud. Soc. Sci. Torun., 7,* 3-12.

LEWIS, W. H. 1961. Chromosome numbers for four North American *Asclepias. Southwest Nat., 6,* 46-47.

*LOPANE, F. 1951. *Caryologia, 4,* 44.

LÖVE, A., AND LÖVE, D. 1949. 1. The Geo-Botanical Significance of Polyploidy. 2. Polyploidy and latitude. *Portug. Acta. Biol. R.B. Gold-Schmidt volume,* 273-352.

LÖVE, A., AND LÖVE, D. 1961. Chromosome numbers of central and northwest European plant species. *Op. Bot. (Lund), 5,* 1-581.

LÖVE, A., AND LÖVE, D. 1964. In IOPB chromosome number reports. *Taxon, 13,* 100-110.

MANGENOT, S., AND MANGENOT, G. 1958. Deuxieme lisle de nombres chromosomiques nouveaux chez diverses dicotyledones et monocotyledones d'Afrique occidentale. *Bull. Jard. Bot., Bruxelles, 28,* 315-329.

MANGENOT, S., AND MANGENOT, G. 1962. Enquete sur les nombres chromosomiques dans une collection deespeces tropicales. *Rev. Cytol. Biol. Veg., 25,* 411-447.

MIEGE, J. 1960. Nombres chromosomiques de plantes d'Afrique occidentale. *Rev. Cytol. Biol. Veg., 21,* 373-384.

MIEGE, J. 1960. Troisieme listle de nombres chromosomique d'especes d'Afrique occidentale. *Ann. Fac. Sci. Univ. Dakar, 5,* 75-86.

MITRA, J. N. 1964. *An Introduction to Systematic Botany and Ecology.* The World Press Private Ltd., Calcutta.

MITRA, K., AND DATTA, N. 1967. In IOPB chromosome number reports. *Taxon, 16,* 459.

*MOORE, R. J. 1946. *Canad. J. Res., 24*: 66.,

MOORE, R. J. 1959. The dog-strangling vine *Cynanchum medium*—its chromosome number and its occurrence in Canada. *Canad. Field Nat., 73,* 144-146.

MOYER, L. S. 1936. Chromosome numbers and electrophoresis of latex in *Asclepias. Bot. Gaz., 97,* 860.

MULAY, B. N., DESHPANDE, B. D., AND TOLANI, U. 1965. Studies in Asclepiadaceae II. Floral morphology and gametogenesis in certain members of the Asclepiadaceae. *J. Ind. Bot. Soc., 44,* 95-104.

NIRULA, R. L. 1945. Embryological and cytological studies in the family Asclepiadaceae. *Proc. Indian Acad. Sci. B, 21,* 174.

*PARDI, P. N. 1934. *Nuovo Gorn. Bot. Ital. N.S., 40,* 560-576.

RAGHAVAN, R. S., AND ARORA, C. M. 1958. Chromosome numbers in Indian medicinal plants. *Proc. Ind. Acad. Sci., 47B,* 352-358.

RILEY, H. P., AND VAN'T HOFF, J. 1961. Chromosome studies in some South African dicotyledons. *Canad. Jour. Genet. Cyt., 3,* 260-271.

SAX, K., AND HUSTED, L. 1936. Polarity and differentiation in microspore development. *Amer. J. Bot., 23,* 606.

*SCHNACK, B., AND COVAS, G. 1947. *Haumania, 1,* 32.

SHARMA, A. K., AND MOOKERJEA, A. 1955. Paradichlorobenzene and other chemicals in chromosome work. *Stain Techn., 30,* 1-7.

SHARMA, A. K., AND SARKAR, S. K. 1955. A new technique for the study of the chromosomes of palms. *Nature, 176,* 261.

SOKOLOVSKAYA, A. P. 1966. Geograficheskoe resperostranenie polyploidnykh vidov rateniy (Issledovanie Flory primorskogo kraya). *Ventnik Leningr. Univ. Ser. Biol., 3,* 92-106.

* Taken from DARLINGTON, C. D., AND WYLIE, A. P. 1955. *Chromosome Atlas of Flowering Plants.* George Allen and Unwin Ltd., London.

POLYPLOIDY AND CHROMOSOME SIZE

A. K. SHARMA

Department of Botany, Calcutta University, Calcutta 19, India

1. Introduction

Considerable debate has already been held regarding the phylogenetic reduction in chromosome size in different groups of plants. Such reduction is not necessarily associated with increase in chromosome number. A number of cases is also on record where there has been an increase in chromosome size with evolutionary advance, the best examples being in certain groups of grasses (Jones and Rees, 1968; Rothfels and Heimburger, 1968; and see Jones, 1970). Polyploidy, which is of wide occurrence in plant genera, is associated with a gradual diminution in chromosome size in the majority of taxa.

In the lower groups of plants, such as algae, where much work has been carried out, particularly on Chlorophyceae, this phylogenetic reduction in chromosome size has been noted in several genera, including the members of the Conjugales (Godward, 1966). In the Charophyceae as well, a similar situation has been met with (Chatterjee, P., 1970) in this laboratory where, along with increase in chromosome number, there has been a slight decrease in the size of the chromosomes.

The study of this correlation has been extensively carried out in Pteridophytes. Litardière (1921) first recorded a phylogenetic reduction in chromosome size in the leptosporangiate ferns (Stebbins, 1960). The situation in Pteridophyta has been extensively reviewed (Stebbins, 1960). The primitive groups of ferns, such as Osmundaceae and Hymenophyllaceae, are characterised by comparatively large chromosomes. The trend of reduction in chromosome size is evidenced in families like Cyatheaceae and Polypodiaceae, where along with evolutionary advance, the chromosome size has undergone a steady diminution. Lastly, in specialised heterosporous families like the Salviniaceae, the chromosomes are very small. The genus *Ophioglossum*, which is regarded as an extremely reduced and specialised genus, borne out also in its very high chromosome number, has extremely short chromosomes.

But in Pteridophytes, certain comparatively rare cases have been recorded of the reverse tendency. The case of *Isoetes coromandeliana* may be cited in which both diploid and triploid forms occur in India having $2n = 22$ and 33 chromosomes respectively. The diploids in this species are characterised by shorter and triploids by longer chromosomes. There are also many cases in Pteridophytes where there is no correlation between evolutionary advance and chromosome size (see Abraham and Mathew, 1962).

In Angiosperms too, though in general the principal tendency has been towards a decrease in chromosome length along with polyploidy, as noted both in monocotyledons and dicotyledons by my colleagues, yet several reports have been published where polyploidy has not been associated with any such decrease (see Research Bulletins, 1968, 1970). A study of chromosome size in diploids and polyploids in various groups of plants leads to the

inference that, in general, polyploidy is mostly associated with a decrease in chromosome size, though there are a number of taxa where no such correlation has been met with.

Before dealing with different views regarding this phylogenetic reduction in chromosome size, it may be mentioned that this decrease in chromosome size is not associated with a decrease in the amount of DNA. The tenet of constancy in the amount of DNA and its proportionate increase along with increase in the number of genomes has been found to be true in all cases.

The different views so far expressed regarding this diminution in chromosome length are outlined below.

Pierce (1937) first suggested that nutritional deficiency, specially of phosphorus in the medium, is responsible for reduction in chromosome size. Bennett and Rees (1967) have also recorded the role of phosphate ions in the medium as a factor in determining chromosome size.

Tobgy (1943) regarded spiralisation as the controlling factor implying thereby that compact spiralisation is responsible for diminution in size. It has also been suggested that diminution or retention of heterochromatin may also influence chromosome size. That heterochromatin may contribute significantly to chromosome size has been shown in several Liliaceous genera such as *Trillium*, *Fritillaria* and *Paris* (Darlington and La Cour, 1940) as well as in species of *Isoetes* where heterochromatin represents about two-thirds of the chromosome size (Abraham and Mathew, 1962).

Darlington (1964) suggested that reduction in chromosome size, which is genotypically controlled, is an adaptation to a decrease in size of the cell or to an increase in the number of chromosomes. This reduction has further been attributed to a premature splitting of chromosome threads which thus enter into mitosis as smaller bodies.

2. Observations

In this laboratory (Research Bulletins, 1968, 1970) in the course of different projects, diminution in chromosome size from diploids to polyploids has been recorded not only in species occurring in nature such as *Vicia*, *Commelina*, *Lens*, *Lathyrus*, *Ophiopogon*, *Chlorophytum*, etc. but even in induced colchitetraploids of several species of *Nigella*, *Spironema* and *Vicia*. The sudden decrease in chromosome size, even in the C_1 generation, indicates the efficiency of the genotypic control in responding quickly to the altered circumstances. It is necessary to have an understanding of the mechanism through which this control is manifested in the chromosome, ultimately resulting in decreased chromosome size. That this decrease is not associated with any change in permanent DNA content has been well established, and, in all cases, polyploids have been shown to be associated with the proportionate increase in DNA content expected in case of genome duplication.

Possibly an insight into the mechanism can be obtained if the role of heterochromatin is taken into account. From a general survey of the chromosome numbers and the accessory chromosomes in the plant kingdom, Darlington (1964) noted that B chromosomes are mostly found in diploids, whereas in polyploids they are rather infrequent. It is also well established that these accessories confer a certain amount of adaptability and have a selective value (Müntzing, 1958 and see p. 208).

In the several cases that have been investigated of plants collected from

the Himalayas as well as from the tropical Indian plains, a similar situation has mostly been met with indicating that B chromosomes are mostly frequent in the diploid species. Of these, two special cases are to be mentioned, which have a direct bearing on the problem of diminution in chromosome size. A species of *Allium*, viz. *A. stracheyii* is found in the Himalayas. This species is characterised by 14A chromosomes and as many as 8B chromosomes. It has already been reported (Sharma and Aiyangar, 1962) that when this species is grown in tropical conditions in Calcutta, the root cells not only lose the B chromosomes but they become completely converted into polyploids. In other words, adaptability afforded by polyploidy is associated with the elimination of B chromosomes. Recently, polyploid individuals of *Allium stracheyii* without B chromosomes have been collected from the Himalayas.

In *Urginea indica* (Sen, 1970), which grows in different parts of Peninsular India, both diploid and polyploid individuals have been collected, especially from the coastal areas. Several populations of diploids growing in ecologically distinct areas are characterised by the presence of up to six B chromosomes. Polyploids on the other hand, show a complete absence of B chromosomes. In both *Allium stracheyii* and *Urginea indica*, polyploids do not show any significant diminution in chromosome size as compared to diploids.

3. Interpretation of data

On the basis of these observations, it appears reasonable to conclude that the selective value in adaptibility conferred by accessories is no longer needed by polyploids where increased gene dosage allows wider tolerance to environmental differences. As accessory chromosomes are often heterochromatic, this conclusion implies that additional heterochromatin, being redundant in polyploids, is eliminated by selection.

This redundancy of heterochromatic chromosomes in polyploids may be applied to heterochromatin segments of other chromosomes as well. Tobgy (1943) suggested that heterochromatic segments may be eliminated in polyploids. But such elimination even in the C_1 generation, involving breakage and deletion, is difficult to reconcile with the proportionate duplication of DNA in polyploids.

On the other hand, it is clear that the need for heterochromatic segments is lessened in polyploids as noted from observations in species of *Allium* and *Urginea* and also from the fact that B chromosomes are found mostly in diploids. It may be suggested therefore that heterochromatic segments, being not needed, are rendered non-functional rather than eliminated in polyploids. Their presence in a non-functional state can be reconciled with the proportionate duplication of DNA in polyploids.

It is not difficult to visualise the mechanism through which this non-functional state can be achieved. It is well established that gene activity involves uncoiling and separation of the polynucleotide columns in the DNA double helix. It is quite possible therefore, that heterochromatic segments needed at the diploid level become condensed in polyploids because of their redundancy.

Such condensation of heterochromatic segments would result in a decrease in chromosome size, without involving a decrease in DNA content.

4. Implications of the inference

This assumption of spiralisation and consequent condensation of heterochromatic segments could explain all the discrepant reports so far obtained in relation to polyploidy and chromosome size. Such cases are:

(*a*) Polyploids, which are not characterised by any difference in chromosome size as compared to diploids: Such cases may represent species where diploids do not have any significant amount of heterochromatin, *e.g. Vicia sativa. V. faba* on the other hand, having prominent heterochromatic segments in diploids, shows significant difference in chromosome size in polyploids. La Cour's (1951) observations in *Trillium tschonoskii* are also significant, where reduction of heterochromatic segments has been noted in tetraploids. Such reduction may reflect a high degree of compactness and as such is not visible under cold treatment. An investigation of comparative chromosome size in polyploids and diploids should include diploids with and without heterochromatin. Induction of tetraploidy in species of *Fritillaria* for such a comparative study may prove to be fruitful.

(*b*) Cases of *Allium stracheyii* and *Urginea indica* where no significant reduction in size is involved in polyploids: In these species, heterochromatic regions are represented in B chromosomes which are absent in polyploids. Consequently no further diminution in chromosome size is expected.

(*c*) Chromosome size in *Isoetes coromandeliana* where triploids have longer chromosomes than diploids: This case can simply be explained if the instability of triploids and the adaptive capacity of heterochromatin are considered together. This species is rather unusual in that, even though it is triploid, it survives through spore production and not apomictic reproduction. The instability consequent to triploidy may have been overcome by the adaptive capacity of heterochromatic segments which are more functional in triploids than in diploids. In other words, it is implied that as an adaptive measure, condensed heterochromatic blocks of diploids are rendered more functional in triploids through uncoiling of their segments. This uncoiling is responsible for increase in chromosome size in triploid *Isoptes coromandeliana* ($2n = 33$) a mechanism needed to meet the unstable situation in this species.

Therefore, on the basis of the evidences and discussions presented above, it can be concluded that the genotypic control of increase or decrease in chromosome size consequent to polyploidy is exerted through the buffering action of heterochromatin—its uncoiling and coiling.

5. References

ABRAHAM, A., AND MATHEW, P. M. 1962. Studies on the cytology and phylogeny of the Pteridophytes. *Jour. Ind. Bot. Soc., 41*, 339-421.

BENNETT, H. D., AND REES, H. 1967. Natural and induced changes in chromosome size and mass in meristems. *Nature, 215*, 93-94.

CHATTERJEE, P. 1970. Cytotaxonomy of Charophyceae (in press).

DARLINGTON, C. D. 1964. *Chromosome Botany*. George Allen and Unwin Ltd., London.

DARLINGTON, C. D., AND LA COUR, L. F. 1940. Starvation of chromosomes of *Trillium*. *J. Genet., 40*, 185-213.

GODWARD, M. B. E. 1966. *The Chromosomes of the Algae*. Edward Arnold and Co., London.

JONES, K. 1970. Chromosome changes in plant evolution. *Taxon, 19*, 172-179.

JONES, R. N., AND REES, H. 1968. Nuclear DNA variation in *Allium*. *Heredity, 23*, 591-605.

LA COUR, L. F. 1951. Heterochromatin and the organization of nucleoli in plants. *Heredity, 5*, 37-50.

LITARDIÈRE, R. D. 1921. Recherches sur l'element chromosomique dans la caryocinese somatique des Filicinees. *La Cellule, 31*, 255-473.

MÜNTZING, A. 1958. A new category of chromosomes. *Proc. Xth Inter. Congr. Genetics. Montreal*, 453-467.

PIERCE, W. P. 1937. The effect of phosphorus on chromosomes and nuclear volume in a violet species. *Bull. Torrey Bot. Club, 64*, 345-354.

RESEARCH BULLETINS, I AND II. 1968, 1970. *Ann Rep. Cytogenetics Lab. Dept. of Bot., Calcutta University* (eds. Archana, Sharma and A. K. Sarkar. Calcutta).

ROTHFELS, K., AND HEIMBURGER, M. 1968. Chromosome size and DNA value in sundews. *Chromosoma, 25*, 96-103.

SEN, S. 1970. Studies on *Urginea indica* with special reference to B chromosomes (in press).

SHARMA, A. K., AND AIYANGAR, H. R. 1961. Occurrence of B chromosomes in diploid *Allium stracheyii* Baker and their elimination. *Chromosoma, 12*, 310-317.

STEBBINS, G. L. (JR.). 1960. *Variation and Evolution in Plants.* Columbia Univ. Press, N.Y., U.S.A.

TOBGY, H. A. 1943. A cytological study of *Crepis fuliginosa, C. neglecta* and their F_1 hybrids and its bearing on the mechanism of phylogenetic reduction in chromosome number. *Jour. Genet., 45*, 67-111.

MEIOSIS AND FERTILITY IN APOGAMOUSLY PRODUCED DIPLOID PLANTS OF *ASPLENIUM TRICHOMANES*

J. BOUHARMONT

Institut Carnoy, Université Catholique de Louvain, Belgium

1. Introduction

Asplenium trichomanes is a polymorphic species with a very broad distribution. Two principal cytotypes are distinguished in Europe: the diploid (subsp. *trichomanes*) grows on siliceous rocks, the tetraploid (subsp. *quadrivalens*) is more frequent and lives on various rocks and walls. The cytotypes are morphologically similar and their origins are certainly identical. Other cytological and geographical evidences indicate the autotetraploid origin of subsp. *quadrivalens* from subsp. *trichomanes*. Nevertheless these plants show several characters of allopolyploidy (Bouharmont, 1968).

The induction of diploid plants from the tetraploid ones was attempted in order to estimate the extent of homology between the genomes and to elucidate the nature of the tetraploid cytotype.

2. Production of diploid plants

Many ferns have a regular apogamous reproduction. Their prothalli do not possess archegonia and the embryo differentiates from the central cushion of the gametophyte. On the mature sporophyte the sporangia contain 32 unreduced spores instead of 64 haploid ones as in the sexual species.

In sexually reproducing species the gametophyte is able to live for several months and, when fertilization is prevented, survival and growth are prolonged (Walp, 1951). In sterile cultures on agar medium, the archegonia are not fertilized for want of water, the growth of the prothalli is unlimited and, sometimes, a few agamous sporophytes are formed (Morel, 1963).

Manton and Walker (1954) obtained apogamous plants of *Dryopteris* from unfertilised gametophytes. Whittier (1964, 1966) and Whittier and Steeves (1960) studied the factors acting on apogamy in cultured prothalli of *Pteridium aquilinum*. Light and succinic acid promote apogamy and the optimum sugar concentrations are 2·5 per cent. for glucose and 5 per cent. for sucrose; the best results were obtained by culturing the gametophytes successively on three different media, the first with a high concentration of sugar (4 per cent.), the second with a high level of sugar and growth substances and the third with only 0·1 per cent sugar. Whittier (1962) also described the principal stages of apogamy in Pteridium: the development started on the cushion bearing the archegonia and the first indications were the formation of a meristematic centre inside and the appearance of sporophytic hairs on the surface of the prothallus.

In our experiments we have used the culture medium of Knudson (1925) with the micronutrients of Heller (1953), 0·8 per cent. agar, various concentrations of sugar (sucrose or glucose), adenin and kinetin, and sometimes some growing substances. The cultures were made in large test tubes. The spores were sterilised for 15 minutes in a 5 per cent. solution of sodium

hypochlorite with saponin. They were then washed three times in sterile distilled water and sowed on the surface of the culture medium with the help of a pipette.

Several *Asplenium* species have been used. For *A. septentrionale*, the first apogamous sporophytes appeared nine months after sowing and several hundred young sporophytes have been obtained. Under the same conditions, a few plants appeared after 17 months in *A. trichomanes* subsp. *quadrivalens*. Sugar was necessary for apogamy, but plantlets differentiated on very different media. Sucrose had been used at concentrations of 1 to 5 per cent. and glucose at 1 to 2 per cent. Several substances have been added without apparent effect on apogamy but they had sometimes an influence on the growth of the prothalli. We have tested vitamins, casein hydrolysate, coconut milk and different proportions of adenin and kinetin.

Apogamous embryos were initiated on the foliaceous prothalli but more often on thickened proliferations. The first external indication of apogamy was the appearance of brownish scales similar to the rhizome and petiole scales. The first apogamous leaf was more dissected and differentiated than that from zygotes. When the plantlets were several months old and had 2-5 leaves, they were transferred to soil in a humid atmosphere. Adventitious roots were not formed before 1-2 months. The early growth of the plants was very slow, several were lost before rooting or later, and sporangia were observed a year after transplantation.

The adult plants were not morphologically different from the native ones. The leaves were smaller than for subsp. *quadrivalens* at the same age and they were indistinguishable from the fronds of the normal diploid subspecies *trichomanes*.

3. Spore fertility

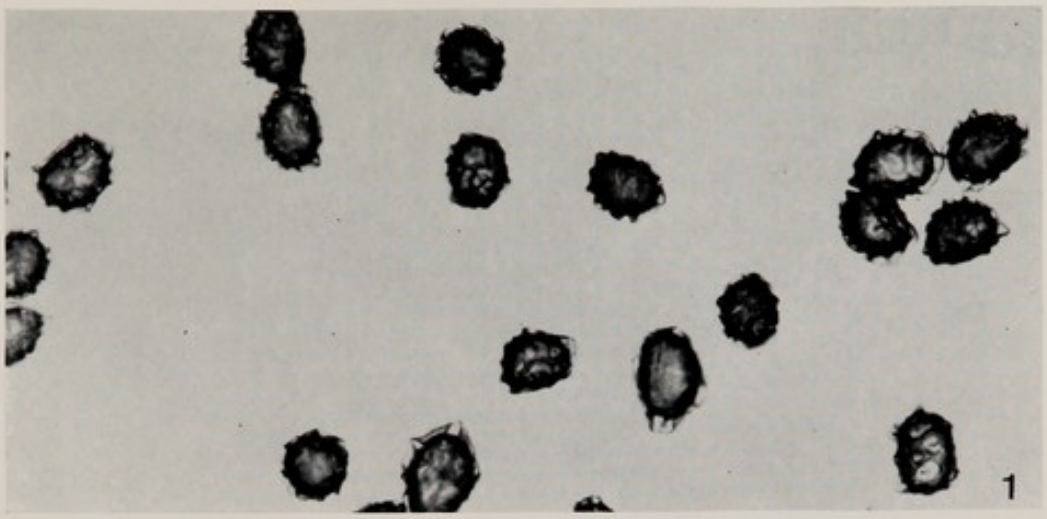
In table 1 are reported the different observations performed on mature spores of four adult diploid plants, compared to the tetraploid parent (subsp. *quadrivalens*) and subsp. *trichomanes*. The differences between the four plants are slight.

Table 1

Spore size and fertility

	Spores of normal size			Larger spores	
Plants	Fertile %	Breadth μ	Coefficient of variation	Frequency %	Breadth μ
1	77·6	16·8	14·6	1·7	31·0
2	82·6	16·6	12·8	0·6	28·3
3	80·8	16·7	11·7	0·4	27·1
4	78·7	16·2	12·8	1·6	29·6
trichomanes		21·4	7·3		
quadrivalens		27·0	7·7		

The large majority of the spores are relatively small, but their epispore is at least as broad as in the parent plants (plate I, figs. 1-3); their sterility, determined for about 1000 spores per plant is relatively low and reaches nearly 20 per cent. The spores with a regular ovate shape were classified as fertile; in sterile spores, the cell is shrunk and shrivelled.



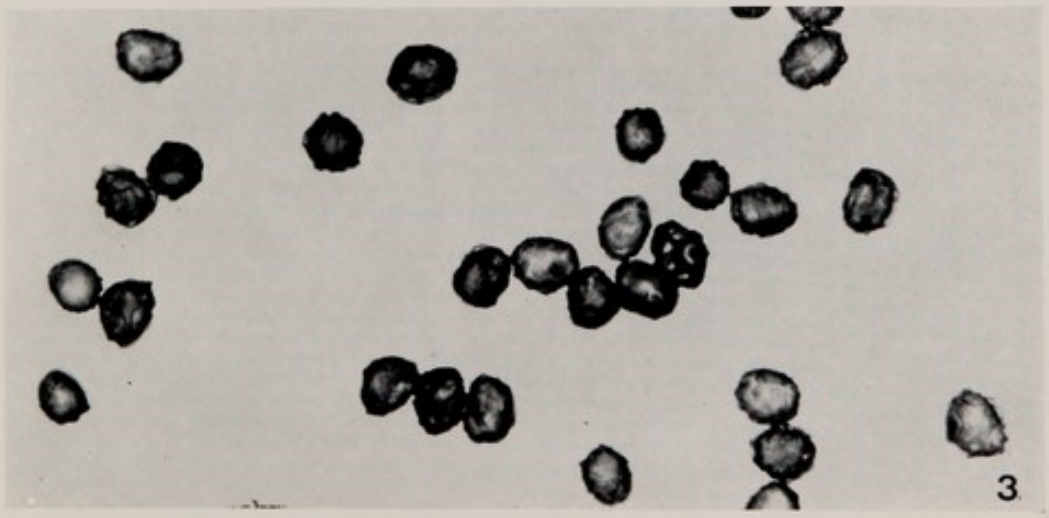
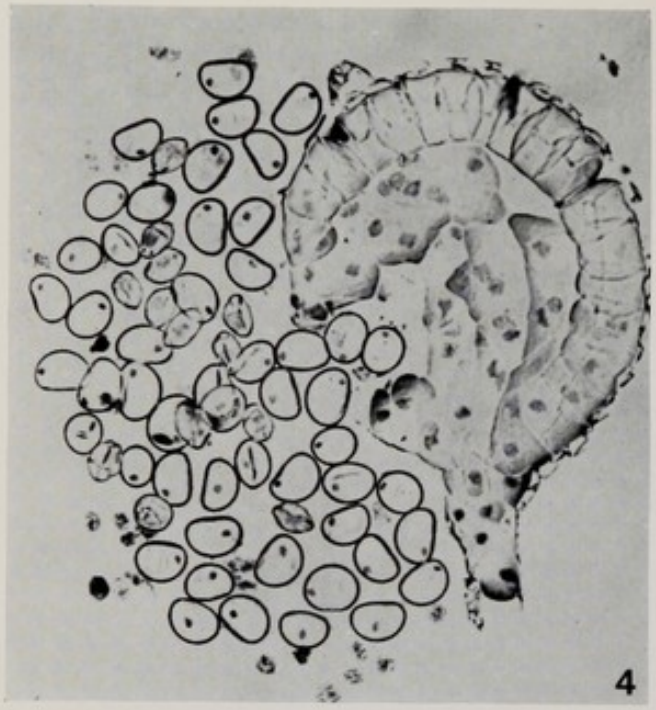
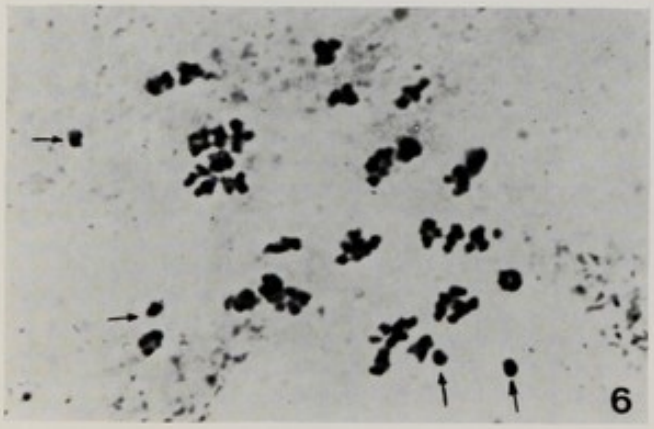
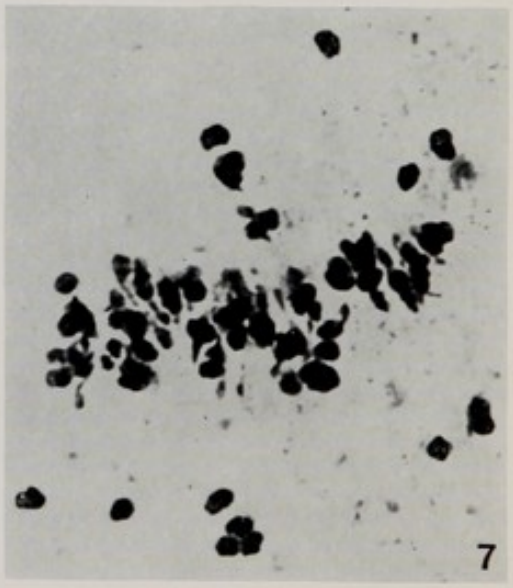
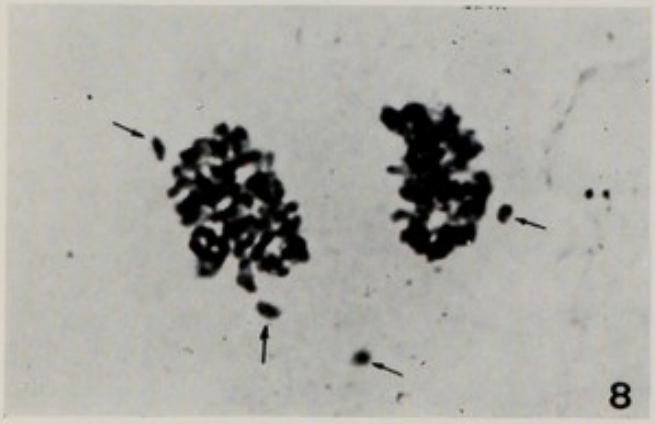


FIG. 1.—Mature spores of *Asplenium trichomanes* subsp. *quadrivalens*.
FIG. 2.—Spores of an induced diploid plant: three of them are unreduced.
FIG. 3.—Spores of subsp. *trichomanes* (×215).









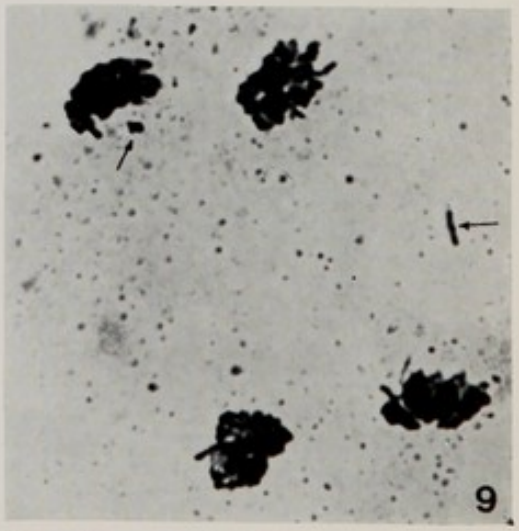


Fig. 4.—Immature sporangium with 50 fertile and 14 sterile spores (×215).
Fig. 5.—Tetrad with two fertile and two sterile spores (×665).
Fig. 6.—Diakinesis: 34 bivalents and 4 univalents (×1330).
Fig. 7.—Metaphase I: many univalents are spread in the cytoplasm (×1330).
Fig. 8 and 9.—Anaphase I and II with several laggards (×1330).

(The enlargement is calculated for a reduction of the plate from 3 to 2)

To measure the breadth of the cells, the spores were dispersed in 50 per cent. acetic acid. The means calculated for 100 cells of the different plants were similar, but very significantly different from the mean determined in the same conditions for the native diploid plants. More generally, the means obtained for many diploid plants from different locations were between 20 and 25 μ (Bouharmont, 1968) and were thus always higher than in our induced diploids. Lower size did not result from the occurrence of a large proportion of very small spores, but from a general shifting of the size classes; the mode of the distribution was 21·0 μ in subsp. *trichomanes* and less than 10 per cent. of the spores reached that level in artificial diploids. On the other hand, the spore size is more variable in induced diploid plants than in diploid and tetraploid subspecies: the coefficient of variation (11·7-14·6 per cent.) is higher than in diploid (7·3 per cent.) and tetraploid (7·7 per cent.).

In natural diploid and tetraploid plants a few large spores are regularly observed: they are unreduced cells, but their frequency is always very low. Large spores occurred also in the four studied plants and their frequency reached about 1 per cent. They were generally well formed and their size corresponded nearly to that of the diploid ones (plate I, fig. 2). Nevertheless, size was rather variable and some cells were clearly larger: the means, calculated for the available cells, are thus often higher than for the spores produced by tetraploid plants. Very small spores were also observed, often attached to larger cells.

On account of the epispore thickness, distinguishing between normally formed and sterile spores is often difficult. Further, spores of different size and density are not necessarily evenly distributed in the mounting medium but we have observed 11 sporangia where it was possible, after squashing, to recover the majority of the 64 spores normally produced (plate II, fig. 4). A little after meiosis, the spores are colourless and without an epispore. Most are egg-shaped, their wall is smooth and their nucleus is small and stained by aceto-carmine. Failed spores are easily distinguished from that stage; the nucleus is diffuse and the wall is a little rough (plate II, fig. 5). Uncertain cases are rarer than at maturity. The frequency of inviable spores in these sporangia was 19·2 per cent. and was thus in good agreement with the sterility level found at maturity during the same period. However, a second sample of 7 sporangia formed during a hot period showed 30·6 per cent. sterility. Some immature spores are larger and their hemispheric or lobed shape indicated an origin from a failure of the second meiotic division or fusion of two cells of the tetrad; several of them showed two nuclei or a single lobed nucleus.

The normal shape of the cells was taken as an indication of fertility. However, these spores have been sowed and their germination was very low. Many " viable " spores were probably unable to germinate. Poor germination, small spore size and the occurrence of fused cells indicate the loss of some chromosomes during meiosis and other irregularities.

4. Meiosis

Observation of the first meiotic division showed that the plants produced by apogamy were diploid with 72 chromosomes as expected. The course of meiosis was relatively regular and seemed identical in the four plants.

Table 2 shows the different numbers of bivalents observed during the first division. The cells are classified according to the stage. These stages are not sharply delimited and the distinction between a bivalent and two univalents was not always certain; but these observations clearly indicate an increase of univalent frequency from diakinesis to prometaphase and metaphase. Chromosome pairing was probably nearly complete in early prophase, for univalents were rare in the earliest observable diakineses, but their frequency increases in late diakinesis and during the following stages (plate II, fig. 6). Many chromosomes remained adjacent, indicating a previous pairing. In metaphase the univalents were rounded (plate II, fig. 7) and generally

TABLE 2

Chromosome pairing

Pairing	Diakinesis	Prometaphase	Metaphase
II+I			
36+0	9	1	3
35+2	7	6	3
34+4	1	4	7
33+6	2	4	3
32+8	3	7	8
31+10	3	4	8
30+12	3	3	8
29+14		2	8
28+16		1	3
27+18		4	4
26+20		1	4
25+22			2
24+24			
23+26			2
22+28			
21+30			1
20+32			1
Total cells	28	37	66
Means	$33{\cdot}7_{II}+4{\cdot}7_{I}$	$32{\cdot}1_{II}+7{\cdot}7_{I}$	$30{\cdot}1_{II}+11{\cdot}8_{I}$

located in the spindle at some distance from the equatorial plate. Another indication of loose pairing was the presence of some linear bivalents with only one terminal chiasma, a very rare configuration in normal plants.

The first observations seemed to show pairing differences between samples fixed at different times and probably related to the observed fertility differences. But it was not possible to confirm these differences because of the variation of pairing pattern according to stage. No differences were detected between the different plants.

During first anaphase many univalents remained in the cytoplasm and they were not included in the interphase nuclei (plate II, fig. 8). Others proceeded toward the poles of the spindle and formed protuberances on the nuclear surface. Lagging chromosomes were frequent in second meiotic division (plate II, fig. 9); some univalents were stretched and formed anaphase bridges, others were lost in the cytoplasm or were responsible for the irregular shape of some nuclei. When the number of bridges and laggards was high, a lobed or irregular restitution nucleus was formed.

The majority of the tetrads were regular but restitution nuclei gave a few large spores and some other spores were united by a bridge. Many normal

spores contained some small chromatic bodies derived from lagging chromosomes outside the nucleus. Other laggards were isolated in very small spores separated from, or attached to, normal ones.

5. Discussion and conclusions

The agreement is very good between the observations on meiosis and on the mature or immature spores. The occurrence of univalents in first prophase and metaphase and their lagging in anaphase are responsible for the failure of a number of spores, their smaller size and their higher variability. Univalents were found in almost all metaphases and it seems that very few spore nuclei receive the whole chromosome complement. The shape of the cell is probably not an appropriate indication of viability and the low germination percentage reflected the high frequency of aneuploid spores. The few plantlets obtained from these spores were similar to the tetraploid plants and they come probably from unreduced spores.

Our purpose in inducing diploid plants by apogamy was to determine the polyploidy nature and origin in *Asplenium trichomanes* subsp. *quadrivalens*. Such a method was previously used by Manton and Walker (1954) for *Dryopteris dilatata* and *D. filix-mas*. They observed only separated chromosomes or a slight pairing confirming the allotetraploid nature of these species.

Intraspecific tetraploid forms have been found in a large number of fern species and they have generally been considered as autopolyploid (Mitui, 1968). This is the case in *Asplenium trichomanes*, *A. ruta-muraria* and *A. septentrionale* (Manton, 1950; Lovis, 1964). The high level of chromosome pairing in early diakinesis is in agreement with the homology of the two chromosome sets in the induced diploid plants and with the autotetraploid origin of subsp. *quadrivalens*. However, the desynapsis indicates a loose pairing and a relatively low chiasma frequency. Thus the genome homology is far from perfect and the original plants were not true autotetraploids. The very frequent occurrence of diploid and tetraploid plants at the same location does not involve a simple and recent chromosome doubling from the diploid.

The tetraploid form is probably derived from crossing between different diploid forms or subspecies followed by a natural chromosome doubling. The very wide distribution of that form should mean a polyphyletic origin from different more or less related progenitors and the level of homology between the genomes is not necessarily identical in the tetraploids from different locations. Another explanation for the reduced chromosome pairing would be diploidisation of a true autotetraploid of ancient origin. At any rate subsp. *quadrivalens* may still be considered as an autotetraploid if the term is taken in a broad sense involving an increase in the number of chromosome sets within the limits of one species.

6. Summary

1. In order to confirm the autopolyploid origin of a tetraploid *Asplenium* ($2n = 144$), diploid sporophytes have been apogamously induced by culture of gametophytes on synthetic media and mature plants have been obtained in *A. trichomanes*.

2. Chromosome pairing in the apogamously produced diploids was relatively regular in early diakinesis but the number of univalents increased later and some chromosomes were lost in anaphase.

3. The spores were smaller than in natural diploids; 80 per cent. were well formed but their germination was very poor.

4. The original plants were autotetraploids in a broad sense, but the homology between the chromosome complements is not perfect.

7. References

BOUHARMONT, J. 1968. Les formes chromosomiques d'*Asplenium trichomanes* L. *Bull. Jard. Bot. Nat.*, *38*, 103-114.

HELLER, R. 1953. Recherches sur la nutrition minérale des tissus végétaux cultivés *in vitro*. *Ann. Sci. Nat., Bot. Biol. Vég.*, Sér. XI, *14*, 1-223.

KNUDSON, L. 1925. Physiological study of the symbiotic germination of orchid seeds. *Bot. Gaz.*, *79*, 345-379.

LOVIS, J. D. 1964. Autopolyploidy in *Asplenium*. *Nature*, *203*, 324-325.

MANTON, I. 1950. *Problems of Cytology and Evolution in the Pteridophyta*. Cambridge Univ. Press.

MANTON, I., AND WALKER, S. 1954. Induced apogamy in *Dryopteris dilatata* (Hoffm.) A. Gray and *D. filix-mas* (L.) Schott *emend.* and its significance for the interpretation of the two species. *Ann. Bot.*, *18*, 377-383.

MITUI, K. 1969. Chromosome and speciation in ferns. *Sci. Rep. Tokyo Kyoiku Daigaku, Sect. B*, *13*, 285-333.

MOREL, G. 1963. Leaf regeneration in *Adiantum pedatum*. *J. Linn. Soc.* (*Bot.*), *58*, 381-383.

WALP, R. L. 1951. Fern prothallia under cultivation for twelve years. *Science*, *113*, 128-129.

WHITTIER, D. P. 1962. The origin and development of apogamous structures in the gametophytes of *Pteridium* in sterile culture. *Phytomorphology*, *12*, 10-20.

WHITTIER, D. P. 1964. The influence of cultural conditions on the induction of apogamy in *Pteridium* gametophytes. *Amer. J. Bot.*, *51*, 730-736.

WHITTIER, D. P. 1966. The influence of growth substances on the induction of apogamy in *Pteridium* gametophytes. *Amer. J. Bot.*, *53*, 882-886.

WHITTIER, D. P., AND STEEVES, T. A. 1960. The induction of apogamy in the bracken fern. *Canad. J. Bot.*, *38*, 925-930.

CYTOGENETIC PATTERNS IN *AMARANTHUS*

T. N. KHOSHOO and MOHINDER PAL
National Botanic Gardens, Lucknow, India

1. Introduction

Although there is considerable disagreement regarding the number of species within the genus *Amaranthus* Linn. (45 by Schinz, 1934; 100 by Aellen, 1961), all taxonomists recognise only two Sections, namely *Amaranthus* Sauer (*Amaranthotypus* Dumort.) and *Blitopsis* Dumort. with nearly equal numbers of species in each. The present study based on 16 species, 13 interspecific hybrids and 4 amphiploids aims at assessing the nature of the cytogenetic mechanisms, the breeding systems and the characteristics of morphology that distinguish the two Sections.

2. Results

The genus is dibasic with $x = 16$ and 17 almost equally distributed in Sect. *Amaranthus*, but there appears to be a preponderance of $x = 17$ in Sect. *Blitopsis* and, therefore, in the genus as a whole (table 1). Only one polyploid species, based on $x = 16$, has been reported so far.

Table 1

Distribution of basic number

	Number of species			
	X = 16		X = 17	
Section	$n = 16$	$n = 32$	$n = 17$	Total
Amaranthus	10	1	9	20
Blitopsis	2½*	—	7½*	10
Total	12½	1	16½	30

* One species (*A. graecizans*) is dibasic.

All the species show normal meiosis with 1 to 3 chiasmata per bivalent (plate, figs. 1 and 3), but the mean number ranges from 1·52 (*A. hybridus*) to 1·73 (*A. hypochondriacus*) in Sect. *Amaranthus*, and 1·73 (*A. gracilis*) to 1·75 (*A. lividus*) in Sect. *Blitopsis* (table 2). Often 1 to 2 bivalents disjunct precociously.

Section *Amaranthus*:

In all, nine interspecific hybrids were studied. Seven of these are between species with $n = 16$ and one each between $n = 17$ and 16, and $n = 17$ and 32. Taking the first group ($n = 16 \times 16$) together, the two combinations *A. caudatus* × *A. hybridus* and *A. edulis* × *A. hybridus*, though successful and with normal seed germination, produced hybrid seedlings that did not proceed beyond the first or at most the second leaf stage. The leaves showed a

TABLE 2

Chiasma frequency in parents and hybrids

Taxon	Average no. of Xta per Cell	Average no. of Xta per Bivalent	Pollen Fertility %
A. hypochondriacus	27·73 ± 0·44	1·733	97·15
A. caudatus	26·56 ± 0·44	1·66	91·96
A. caudatus var. *atropurpureus*	26·36 ± 0·02	1·647	28·9 to 83·5
A. edulis	25·64 ± 0·35	1·602	93·1
A. hybridus	24·32 ± 0·36	1·52	92·41
A. edulis × *hypochondriacus*	24·52 ± 0·38	1·532	Nil
A. caudatus × *hypochondriacus*	25·36 ± 0·30	1·585	3·4
A. edulis × *caudatus*	24·82 ± 0·37	1·551	24·9
A. edulis × *caudatus* var. *atropurpureus*	26·0 ± 0·64	1·625	56·4
A. hybridus × *hypochondriacus*	26·12 ± 0·58	1·633	55·0
A. gracilis	29·4 ± 0·312	1·729	Normal
A. lividus var. *lividus*	29·8 ± 0·334	1·752	Normal

virus-like syndrome and became thick and distorted. There was a complete dissolution of the apical meristem and seedlings died soon afterwards.

Some of the remaining five combinations also show virus-like syndromes, genetic tumours and a peculiar twining of stem in the adult plants. In these, the chromosomes generally did not pair along their entire length at pachytene and a number of unpaired regions were apparent. In some bivalents terminal differential segments and deletions could also be made out. The highest number of such abnormalities were found in *A. edulis* × *A. hypochondriacus* followed by *A. edulis* × *A. caudatus* and *A. caudatus* × *A. hypochondriacus*; the least number were in *A. edulis* × *A. caudatus* var. *atropurpureus* and *A. hybridus* × *A. hypochondriacus*. However, in spite of such abnormalities, there are always 16 bivalents both at diakinesis and metaphase I (plate, fig. 2) accompanied by a drop in the mean chiasma frequency per cell as compared to the parents (table 2) in *A. edulis* × *A. hypochondriacus*, *A. edulis* × *A. caudatus* and *A. caudatus* × *A. hypochondriacus* indicating that differential regions exert an influence on the realisation of cross overs. Chiasma frequency is intermediate between that of the parents in *A. edulis* × *A. caudatus* var. *atropurpureus* and *A. hybridus* × *A. hypochondriacus*.

Precocious disjunction and heteromorphic bivalents (plate, fig. 2) were also noted at metaphase I. The subsequent course of meiosis is generally normal barring a few exceptional cells with lagging univalents, late disjunctional bridges and dicentric bridge-fragment associations.

Pollen fertility ranges from 0, 3·4, 24·9 to 56·4 per cent. in different crosses (table 2). Seed fertility was reasonable (49-68 per cent. threshable seed) in three of the five hybrids. The F_2, studied in two crosses, showed a good deal of seedling mortality, about 11-18 per cent. plants showing retarded growth and a virus-like syndrome. The course of meiosis was normal and pollen fertility ranged from 40 to 84 per cent. There was no correlation between pollen and seed fertility.

A. bouchoni × *A. hypochondriacus* involves two basic numbers, $x = 17$ and 16, and the most common association found in 62·5 per cent. cells is 15 bivalents plus one chain of 3 chromosomes, while in the remaining cells there are 16 II + 1 I. The hybrid is totally sterile on selfing but backcrosses with both parents succeed.

The parents, *A. spinosus* and *A. dubius*, of the triploid dibasic hybrid contain 17 and 32 bivalents respectively with 1 or 2 (rarely 3) chiasmata in each bivalent. An analysis of a very large number of pollen mother cells of the tetraploid *A. dubius* does not show any multivalent formation. This is also clear from the earlier observations of Grant (1959), Clifford (1959) and Pal and Khoshoo (1965). These observations are further corroborated by the presence of only 2·24 bivalents per cell in the polyhaploid of *A. dubius* (Pal, unpublished).

In the triploid, however, nearly 64 per cent. cells have either 17 II + 15 I or 1 IV + 15 II + 15 I. The range of different associations is very narrow. At anaphase there may be late disjunction bridges and 0-6 lagging chromosomes leading to unequal distribution. The number at each pole varies from 19 to 29, otherwise the course of meiosis is normal with 8-12 per cent. pollen fertility and no seed set on selfing but about 4 per cent. fertility when backcrossed.

Amphiploids: Three amphiploids (2 tetraploid and 1 hexaploid) were raised by colchicine treatment of the relevant F_1 hybrids. The tetraploids ($2n = 64$), *A. edulis-caudatus* and *A. hybridus-hypochondriacus* (plate, fig. 6), show at metaphase I on average 5·84 and 6·7 quadrivalents per cell that range from 4 to 8 and 2 to 11 per cell respectively (table 3). The remaining

TABLE 3

Average number and range of associations at metaphase I

	Quadrivalents	Trivalents	Bivalents	Univalents	Average fertility%	
					Pollen	Seed
Sect. *Amaranthus*						
Amphiploids						
A. edulis-caudatus	5·84 ± 0·32 (4-8)	0·44 ± 0·02 (0-5)	18·6 ± 0·2 (14-24)	2·12 ± 0·21 (0-6)	54·1	—
A. hybridus-hypochondriacus	6·727 ± 0·41 (2-11)	0·122 ± 0·06 (0-2)	18·272 ± 0·47 (10-28)	0·182 ± 0·01 (0-3)	52·6	—
Autoploids						
A. hypochondriacus	7·538 ± 0·38 (3-10)	0·076 ± 0·01 (0-1)	16·653 ± 0·39 (11-24)	0·307 ± 0·02 (0-2)	94·8	88·1
A. edulis	6·0 ± 0·27 (4-8)	0·059 ± 0·02 (0-1)	19·529 ± 0·23 (16-24)	0·765 ± 0·06 (0-4)	86·3	88·3
A. caudatus	6·80 ± 0·29 (4-9)	0·240 ± 0·02 (0-2)	17·320 ± 0·07 (13-23)	1·440 ± 0·03	82·4	67·0
Sect. *Blitopsis*						
Amphiploid						
A. lividus-tricolor	0·8 (0-2)	0·1 (0-1)	32·0 (30-34)	0·5 (0-2)	63·7	Near normal
Autoploid						
A. tricolor var. *salicifolius*	6·920 ± 0·31 (5-11)	0·160 ± 0·01 (0-1)	19·560 ± 0·37 (12-24)	0·720 ± 0·02 (0-3)	81·4	68·0

associations are bivalents. Trivalents and univalents are very low, in fact the latter may represent precociously disjoined bivalents as are also seen in parents. Pollen fertility was 54·1 and 52·6 per cent. respectively.

The meiotic situation in the amphidiploids is reminiscent of the autotetraploids of the four species (Pal and Khoshoo, 1968). In the C_0 generation the average frequency of quadrivalents varies from 6 (*A. edulis*), 6·8 (*A. caudatus*) to 7·5 (*A. hypochondriacus*) with ranges of 4-8, 4-9 and 3-10 respectively. As in the amphidiploids the number of trivalents and univalents is low and the majority of the associations are bivalents. However, pollen fertility in autoploids is higher than the amphidiploids (table 3).

Amphiploid *A. spinosus-dubius* ($2n = 98$) from the dibasic triploid hybrid ($2n = 49$) had on average in the C_0 generation only 1·2 IV which range from 0-2, while the majority of the associations are in the form of bivalents (46·2 out of a maximum of 49 expected) with 92 per cent. pollen fertility and near normal seed fertility.

Section *Blitopsis*:

Out of the four hybrids, in one (*A. graecizans* var. *graecizans* × *A. lividus* var. *lividus*) the inflorescences were exclusively composed of very poorly developed female flowers and only a few stamens were found in the entire plant, thus no meiotic studies could be undertaken.

In strong contrast to parental species, meiosis in the three interspecific hybrids shows chains and/or rings of 4 to 10 chromosomes at metaphase I. On the one hand there is the simple case of *A. gracilis* × *tricolor* cv. " Purple Leaf " which shows R4+15 II followed by *A. graecizans* var. *graecizans* × *tricolor* cv. " Purple Leaf " in which 95 per cent. cells contain 4 to 6 chromosomes in each complex. A common arrangement (46·7 per cent. cells) is a complex of 4 chromosomes while the maximum number of chromosomes involved are 8 (2 chains or 1 ring and 1 chain of 4) or 10 (1 chain of 6+1 ring of 4). The maximum association of 10 chromosomes is rarely realised perhaps due to small homologous chromosomal segments.

In *A. lividus* × *tricolor* 20 per cent. cells have C8+12 II+2 I (plate, fig. 5) followed by C6+C4+12 II in 16 per cent., C10+12 II in 12 per cent. and C8+C4+10 II+2 I and C5+14 II+1 I each in 8 per cent. cells. There are 9 other arrangements ranging from C10+C4+9 II+2 I, R8+11 II+4 I (plate, fig. 4) to C4+15 II, all together constituting 38 per cent. cells. The two extremes are C10+C4+9 II+2 I and C4+15 II. In all, 14 chromosomes may be involved in 2 complexes, followed by 12 in 2 or 3 complexes and 11 or 10 chromosomes in 1 or 2 complexes. Probably the chain of 11 chromosomes may actually be part of a complex of 12 chromosomes.

The number of bivalents in the three hybrids is 15 in *A. gracilis* × *tricolor* and 9 to 15 in the other two hybrids some of which are distinctly heteromorphic.

The orientation of most rings was adjacent while smaller chains were mostly alternate. The orientation of larger complexes is variable, adjacent and alternate (plate, fig. 5). Such orientation may be due to the small size of the chromosomes involved in the interchanges (John and Lewis, 1965; Khoshoo and Mukherjee, 1966; Sybenga, 1968; Khoshoo and Raina, 1967; Zadoo and Khoshoo, 1968).

During anaphase I and II, 1-2 bridge-fragment configurations are

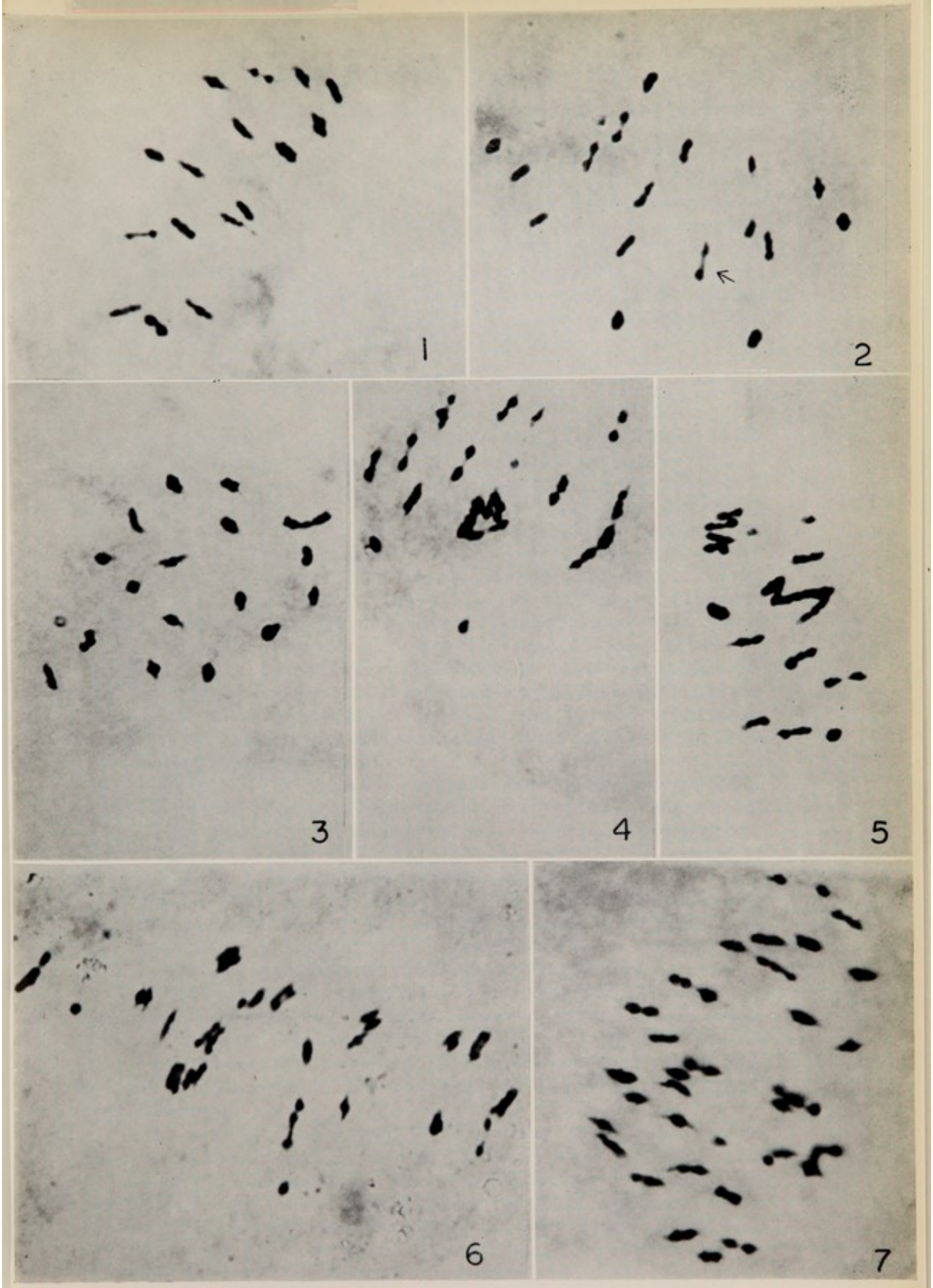


FIG. 1.—*A. edulis*. Metaphase I, $n = 16$.
FIG. 2.—*A. edulis* × *hypochondriacus*. Metaphase I, $n = 16$. One bivalent each shows heteromorphicity and early disjunction.
FIG. 3.—*A. graecizans*. Metaphase I, $n = 17$.
FIGS. 4-5.—*A. lividus* × *tricolor*. Metaphase I, C8+12 II+2 I and R8+11 II+4 I.
FIG. 6.—Amphiploid *A. hybridus-hypochondriacus*. Metaphase I, 9 IV+1 III+11 II+3 I.
FIG. 7.—Amphiploid *A. lividus-tricolor*. Metaphase I, 1 IV+30 II+4 I.
All ×1680

found in 28-57 per cent. cells and chromosome number at each pole varies from 15 to 18.

All four hybrids were completely pollen and seed sterile.

Amphiploids: A. lividus var. *lividus-tricolor* var. *viridus* ($2n = 68$) contains 30 to 34 bivalents. In the former, the remaining 8 chromosomes may be associated in the form of rather loose quadrivalents or trivalents and univalents. Average associations are 0·8 IV + 0·1 III + 32 II + 0·5 I (table 3). A most noteworthy feature is the total absence of interchange complexes so characteristic of the F_1 hybrid. Anaphase is regular with 34 : 34 distribution with pollen fertility 63·7 per cent. and normal seed fertility. This is in contrast to the autoploids of this Section (table 3).

3. Discussion

If the present results are any indication of the prevailing situation in the two Sections, then in interspecific hybrids within Sect. *Amaranthus* bivalent pairing at diakinesis and metaphase I is accompanied by varying degrees of pollen and seed fertility ranging from almost nil to 56 per cent. The fertility of different interspecific hybrids recorded in the literature follows the same pattern (Murray, 1940; Sauer, 1957; Aellen, 1961). Such a situation is characteristic of a number of hybrids and Stebbins (1950) has explained it on the basis of cryptic structural hybridity. The only way to unravel the nature and extent of such hybridity is through detailed analysis at pachytene.

While pachytene pairing in the parental species is regular, that in interspecific hybrids is incomplete with evidence for structural changes like deletions, differential segments and possible inversions. Such differentiation has accumulated at the interspecific level as a result of isolation including, in some cases, long periods under cultivation. While no differential index (*sensu* Shastry and Misra, 1961) was calculated in the present studies, it may be mentioned that F_1 *A. edulis* × *hypochondriacus* showed the maximum abnormalities and was completely sterile. Where sterility was not accompanied by structural differentiation as in *A. hybridus* × *A. hypochondriacus*, it is probable that either differential segments are not readily visible at pachytene, or the differentiation is at a genic level.

The chiasma frequency in hybrids was either lower than that in both the parents or was intermediate. The former is true of *A. edulis* × *hypochondriacus*, *A. edulis* × *caudatus* and *A. caudatus* × *hypochondriacus* and may indicate reduced homology between the pairing chromosomes.

Sterility of pollen and seed may offer a good index of differentiation. *A. caudatus* × *hypochondriacus* and *A. edulis* × *hypochondriacus* which are totally sterile show several types of developmental sterility, viz. a virus-like syndrome in leaves, small deformed indehiscent stamens, tumourous stems and roots, splitting of cortical regions of the stem and twining of the stem and inflorescence axes.

In the partially sterile group (25-56 per cent. sterile pollen; 49-68 per cent. threshable seed) containing *A. edulis* × *caudatus* var. *atropurpureus* and *A. hybridus* × *hypochondriacus* there is heterotic vigour and seasonally controlled developmental sterility but not to the extent enumerated above. Evidently species involved in the first group are more strongly differentiated genetically than those in the second group. However, in all the hybrids in this section there is differentiation at the genetic level together with cryptic

structural hybridity. This permits bivalent formation which results in disharmonious combinations and total or partial sterility, which depends upon, and also may be an index of, the extent of differentiation of genomes of the parents. Thus, the extent of pachytene abnormalities and fertility may indicate the extent of phylogenetic relationship.

A study of the interspecific hybrids in *Blitopsis* shows that differentiation is chiefly due to segmental differences together with possible inversions. Whatever the reason, the presence of multiple associations in interspecific hybrids within this Section contrasts with the bivalent pairing in Sect. *Amaranthus*. If interchanges are the basis of differentiation, then *A. gracilis* and *A. tricolor* differ by one interchange. However, between *A. graecizans* and *A. tricolor* and particularly between *A. lividus* and *A. tricolor*, the differentiation is rather more complex and up to 14 chromosomes are involved. The first two differ by a maximum of 3 and the second pair by 5 to 6 interchanges which may form one or, at the most, two series. Further, one or two bridge-fragments were also seen during anaphases perhaps due to paracentric inversions. Which of the arrangements is ancestral, can be worked out only after systematic analysis of intra- and interspecific hybrids within the Section *Blitopsis* for their crossability, interfertility and translocation patterns. This may give a clear picture even though the chromosomes are small and not individually identifiable. However, all the three hybrids are totally male and female sterile and a perusal of the fertility of hybrids reported earlier in Sect. *Blitopsis* (Aellen, 1961) also shows that although many of them have been given taxonomic names, most, if not all, are totally sterile.

In general multiple associations in hybrids are interpreted as due to interchange hybridity particularly when the parents do not show them. However, White (1961) is of the opinion that such multiple complexes in F_1 hybrids may not be due to interchanges but to end homologies of short terminal segments in otherwise non-homologous chromosomes, which express themselves in the absence of competition for pairing. Even if this be the case, a difference between the two Sections is still apparent, because in Sect. *Amaranthus* such chromosome complexes were not encountered. Terminal or nearly terminal associations are predominant in multiples but interstitial chiasmata are by no means uncommon and such associations appear to be interchanges. Similarly whether the bridge-fragments are due to inversion heterozygosity or chromosome breakage induced by foreign cytoplasm or genotypic unbalance (John and Lewis, 1965) is also not known.

An important aspect of this study has been to investigate, as far as possible, the extent and innate nature of the bivalent pairing in Sect. *Amaranthus* and possible interchange complexes in *Blitopsis*. Further, it was to be ascertained whether the partial or complete sterility in interspecific hybrids of the former and the total sterility in the latter is due to disharmonious recombinations and irregular segregation or due to genic causes. In order to clarify some of these points three amphiploids in Sect. *Amaranthus* and one in Sect. *Blitopsis* were studied.

In Sect. *Amaranthus*, two amphiploids at the tetraploid level showed typical autoploid or segmental alloploid characteristics during meiosis, *i.e.* there were quadrivalents comparable with those of typical autoploids (table 3). Further, amphiploid fertility was reduced in comparison to the autoploids. However, in the hexaploid *A. spinosus-dubius*, general bivalent pairing and total fertility were restored although there was bivalent pairing

and sterility in the triploid progenitor. These data indicate that in general species are not strongly differentiated and there are reasons to believe that differentiation is due to genic and cryptic structural differences.

On the other hand in *Blitopsis*, the amphiploid *A. lividus-tricolor*, whose parents show chromosomal differentiation involving 4 to 14 chromosomes, has predominant bivalent formation with an occasional very loosely joined quadrivalent (not of the translocation but autotetraploid type) and there is restoration of full fertility. In this sense, in *Blitopsis* interspecific crossing recreates genomic conditions which may have been present before the establishment and differentiation of species. However, the complete preferential pairing in amphiploids reflects the small size of the segments which, though enough to bring about pairing in terminal segments in absence of competition for pairing in the F_1 hybrid, are not enough to give multiple pairing when completely homologous mates are available in the amphiploids. The restoration of fertility in the amphiploid indicates that the total sterility in F_1 is entirely segregational. Thus, this amphiploid has alloploid characteristics.

In both Sections sterility is mostly segregational, attributable to recombination of genes and cryptic differences in *Amaranthus*, and to larger non-homologous segments in *Blitopsis*. Recombination between parental genomes is not suppressed by polyploidy in the former, while it is totally suppressed in the latter and so the former continue to be only semifertile, while in the latter full fertility is restored.

The difference between cytogenetic patterns in the two Sections is clear from the foregoing account. If the present work is an indication of the prevailing situation in the two Sections of the genus, then correlated with the above difference there are some distinctive traits of the breeding system.

The Section *Amaranthus* lacking multiple associations of chromosomes in interspecific hybrids, is predominantly cross pollinated because the glomerules in the huge often showy inflorescences possess a single male flower, and their percentage per glomerule varies from 0·4 to 1. Further, although there are no nectaries, honey bees visit in October-February, probably for collecting pollen. On the other hand, in *Blitopsis* where complex associations are commonly found in interspecific hybrids, the inflorescences are never showy but are generally axillary, and glomerules contain 10-25 per cent. male flowers and are never visited by insects. This Section is predominantly self pollinated and its members have narrow ecological preferences. A correlation between inbreeding and chromosomal repatterning is understandable in as much as such a breeding system helps not only in the accumulation of interchanges and inversions, but the chances of rendering them homozygous are also very high. Thus inbreeding is likely to help in the establishment and maintenance of homozygotes. Very likely such rearrangements are associated with linkage of some adaptive gene combinations and with continued inbreeding, sterility barriers appear. As seen in the present hybrids, total sterility results from the recombination of such repatterning. With continued isolation and differential selection, the possibility of gene exchange is almost totally restricted and evolutionary divergence from the ancestral type is expected. A close parallel between the situations in the two Sections of the genus *Amaranthus* is seen in the genus *Oenothera* in which there is a high incidence of structural heterozygotes in inbred species in comparison to the outbred species of California (Cleland,

1949; Rees, 1961). While occurrence of interchange heterozygotes intraspecifically is well known in a large number of species in *Clarkia* (Lewis and Raven, 1958), cockroaches (Lewis and John, 1957), etc. (Burnham, 1956), the evidence of this mechanism as a means of cytogenetic differentiation at interspecific level is found in *Datura* (Avery, Satina and Rietsema, 1959), *Clarkia* (Lewis, H., 1953) *Secale* (Riley, 1955; Khush, 1962), *Avena* (Holden, 1966), *Linum* (Gill and Yermanos, 1967), *Epilobium* (Mosquin, 1968), *Gossypium* (Gerstel, 1953), *Gilia* (Grant, 1956), *Collinsia* (Garber and Dhillon, 1962), etc.

Lastly, in consonance with the above differences, the species of the two Sections have been exploited by man for altogether different purposes, those in Sect. *Amaranthus* for nutritive grain and/or ornamental inflorescences and those in *Blitopsis* as pot herbs very rich in carotene content and as foliage ornamentals. This selection has taken place in two different continents, the New World and the Indian Sub-continent respectively.

4. Summary

1. Species differentiation in the generally cross pollinated Section *Amaranthus* Sauer involves gene differences and cryptic structural hybridity and results in bivalent pairing and varying degree of sterility in the interspecific hybrids, whose amphiploids show autoploid or segmental alloploid characters.

2. In the generally self pollinated Section *Blitopsis* Dumort., interspecific hybrids show interchange heterozygosity involving long segments of 4 to 14 chromosomes accompanied by total sterility. However, complete fertility is restored in amphidiploids.

3. The naturalness of the two Sections is evident from these patterns together with the lack of hybrids between them.

Acknowledgement.—Our thanks are due to Mr T. K. Sharma for photographs illustrating this paper.

5. References

AELLEN, P. 1961. Die Amaranthaceen Mittleleuropas Carl Hanser Verlag, Munchen.

AVERY, A. G., SATINA, S., AND RIETSEMA, J. 1959. Blakeslee: The genus Datura, New York.

BURNHAM, C. R. 1956. Chromosomal interchanges in plants. *Bot. Rev., 22*, 419-552.

CLELAND, R. E. 1949. Phylogenetic relationships in *Oenothera*. *Proc. 8th Intern. Congr. Genet. Stockholm. Hereditas Suppl., 35*, 173-188.

CLIFFORD, H. T. 1959. On hybridization between *Amaranthus dubius* and *A. spinosus* in the vicinity of Ibadan, Nigeria. *J. West. Afric. Sci. Assoc., 4*, 112-116.

GARBER, E. D., AND DHILLON, T. A. 1962. The genus *Collinsia* XVII. Preferential pairing in four amphiploids and three triploid interspecific hybrids. *Can. J. Genet. Cytol., 4*, 6-13.

GERSTEL, D. U. 1953. Chromosomal translocation in interspecific hybrids of the genus *Gossypium*. *Evolution, 7*, 234-244.

GILL, K. S., AND YERMANOS, D. M. 1967. Cytogenetic studies on the genus *Linum* I and II. *Crop. Sci., 7*, 623-631.

GRANT, V. 1956. Chromosome repatterning and adaptation. *Advances in Genet, 8*, 89-107.

GRANT, W. F. 1959. Cytogenetic studies in *Amaranthus* II. Natural interspecific hybridization between *Amaranthus dubius* and *A. spinosus*. *Canad. J. Bot., 37*, 1063-1070.

HOLDEN, J. H. W. 1966. Species relationships in Avenae. *Chromosoma Berl., 20*, 75-124.

JOHN, B., AND LEWIS, K. R. 1965. The meiotic system. *Protoplasmatologia, 6*, 1-335.

KHOSHOO, T. N., AND MUKHERJEE, IVA. 1966. A translocation heterozygote in garden Canna. *Genetica, 37*, 255-258.

KHOSHOO, T. N., AND RAINA, S. N. 1967. An interchange heterozygote in garden *Crinum*. *Jour. Cytol. Genet.*, *2*, 6-9.

KHUSH, G. S. 1962. Cytogenetic and evolutionary studies in *Secale* II. Interrelationships of the wild species. *Evolution*, *16*, 484-496.

LEWIS, H. 1953. The mechanism of evolution in the genus *Clarkia*. *Evolution*, *5*, 142-157.

LEWIS, H., AND RAVEN, PETER H. 1958. Rapid evolution in *Clarkia*. *Evolution*, *12*, 319-336.

LEWIS, K. R., AND JOHN, B. 1957. Studies in *Periplaneta americana* II. Interchange heterozygosity in isolated populations. *Heredity*, *11*, 11-21.

MOSQUIN, THEODORE. 1968. Chromosome translocation in *Epilobium*. *Can. J. Genet. Cytol.*, *10*, 794-798.

MURRAY, M. J. 1940. The genetics of sex determination in the family Amaranthaceae. *Genetics*, *25*, 409-431.

PAL, M., AND KHOSHOO, T. N. 1965. Origin of *Amaranthus dubius*. *Curr. Sci.*, *34*, 370-371.

PAL, M., AND KHOSHOO, T. N. 1968. Cytogenetics of the raw autotetraploid *Amaranthus edulis*. *NBG Tech. Comm.*, 25-36.

REES, H. 1961. The consequences of interchange. *Evolution*, *15*, 145-152.

RILEY, R. 1955. The cytogenetics of the differences between some *Secale* species. *J. Agric. Science*, *46*, 377-383.

SAUER, J. D. 1957. Recent migration and evolution of dioecious amaranths. *Evolution*, *11*, 11-31.

SCHINZ, H. 1934. Amaranthaceae. In *Die naturlichen pflanzenfamilien*. (ed. by A. Engler and K. Prantl), *16C*, 7-85.

SHASTRY, S. V. S., AND MISRA, R. N. 1961. Pachytene analysis in Oryza II. Sterility in *Japonica indica* rice hybrids. *Chromosoma* Berl., *12*, 248-271.

STEBBINS, G. L. 1950. *Variation and Evolution in Plants*. Columbia Univ. Press, New York.

SYBENGA, J. 1968. Orientation in interchange multiples in *Secale cereale*. *Heredity*, *23*, 73-79.

WHITE, M. J. D. 1961. The role of chromosomal translocations in urodele evolution and speciation in the light of work on grass hoppers. *Amer. Nat.*, *95*, 315-321.

ZADOO, S., AND KHOSHOO, T. N. 1968. Cytogenetical studies on *Bougainvillea*. 1. A case of interchange hyterozygosity. *Genetica*, *39*, 353-359.

INTERCHANGES AND INTERPOPULATIONAL GENE EXCHANGE IN *CLARKIA SPECIOSA*

WILLIAM BLOOM* and HARLAN LEWIS
University of California, Los Angeles

1. Introduction

Clarkia speciosa (Onagraceae) is an outcrossing annual consisting of several morphologically recognisable ecogeographic races in the South Coast Ranges of California and two that occur on the eastern margin of the San Joaquin Valley in the foothills of the Sierra Nevada (fig. 1). The latter were once considered members of different species, *C. nitens* and *C. speciosa polyantha* (Lewis and Lewis, 1955) based on numerous floral and vegetative characteristics by which they differ, the very low fertility of hybrids produced in the garden, and structural differences in their chromosomes. We now know that these two taxa, which replace each other from north to south respectively, are fully interfertile and intergrade morphologically where they meet in the Kings River drainage and are, therefore, conspecific. In this paper these two taxa will be referred to as " nitens " and " polyantha."

Two allopatric diploid chromosome races ($2n = 18$) have been found within *C. speciosa* which differ by at least seven interchanges such that hybrids between them form a ring of 16 chromosomes and one pair during meiosis. Nondisjunction is frequent in these large rings and the result is low fertility. One of the chromosome races, which we will call " South ", characterises the coastal races of *C. speciosa* and the southern populations of polyantha; the other, which we will call " North ", characterises populations in the northern half of the range of polyantha and all of nitens (Mosquin and Lewis, 1959). Although polyantha includes populations of both chromosomal races, no morphological criteria have been found that will distinguish them.

The area of contact between the two chromosome races, as determined by interpopulational crosses, has been found to occur in southern Tulare County between California Hot Springs and the Tule River (Mosquin and Lewis, 1959). The purpose of this paper is to examine the effect of chromosomal rearrangement on the ability of the two races to exchange genetic material, using field samples and hybridisation experiments involving populations in the area of contact.

2. Methods

A transect which included 15 study populations was established in the contact area along the 22-mile-long Deer Creek Road between California Hot Springs and the Tule Indian Reservation Road in Tulare County, California (fig. 1). Transect populations were numbered from mile 0·0 at the north end to mile 22·0 at the south end near California Hot Springs and will be referred to according to their positions on the transect. Two additional populations chosen to represent the two chromosome races were used in an interpopulational crossing programme, one located about ten

* Present address: Department of Botany, University of Kansas, Lawrence, Kansas 66044, U.S.A.

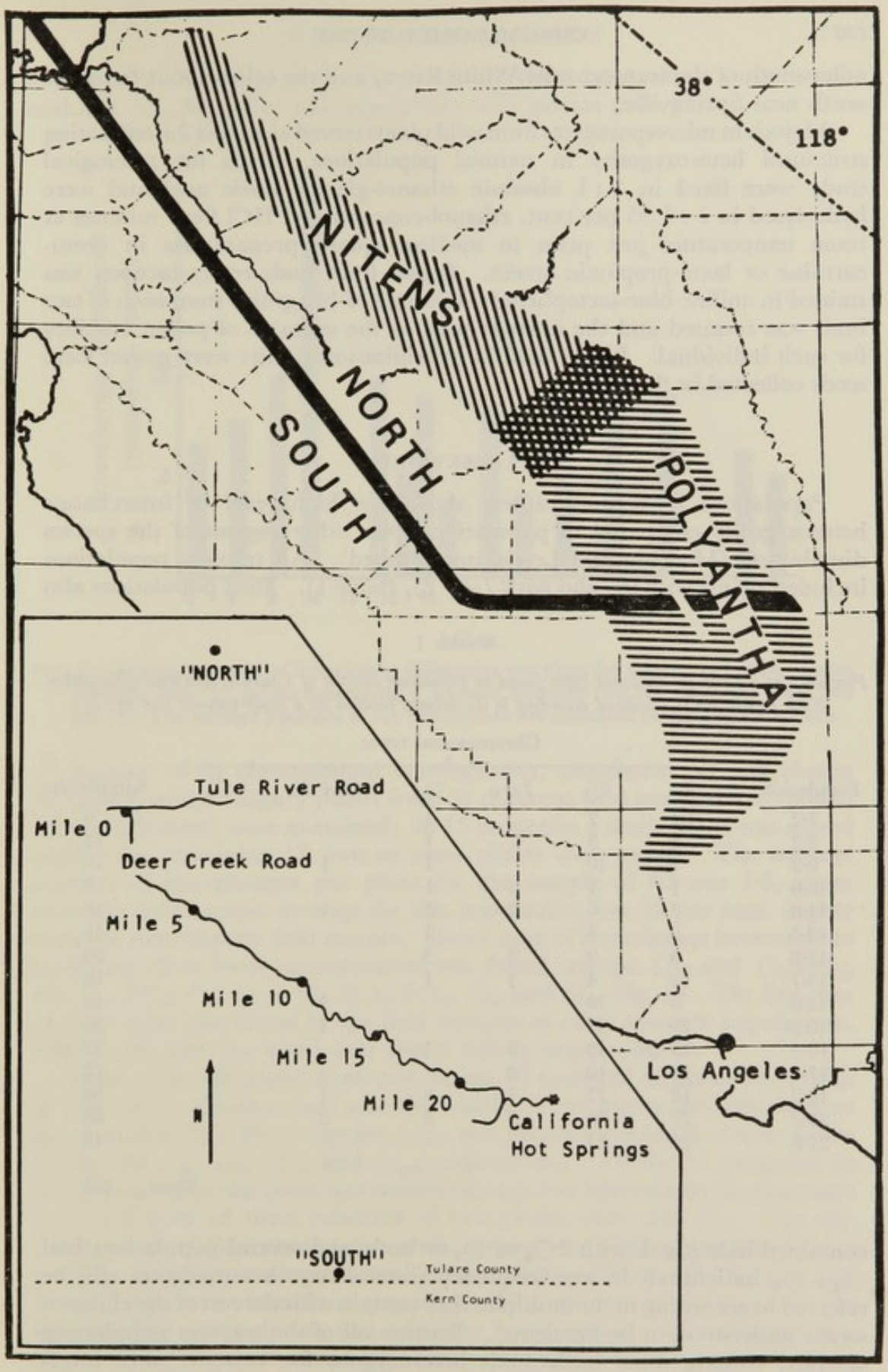


FIG. 1.—Distribution of the ecogeographic races *Clarkia speciosa nitens* and *Clarkia speciosa polyantha* and the chromosomal races North and South. The region of morphological intergradation between nitens and polyantha is indicated by the overlapping patterns. The heavy black line separates the North and South races. The white line crossing the cytological boundary shows the location of the Deer Creek Transect. Inset shows details of the transect and the location of the populations "North" and "South" used to represent the two chromosomal races.

miles south of the transect near White River, and the other about ten miles north near Springville.

Meiosis in microsporocytes from wild plants served as a basis for estimating structural heterozygosity in natural populations. Buds for cytological study were fixed in 3 : 1 absolute ethanol-glacial acetic acid and were hydrolysed in 1 : 1 95 per cent. ethanol-concentrated HCl for 7 minutes at room temperature just prior to making squash preparations in aceto-carmine or lacto-propionic orcein. Pollen from buds ready to open was stained in aniline blue-lactophenol; a sample of 100 grains from each of two buds was counted and the average used as the estimate of pollen viability for each individual. Plants used in hybridisation studies were grown from seeds collected in the wild.

3. Observations

Populations from the transect showed a high level of interchange heterozygosity compared to populations from other regions of the species distribution (Mosquin and Lewis, unpublished). All transect populations included plants with 9_{II} and with $7_{II}+\bigcirc_4$ (table 1). Most populations also

Table 1

Frequency of meiotic chromosomal types found in population samples of Clarkia speciosa polyantha. *Each population is numbered according to its mileage position on a study transect (see fig. 1)*

	Chromosomal types						
Population	9_{II}	$\bigcirc_4$	$2\bigcirc_4$	$\bigcirc_6$	$\bigcirc_4+\bigcirc_6$	$\bigcirc_8$	Sample size
7·2	3	2		1			6
8·2	5	5	1	1			12
9·2	4	10		1	1		16
10·0	1	1	2	1		3	8
11·8	7	6	2	2	1		18
12·5	9	6		1			16
12·8	9	9	3	3			24
13·7	12	4	1	1			18
14·6	5	10			1		16
15·5	19	11			1		31
16·6	15	13	8	1			37
17·8	10	10	8				28
19·2	18	27	7	3	1		56
21·2	27	25	9	3	2		66
21·8	23	23					46
						Total	398

contained individuals with $2\bigcirc_4$ or $\bigcirc_6$ or both, and several populations had $\bigcirc_4+\bigcirc_6$ individuals in low frequency (interchange heterozygotes will be referred to according to the multiples they contain with the rest of the chromosomes understood to be bivalents). Because all of the transect populations sampled contain some individuals heterozygous for two or more interchanges, the level of heterozygosity is expressed in terms of the average number of interchange differences per plant rather than the frequency of interchange heterozygotes. The averages ranged from 0·4 to 1·0 interchange per plant for all but one, population 10, which had an average of

2·0 (fig. 2). Population 10 also produced the largest single interchange multiple, $\odot_8$, found in wild populations of *C. speciosa* (table 1).

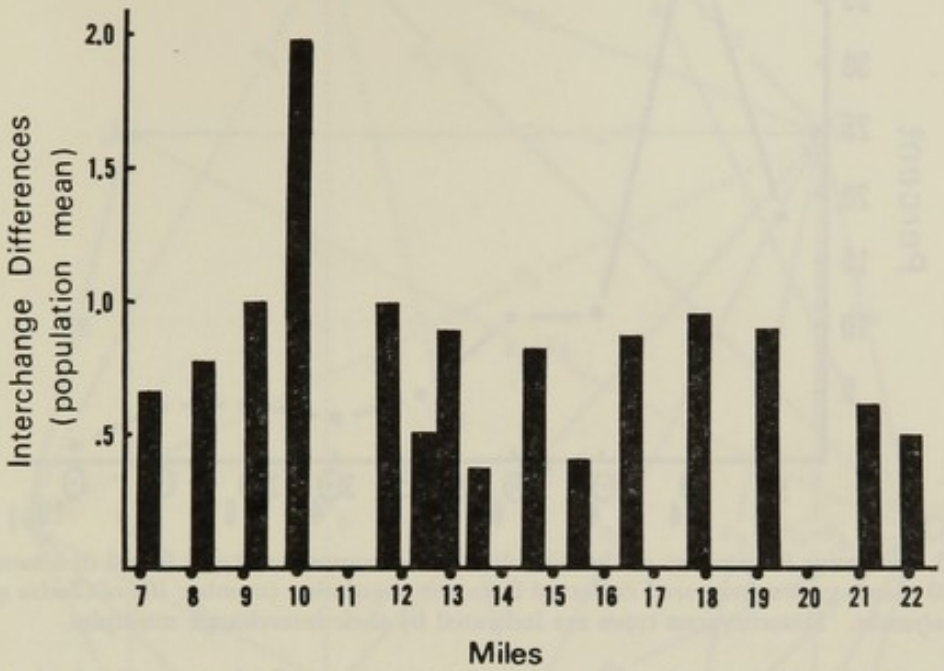


FIG. 2.—Average number of interchange differences per plant for samples of *Clarkia speciosa polyantha* from 15 populations along a transect between two chromosomal races (see fig. 1). The relative positions of the populations are indicated on the horizontal axis.

Because of its chromosomal heterogeneity, population 10 was chosen for special study. Eighty plants from 32 different wild seed parents (pollen parent unknown) were examined; in 15 progenies a single plant was scored and in the remaining 17, two or more plants were scored. The average number of interchanges per plant for the sample of 80 was 1·5, lower than the field sample average for this population, but 50 per cent. higher than the next highest field sample. Every type of interchange heterozygote involving 12 or fewer chromosomes was found, except $\odot_{12}$ and $\odot_4 + \odot_8$, viz. $\odot_4$, $2\odot_4$, $\odot_6$, $\odot_4 + \odot_6$, $3\odot_4$, $2\odot_6$, $\odot_8$, and $\odot_{10}$ (fig. 3). The first four of these were also found in the field samples of other transect populations, whereas the last four types were found only in population 10.

Most of the progenies from population 10 consisted of plants with rings of four or six chromosomes and the highly heterozygous individuals were found mainly in a few progenies (*e.g.*, one progeny consisted of four plants which had $\odot_6$, $\odot_8$, $3\odot_4$ and $\odot_{10}$, respectively). Of the 17 progenies in which more than one plant was examined, only two had a single chromosomal type and both of these consisted of two plants each with 9_{II}. The distribution of chromosomal types with respect to progeny suggests that a number of different chromosomal arrangements, differing by one or two interchanges, are common in population 10. As a consequence, most individuals have a small ring and a few are homozygotes. The relatively few highly heterozygous individuals are probably produced by infrequent arrangements which differ considerably from both the common arrangements and each other.

In order to obtain more precise information about the number of chromosomal arrangements in population 10 and their relationship to one

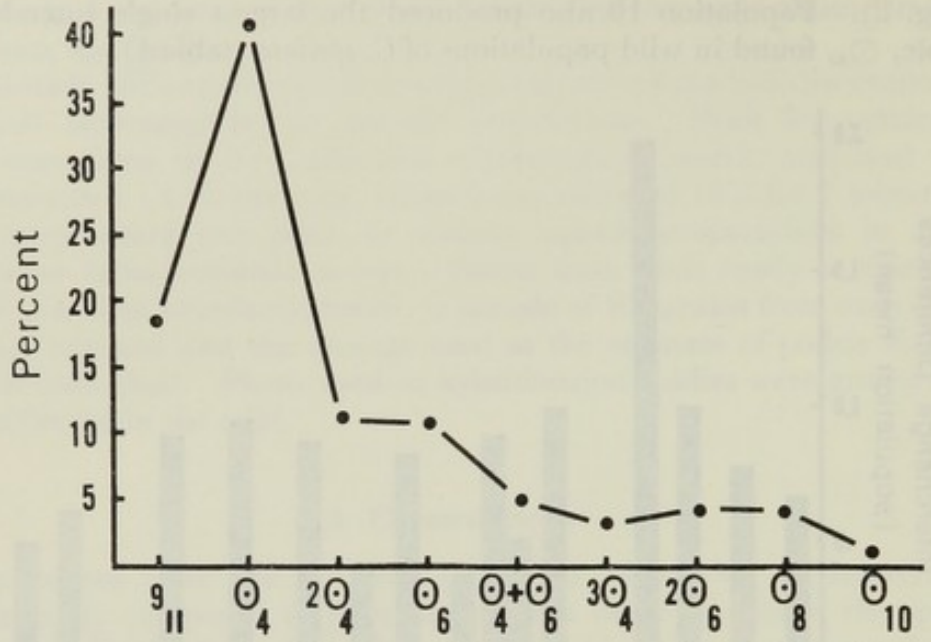


FIG. 3.—Relative frequencies of the nine different chromosomal types found in a sample of 80 plants grown from seed collected from one population (number 10) of *Clarkia speciosa polyantha*. Heterozygous types are indicated by their interchange multiples.

another, 22 plants, selected as representative of the cytological range of the population, were hybridised with one another. The resulting hybrids included all the chromosomal types found in the earlier samples from population 10 plus an additional one, $\odot_4 + \odot_8$. Analysis of these hybridisation data indicate the 22 parental plants involved ten different chromosomal arrangements (Bloom, 1970). The arrangements have been designated A to J according to the order in which they were first used in the hybridisation studies and the letters do not reflect chromosomal similarities. The hybrid combinations of the ten arrangements which were examined and used to determine their relationships are shown in fig. 4.

The relative frequency of each of the ten arrangements of population 10 varied from a high of about 30 per cent. for arrangement C to a low of less than 3 per cent. for arrangements A and I (fig. 5). These frequencies are only approximate, however, since the sample of 22 plants used for the hybridization experiments was not random, specifically, proportionately more homozygotes were used than are found in population 10, and all of the $\odot_8$ (three) and $\odot_{10}$ plants (one) found were selected for testing.

The five most common arrangements, C, D, E, G, and H, together comprising over 80 per cent. of the population 10 sample, are all very similar, differing mostly by one or two interchanges whereas the five infrequent arrangements show much larger differences on the average. Consequently, plants tested from the 9_{II} class and the $\odot_4$ class were found to involve only the most common arrangements, whereas the few individuals with large rings involved the infrequent arrangements.

Designation of chromosomal end sequences for each of the arrangements found in population 10 was guided by two restrictions in addition to the crossing data: (1) the rearrangements were limited to a set of 12 chromosomes because the maximum number of chromosomes involved in interchange multiples in any single plant was 12 (*cf.* $3\odot_4$, $2\odot_6$ and $\odot_4 + \odot_8$); and (2) the lowest possible number of rearrangements consistent with the

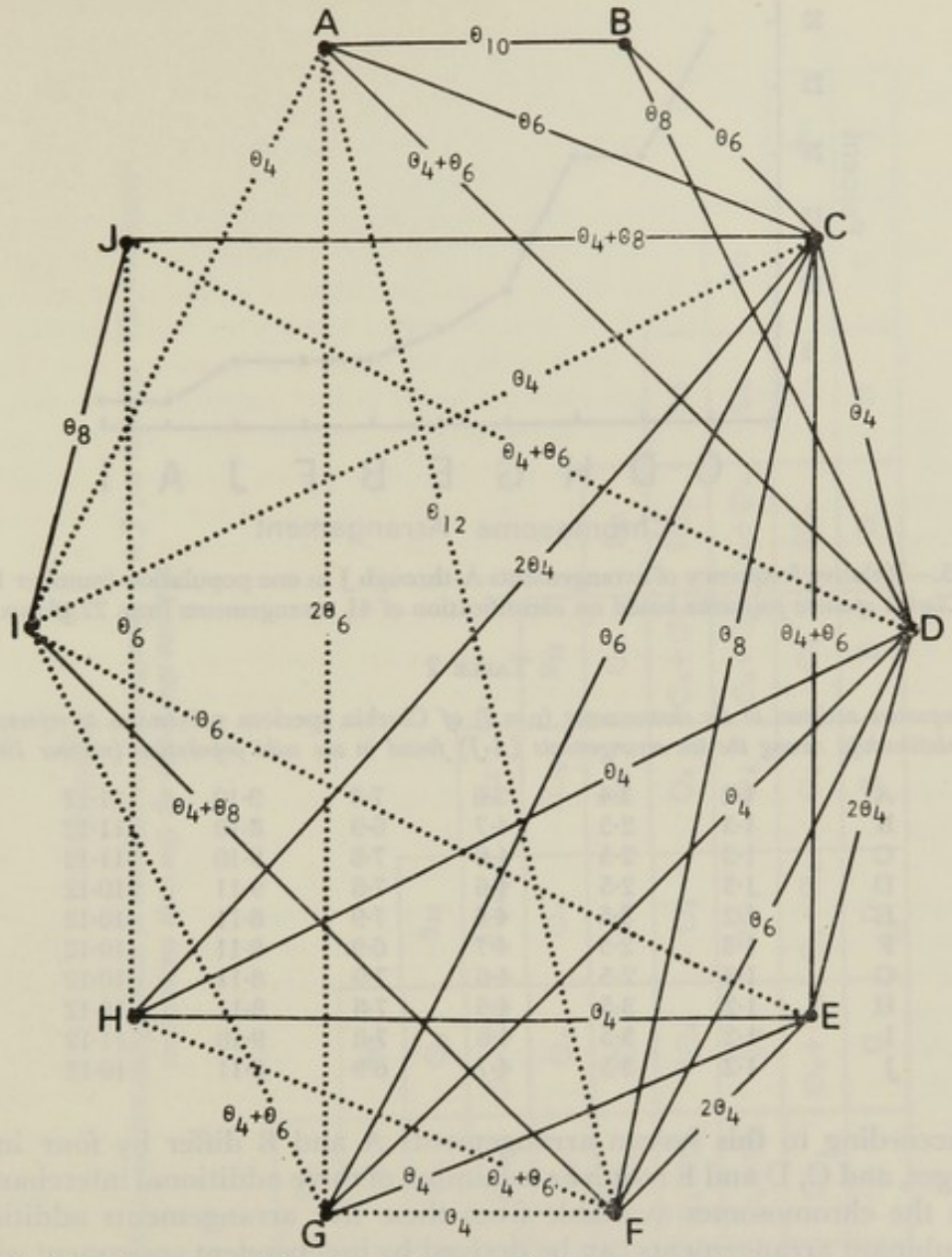


FIG. 4.—Interchange multiples formed by combinations of ten chromosome arrangements (A-J) from one population of *Clarkia speciosa polyantha*. Solid lines indicate intrapopulation combinations used in assigning relative chromosome end relationships; dotted lines indicate combinations obtained subsequently from interpopulation crosses.

crossing data was used. Arrangement A was arbitrarily given the sequence 1·2, 3·4, 5·6, 7·8, 9·10, 11·12 where each number represents a pairing end and the dot represents the centromere (table 2). The hybrid AB has a $\odot_{10}$ and the end sequence assigned to arrangement B meets this criterion, but is only one of a number of end sequences that would give a $\odot_{10}$ with A. Arrangements C, D and E were designated according to their relationship to the sequences assigned to A and B and to each other. This was done by trial and error. The system finally adopted fulfils all the crossing requirements, uses no more than 12 chromosomes, and requires the lowest number of new chromosomal translocations possible. The meiotic configurations expected in each of the 45 possible heterozygous combinations of the ten arrangements are shown in table 3.

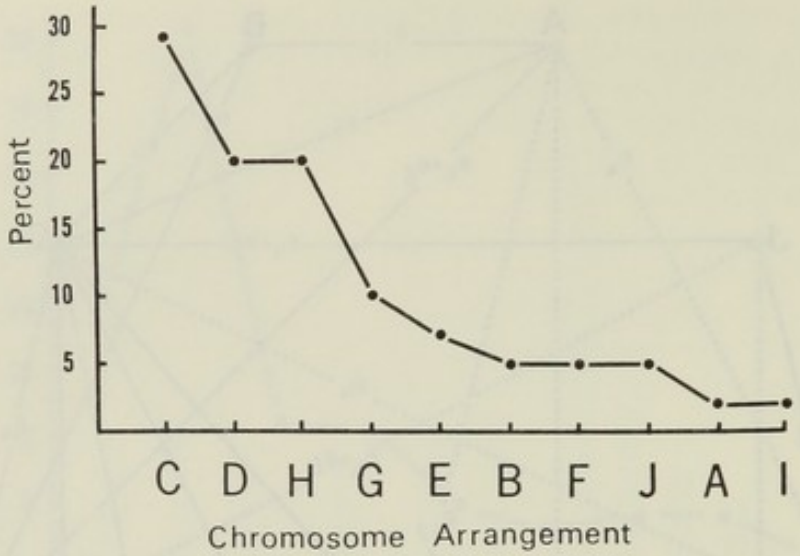


FIG. 5.—Relative frequency of arrangements A through J in one population (number 10) of *Clarkia speciosa polyantha* based on identification of 41 arrangements from 22 plants.

TABLE 2

End sequences assigned to six chromosomes (n = 9) *of* Clarkia speciosa polyantha *to represent the relationships among the ten arrangements* (*A-J*) *found in one wild population* (*number 10*)

A	1·2	3·4	5·6	7·8	9·10	11·12
B	1·3	2·5	4·7	6·9	8·10	11·12
C	1·3	2·5	4·6	7·8	9·10	11·12
D	1·3	2·5	4·6	7·8	9·11	10·12
E	1·2	3·5	4·6	7·9	8·11	10·12
F	1·3	2·5	4·7	6·9	8·11	10·12
G	1·3	2·5	4·6	7·9	8·11	10·12
H	1·2	3·5	4·6	7·8	9·11	10·12
I	1·2	3·5	4·6	7·8	9·10	11·12
J	1·2	3·5	4·7	6·9	8·11	10·12

According to this system arrangements A and B differ by four interchanges, and C, D and E require a minimum of three additional interchanges. With the chromosomes available from these five arrangements additional recombinant arrangements can be derived by independent assortment which will satisfy all the requirements for the five remaining arrangements F, G, H, I, and J.

The combinations of arrangements used in assigning chromosomal end sequences to the ten arrangements A to J include 20 of the 45 possible heterozygous combinations. Crosses involving plants from population 10 and several adjacent populations analysed subsequent to designation of the end sequences yielded an additional 11 heterozygous combinations, all of which gave the predicted results based on the end sequences proposed (fig. 4). For example, the two arrangements A and F were assigned end sequences independently of each other on the basis of crosses with other arrangements in population 10. The resulting end sequences of arrangements A and F differed from one another by five interchanges involving 12 chromosomes, and the hybrid between arrangements A and F as defined, would be expected to exhibit a $\odot_{12}$. The actual AF combination was not available from population 10 to test this predicted relationship and a $\odot_{12}$ has never been found in population 10 (the expected frequency of the AF

TABLE 3

Expected meiotic chromosome configurations in combinations of arrangements A to J (see table 2). The heterozygous combinations show only the interchange multiples

A	9_{II}									
B	$\odot_{10}$	9_{II}								
C	$\odot_{6}$	$\odot_{6}$	9_{II}							
D	$\odot_{4}+\odot_{6}$	$\odot_{8}$	$\odot_{4}$	9_{II}						
E	$2\odot_{6}$	$3\odot_{4}$	$\odot_{4}+\odot_{6}$	$2\odot_{4}$	9_{II}					
F	$\odot_{12}$	$\odot_{4}$	$\odot_{8}$	$\odot_{6}$	$2\odot_{4}$	9_{II}				
G	$2\odot_{6}$	$2\odot_{4}$	$\odot_{6}$	$\odot_{4}$	$\odot_{4}$	$\odot_{4}$	9_{II}			
H	$2\odot_{4}$	$\odot_{4}+\odot_{8}$	$2\odot_{4}$	$\odot_{4}$	$\odot_{4}$	$\odot_{4}+\odot_{6}$	$2\odot_{4}$	9_{II}		
I	$\odot_{4}$	$\odot_{4}+\odot_{6}$	$\odot_{4}$	$2\odot_{4}$	$\odot_{6}$	$\odot_{4}+\odot_{8}$	$\odot_{4}+\odot_{6}$	$\odot_{4}$	9_{II}	
J	$\odot_{10}$	$2\odot_{4}$	$\odot_{4}+\odot_{8}$	$\odot_{4}+\odot_{6}$	$\odot_{4}$	$\odot_{4}$	$2\odot_{4}$	$\odot_{6}$	$\odot_{8}$	9_{II}
	A	B	C	D	E	F	G	H	I	J

heterozygote is less than ·001). The interpopulational crosses did, however, yield hybrids between arrangements A and F (identified as such by independent crosses with other arrangements of population 10) and the AF hybrid did, as predicted, form a $\bigcirc_{12}$. In total, 31 of the 45 possible heterozygous combinations of arrangements A through J have been found (fig. 4).

To estimate the effect of interchange heterozygosity on fertility, pollen samples from plants with various chromosome combinations were scored for stainability. These data show a constant decrease in mean stainable pollen with an increase in the size and number of interchange multiples. Small multiples of four or six chromosomes score very high, having 96·1 per cent. and 91·7 per cent. respectively, for structural heterozygotes. Larger multiples, however, have considerably lower pollen stainability, for example, the $\bigcirc_8$ scores 80 per cent. and the largest multiple with 16 chromosomes has 43 per cent. mean stainable pollen. The pollen data indicate that in heterozygote classes with more than one ring the effect of each ring on fertility is independent. In no case is the per cent. unstained pollen in a multiple-ringed class appreciably greater than the product of each component ring, indicating the rings are not genetically interdependent.

The effect of interchange heterozygosity on fertility can also be estimated from data on chromosomal disjunction in interchange heterozygotes since disjunction is required for genetically balanced products. Disjunction data have been obtained by scoring alternate versus adjacent orientation in multiples just prior to separation at anaphase I. Plants with two independent multiples were scored in all or none fashion, *i.e.* if one multiple was scored adjacent the cell was scored adjacent; in order to be scored alternate both multiples had to be alternate. The frequency of alternate orientation was found to decrease as the size of the multiple increased. Multiples of four or six chromosomes are characterised by a high frequency of alternate orientation —97 per cent. and 90 per cent. respectively—yielding fertility estimates very similar to those derived from pollen stainability. However, in larger rings the disjunction percentages drop much more abruptly than does pollen stainability. For example, the $\bigcirc_8$ has a disjunction mean of 58 per cent. (compared to a mean stainable pollen of 80 per cent.) and the $\bigcirc_{16}$ disjunction mean is 11 per cent. (compared to a mean stainable pollen of 43 per cent.). Thus, while the disjunction data for rings of four and six yield fertility estimates very similar to those obtained from pollen staining, the disjunction data for the larger rings suggest much lower fertility than does pollen staining. The discrepancy between the pollen staining estimate and the disjunction frequency estimate of fertility increases with an increase in the size of the multiple and is undoubtedly attributable to the stainability of some genetically unbalanced pollen grains. Thus, the disjunction frequencies appear to be a more informative estimate of fertility than is pollen stainability.

The relationships between the arrangements of population 10 and those of other populations in the transect were determined by meiotic analysis of interpopulational hybrids. Six populations were tested, three north of, and three south of population 10, ranging between mile 6·4 and 14·6 of the transect. Each population was represented by from one to four individuals and each was crossed with arrangements of population 10, primarily C, D, and E (table 4). Each of the arrangements so tested yielded meiotic configurations identical to those expected from one and only one, of the ten

TABLE 4

Identification of chromosome arrangements in plants from six populations of Clarkia speciosa polyantha *based on test crosses with arrangements from population 10·0. Populations are designated by their position along the study transect (fig. 1). Structural homozygotes are indicated as* 9_{II}, *heterozygotes by their interchange multiple(s)*

Population	Plants tested	Test arrangement	Progeny	Conclusion for plant tested
6·4	9_{II}	C	$\odot_8$	FF
		D	$\odot_6$	
		E	$2\odot_4$	
8·2	9_{II}	F	9_{II}	FF
	$\odot_6$	CG	$\odot_4$, $\odot_8$	FD
	$\odot_4$	C	$\odot_6$	FG
		D	$\odot_4$, $\odot_6$	
	9_{II}	C	$\odot_8$	FF
		E	$2\odot_4$	
9·2	9_{II}	C	$\odot_6$	
		D	$\odot_4$	GG
		E	$\odot_4$	
11·6	$\odot_6$	C	9_{II}	
		D	$\odot_4$	CG
		E	$\odot_4$	
	9_{II}	C	$2\odot_4$	
		D	$\odot_4$	HH
		E	$\odot_4$	
12·5	$\odot_4$	C	$\odot_4$, $\odot_6$	
		D	$\odot_4+\odot_6$	AI
		E	$\odot_6$	
	9_{II}	C	$\odot_6$	
		H	$2\odot_4$	AA
		South	$\odot_6$	
14·6	$\odot_6$	D	$\odot_4$, $\odot_4+\odot_6$	
		E	$\odot_4+\odot_6$	AC
		G	$\odot_6$	

arrangements described from population 10. For example, the plant from mile 6·4 yielded a $\odot_8$ with arrangement C, and $\odot_6$ with arrangement D and $2\odot_4$ with arrangement E. These results are identical to those expected from arrangement F and since no other arrangements known will give these results (*cf.* table 3) the tested arrangement is assumed to be F. Comparisons of this sort were used to evaluate each of the arrangements tested from the six populations.

The identification of arrangements from the seven populations studied reveals that a definite pattern exists in the distribution of the chromosomal arrangements along the transect. The centre of the transect at population 10 is the most diverse in terms of the number of chromosome arrangements present. In population 10, ten different arrangements have been found, but five of these (C, D, G, H and E) make up over 80 per cent. of the total, and the remaining five occur with a frequency of 5 per cent. or less—perhaps much less. Two of these low frequency arrangements, A and F, were found to be the dominant arrangements in other areas of the transect.

Specifically, arrangement A is the most frequent arrangement in the southernmost populations tested, whereas arrangement F dominates the northernmost populations tested. The additional rearrangements in these northern and southern populations were invariably very similar to the dominant arrangement. Thus, from population 10, arrangement A apparently increases in frequency toward the south, arrangement F increases toward the north, and each is associated with those arrangements of population 10 which form rings of four or six with them.

The relationship between the arrangements found in the transect populations and those which characterise the North and South chromosomal races was also investigated via interpopulational hybrids. The number of different arrangements tested against the North and South types is not sufficient to define the chromosomal end sequence of North or South; however, it was determined that none of the arrangements tested are identical to either North or South. Arrangement A, the most frequent one in the southern transect populations, is more similar to South than any other transect arrangement, the A-South hybrid having a $\odot_6$, while the A-North hybrid has a $\odot_{16}$. Arrangement F, characteristic of the northern transect populations and apparently the transect arrangement most like North, forms $2\odot_6$ with North whereas the F-South hybrid displays a $\odot_{16}$. The arrangements most common in population 10 give large rings when crossed with both North and South. For example, one homozygote (probably arrangement G) formed a $\odot_{16}$ with North and a $\odot_6 + \odot_{10}$ with South. Thus, this one arrangement involves at least six chromosomes not found in either North or South arrangements.

The minimum number of interchanges required to establish the transect system of arrangements can be estimated as follows: arrangement A differs from South by at least two interchanges ($\odot_6$), F differs from North by at least four ($2\odot_6$), and three interchanges are required to account for the transect arrangements other than A and F. The minimum number of interchanges required for all of the known arrangements, therefore, is nine.

One additional cross was analysed which is pertinent to the interpopulational chromosomal relationships: viz. a cross between a homozygote of population 21·1 and the South arrangement which yielded a 9_{II} individual. Thus, population 21·1 represents the most northerly location known for the South arrangement.

Fifteen transect populations between the North and South chromosomal races have been studied in two different ways: first, the field samples were analysed to determine what types of chromosomal configurations are present in each population and in what frequency, and second, a smaller number of plants from these populations were used in a controlled hybridisation programme to identify the specific arrangements present in each. These two sets of information used together allow a more complete evaluation of the distributions and frequencies of the different arrangements than does either set alone (Bloom, 1970). Such an evaluation allows a number of inferences to be made about the distribution and frequency of major arrangements in the transect (fig. 6).

Arrangement A, found at a frequency of less than 3 per cent. at mile 10, increases toward the south to over 70 per cent. at mile 12·5 and drops to 0 per cent. at mile 21·8. Arrangement F was also found in low frequency (5 per cent.) at mile 10, but increases toward the north to over 60 per cent.

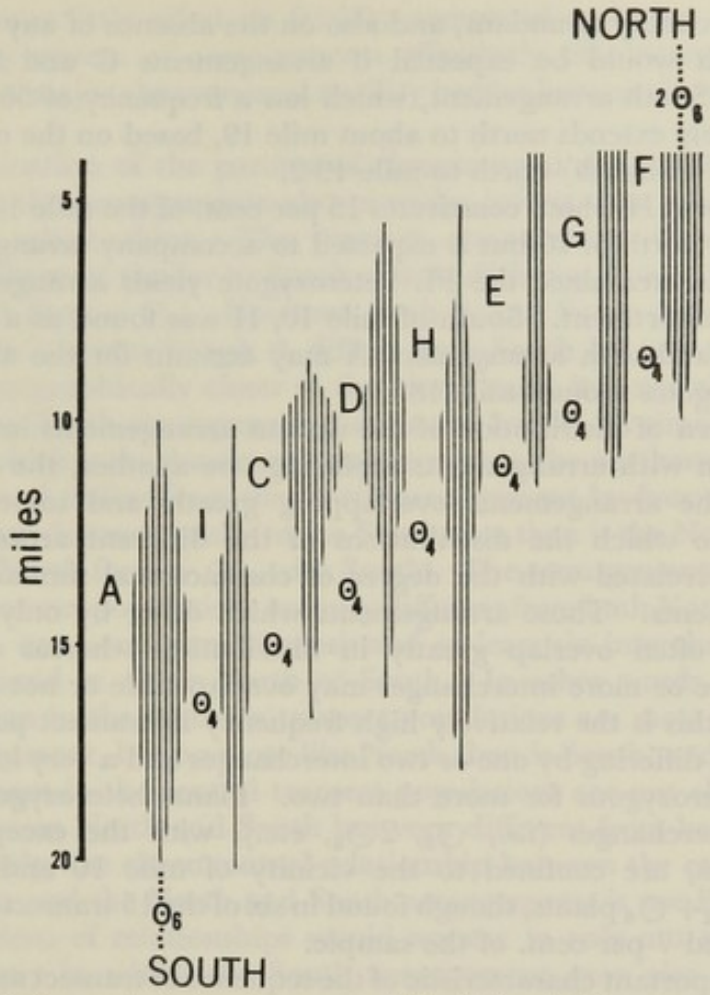


FIG. 6.—Distribution and relative frequency of the indicated chromosome arrangements along the Deer Creek Transect (see fig. 1). The vertical scale along the left indicates position on the transect. The chromosomal relationship between the adjacent arrangements is indicated, and the width of the pattern for each arrangement indicates its approximate frequency at the position indicated.

at mile 7·8; its northern limit is unknown. Arrangements G and E were found at about equal frequencies (10 per cent. and 7 per cent. respectively) at mile 10. The region from mile 11 to 12 likely represents their southern limit since they both form $2\odot_6$ with A, which was abundant in the sample at mile 12·5, and no $2\odot_6$ heterozygote was found in this region. E and G were also found north of mile 10. G, in fact, may reach its maximum frequency just north of mile 10, judging from its appearance as a homozygote at mile 9·2. The northern limits of G and E are unknown but they undoubtedly occur at least north to mile 6·4 since they give a $\odot_4$ and $2\odot_4$ respectively with F which is abundant in this area.

Arrangement D extends northward probably beyond mile 7, whereas C drops abruptly from its frequency of 30 per cent. at mile 10 to 0 per cent. in the region mile 9 to 8. The decrease in C corresponds with the increase in frequency of F in that area undoubtedly because the CF hybrid forms a $\odot_8$ with a considerable loss of fertility; D on the other hand forms a $\odot_6$ with F and the $\odot_6$ type is common at the north end of the transect.

Both C and D, the two most abundant arrangements at mile 10, apparently occur no further south than about mile 17. This conclusion is based on the absence of $\odot_6$ (AC) types and $\odot_4+\odot_6$ (AD) types at mile 17·8

where A is probably abundant, and also on the absence of any $2\odot_6$ heterozygotes which would be expected if arrangements C and South made contact; the South arrangement, which has a frequency of 50 per cent. at mile 21·8, likely extends north to about mile 19, based on the occurrence of the $\odot_6$ types (A-South) north to mile 19·2.

Arrangement H which constitutes 15 per cent. of the mile 10 sample was not identified north of 10 but is expected to accompany arrangements F, E and D in this area since the FE heterozygote yields arrangement H via independent assortment. South of mile 10, H was found as a homozygote at mile 11·6 and with arrangement A may account for the abundance of $2\odot_4$ heterozygotes around mile 18.

The pattern of distribution of the various arrangements in the transect is a succession with arrangements replacing one another, the distributions of some of the arrangements overlapping greatly and some not at all. The extent to which the distributions of the different arrangements do overlap is correlated with the degree of chromosomal similarity between the arrangements. Those arrangements which differ by only one or two interchanges often overlap greatly in distribution whereas those which differ by three or more interchanges may overlap little or not at all. Correlated with this is the relatively high frequency in transect populations of heterozygotes differing by one or two interchanges and a very low frequency of plants heterozygous for more than two. Plants heterozygous for more than two interchanges (*i.e.*, $\odot_8$, $2\odot_6$, etc.), with the exception of the $\odot_4+\odot_6$ class, are confined to the vicinity of mile 10 and uncommon there; the $\odot_4+\odot_6$ plants, though found in six of the 15 transect populations, never exceeded 7 per cent. of the sample.

A most important characteristic of the sequence of transect arrangements, relative to their interpopulational effect, is the gradual nature of the chromosomal changes from the South arrangement to the North arrangement. For example, the South arrangement forms a $\odot_6$ with arrangement A, and at least one unidentified arrangement exists in the southernmost transect populations which forms a $\odot_4$ with South. Toward the north, arrangement A overlaps arrangement I which in turn overlaps C which in turn is succeeded by D, H, E, G and finally F, the dominant arrangement in the most northerly transect population tested. Each of these arrangements differs from the adjacent ones in the sequence by one translocation, whereas the two extremes, A and F, form a $\odot_{12}$. Arrangement F differs from the North race by $2\odot_6$ but additional intermediate arrangements undoubtedly exist between the transect and the North race at Springville because population samples in that area revealed $\odot_4$ and $\odot_6$ heterozygotes but none with $2\odot_6$.

4. Discussion

The boundary region between the South and North chromosomal races of *C. speciosa* was found to be occupied by a minimum of ten different chromosomal arrangements—different from the North and South races and from each other. These boundary arrangements are distributed in such a fashion that crosses within populations and between adjacent populations rarely yield hybrids heterozygous for more that two translocations. The meiotic behaviour of these small rings of four or six chromosomes has been shown to be very regular, with a high frequency of disjunction, and as a

result they have little effect on fertility compared to larger rings. Thus, the boundary system of arrangements effectively eliminates what would otherwise be a major chromosomal sterility barrier between North and South races.

An examination of the pattern of chromosomal similarities and differences among the arrangements also provides information concerning their phylogenetic relationships. The transect arrangement determined to be chromosomally most similar to South was A, differing from it by two translocations that form a $\odot_6$. However, both A and South form a $\odot_{16}$ with North. That is, even though A differs from South by two translocations and occurs geographically closer to the North race, it is not closer chromosomally to the North arrangement than is the South. A similar example is found in F, which is the dominant arrangement in the northernmost transect populations and differs from the North arrangement by four translocations but is no closer chromosomally to the South race than is the North. That is, both F and North form a $\odot_{16}$ with South. The arrangements dominant in the central transect populations are very different from both North and South, for example, one arrangement consists of at least six interchange chromosomes not found in either North or South. In other words, the arrangements common in the southern transect populations are most similar to the South arrangement, but no more like North than is South, and the arrangements dominant in the central transect populations are not chromosomally midway between North and South but very different from both North and South. Clearly, the chromosomal relationship between the central transect arrangements and the North and South arrangements is not linear.

This pattern of relationships would appear to rule out the possibility of a phylogeny in which the South arrangement gave rise to the North arrangement (or *vice-versa*) by a gradual accumulation of interchanges, with the transect arrangements representing the intermediate steps. Two alternatives exist: (1) the transect system of arrangements gave rise to both the North and South races, or (2) the transect arrangements are a consequence of hybridisation between the North and South races. The first model would appear to be as untenable as the linear model. It requires an ancestral arrangement to give rise to new arrangements toward the south and others toward the north, and that these in turn give rise to still additional arrangements, ultimately leading to North and South. Each derivative arrangement must be able to coexist with its progenitor in a polymorphic population but become dominant toward the periphery of its distribution only to give rise to another arrangement which does the same; the ultimate products of this succession, the North and South arrangements, however, are required to establish themselves over the majority of the range of the species as essentially monomorphic races.

The chromosomal relationships among the arrangements are most readily explained as a consequence of hybridisation between the North and South races. According to this hypothesis the two chromosomal races came into contact and produced $\odot_{16}$ hybrids with very low fertility. In such a hybridising population new interchanges which resulted in the production of smaller multiples would be selectively advantageous because of the higher fertility of smaller rings. For example, a single interchange within the North-South hybrid could yield a new chromosome arrangement which gives a $\odot_8$ with one parental arrangement and a $\odot_{10}$ with the other. These

$\odot_8$ and $\odot_{10}$ multiples would have a fertility about three or four times as high as the $\odot_{16}$. Because of its much higher fertility such an arrangement would increase in frequency in the hybrid populations. In fact, every new interchange that resulted in arrangements which gave smaller rings with increased fertility would have a strong selective advantage.

As the number and frequency of new translocations increased the selection pressures would shift such that the frequencies of the new arrangements would be determined by their chromosomal similarity to one another as well as by their interaction with the North and South parental arrangements. Eventually this would lead to the elimination of North and South from the initial hybrid populations because of the low fertility of combinations involving them, and a series of new arrangements which yielded only small rings with one another would come to dominate. Continued interaction between the new arrangements of the hybrid populations and parental arrangements in adjacent populations would result in the establishment of a spatial succession of chromosomal arrangements. Those new arrangements of the hybrid populations which were most similar to South would have a selective advantage in populations intermediate between the South race and the hybrid populations because of their effect on fertility. The large number of interchanges available from the hybrid populations would make possible a smooth transition from the hybrid populations to the South race via a series of intermediate arrangements, and between the hybrid populations and the North race via a different series of intermediate arrangements. Hence, the situation that exists in the boundary region today with the central populations having a number of arrangements similar to one another and different from both North and South arrangements, whereas populations on either side of the central area are dominated by arrangements selected for their chromosomal similarity to the central arrangements on the one side and the parental arrangement on the other.

The boundary system of arrangements linking the North and South races would appear to be stable geographically. The limits of distribution of each of the boundary arrangements is dependent on its relationship to other arrangements. For example, arrangement C is abundant in population 10 where it forms small rings with the other common arrangements there; north of mile 10, however, it is limited because it forms a $\odot_8$ with arrangement F which is common there, and southward C is restricted, for example, by the $2\odot_6$ it forms with the South arrangement. The distribution of each of the arrangements in the boundary area is similarly restricted by other arrangements. The ultimate result is a system of arrangements in a spatial equilibrium.

The system of interchanges that has evolved in the boundary area offers no restriction to gene exchange between populations and no restriction to gene recombination except for genetic information restricted to differential segments that may exist adjacent to the centromeres of interchange chromosomes. Consequently, most of the genetic material is able to recombine and move freely across the boundary area, the extent and direction being a function of selection pressures which are different from those operating to maintain the system of chromosome arrangements. This undoubtedly accounts for the lack of apparent morphological or physiological differences among populations within or adjacent to the boundary area between the two chromosome races. The two races were, however, undoubtedly

differentiated morphologically and ecologically when they initially came into contact and formed hybrids of extremely low fertility.

The northern race at the time of contact was probably comparable in all respects to nitens which as a morphological and ecological entity now occurs many miles to the north of the cytological boundary (fig. 1); the southern race was presumably comparable to polyantha. On this assumption, the present pattern came about as a result of factors that favoured the northward migration of polyantha where it came into contact with nitens at the ecological limit of its distribution. Although one might have expected the chromosomal barrier between them to be reinforced by other barriers to gene exchange, the evolution of the boundary system of arrangements had the effect of increasing recombination and gene exchange. As a result, polyantha-like genotypes are assumed to have become reconstituted on the nitens arrangement of chromosomes because the selective forces responsible for the northward migration of polyantha were still operating to favour polyantha-like genotypes. Once the chromosomal barrier was overcome, the interaction between nitens and polyantha genotypes became comparable to that between any fully interfertile ecogeographic races. The present distribution of nitens and polyantha phenotypes and the zone of intergradation between them is determined by selection operating on these phenotypes independent of any factors operating in the boundary area between the two chromosome races.

5. Summary

1. *Clarkia speciosa* ($2n = 18$) consists of two allopatric chromosomal races, " North " and " South ", which differ by a minimum of seven interchanges and are morphologically indistinguishable where they come together in the Sierra Nevada foothills of California. Artificial hybrids between the North and South races have very low fertility due to nondisjunction.

2. A series of 15 populations transecting the North-South boundary was sampled cytologically:

(i) The average number of interchanges per plant ranged from 0·4 to 1·0 for all populations except one (number 10) which had an average of 2·0.

(ii) Rings of four and six chromosomes are common in all the transect populations whereas rings of eight and ten chromosomes are found only in population 10.

3. Rings of four and six are highly regular in their meiotic behaviour and have little affect on fertility whereas larger rings greatly reduce fertility due to nondisjunction.

4. Hybridisation studies within population 10 revealed a minimum of ten different arrangements involving 12 of the 18 chromosomes, and which differed from one another by one to five interchanges; in combination they produce the following types of interchange heterozygotes—$\odot_4$, $2\odot_4$, $\odot_6$, $\odot_4+\odot_6$, $3\odot_4$, $\odot_8$, $\odot_4+\odot_8$, $2\odot_6$, $\odot_{10}$ and $\odot_{12}$.

5. Interpopulational hybridisation studies show that the arrangements are distributed through the boundary region such that crosses within populations and between adjacent populations usually yield either structural homozygotes or heterozygotes with rings of four or six chromosomes.

6. The pattern of distribution of the boundary arrangements, together

with the high fertility of the small rings, effectively eliminate what would otherwise be a major barrier to gene exchange between the two chromosomal races.

7. A comparison of the chromosomal relationships between the boundary arrangements and the North and South arrangements suggests that the boundary arrangements arose following hybridisation between the two chromosome races.

8. *Clarkia speciosa* provides an example of chromosomal interchanges functioning to increase the interfertility of two chromosomally differentiated races.

6. References

BLOOM, W. L. 1970. The effect of chromosomal translocations on genetic recombination within and between populations of *Clarkia speciosa* Lewis and Lewis. Ph.D. Thesis, University of California, Los Angeles, Library, U.S.A.

LEWIS, H., AND LEWIS, M. E. 1955. The genus *Clarkia*. *Univ. of California Pubs. Botany*, *20*, 251-392.

MOSQUIN, T., AND LEWIS, H. 1959. (Abstract) Variation in relation to reciprocal translocations in a diploid species of *Clarkia* (Onagraceae). *Proc. IX Int. Bot. Congr.*, *2*, 272.

ABSTRACTS OF PAPERS

I. CHROMOSOME STRUCTURE AND SYNTHESIS

ULTRASTRUCTURE OF THE INDUCED LAMPBRUSH STAGE IN POLYTENE CHROMOSOMES

VEIKKO SORSA
Department of Genetics, University of Helsinki, Finland

Comparative studies were carried out by light and electron microscopy of the transition in the normal salivary chromosome banding into a lampbrush type of organisation as a result of urea-alkali treatment. The chromosomes were prepared for electron microscopy by the squash-thin sectioning method. Both uranyl acetate and indium chloride electron stains were used to indicate the position of the DNA and the protein components during different phases of the transition process. An essential feature in the process is the gradual uncoiling of the chromomeric fibrils and the formation of a distinct multistranded central axis with linearly differentiated regions. The induced lampbrush stage provides an opportunity for a closer investigation of the structural organisation of the connecting points between interband and chromomeric fibrils and their interrelationships.

CONSTITUTIVE HETEROCHROMATIN IN *D. MELANOGASTER*

CLAUDIO BARIGOZZI and CARLOTTA HALFER
Istituto di Genetica, The University, Milan, Italy

The structure of the Y chromosome has been studied by several authors especially in relation to the genetical problems raised by this element. *Drosophila* is especially interesting in this sense, since the genetical functions of the Y chromosome have been better analysed in this genus than in any other. The use of embryonic cells cultured *in vitro* allows a higher resolving power which, combined with autoradiography, permits a study of DNA replication in relation to the structural differentiation (blocks of heterochromatin) which can be seen in it. The use of some translocations involving portions of the Y and eu- or hetero-chromatic segments of other chromosomes (both X and autosomes) has revealed a region in the long arm of the Y which seems to be responsible for determining the relative replication time of this chromosome.

DIFFERENTIAL DNA REPLICATION OF THE Y CHROMOSOME IN SPERMATOGONIA OF THE INDIAN HOUSE SHREW, *SUNCUS MURINUS*

S. R. V. RAO, S. G. A. RAO, V. K. SHARMA and V. C. SHAH
Department of Zoology, University of Delhi, Delhi 7, India

Terminal stages of DNA synthesis in the metacentric duplicate-type X and submetacentric Y chromosome of *Suncus murinus* in the bone marrow cells and spermatogonia were studied autoradiographically. The X chromosome synthesises DNA synchronously with the autosomes in both the bone marrow cells and spermatogonia. The Y replicates early in the bone marrow cells but its long arm replicates relatively later (differential pattern) in spermatogonia. It is concluded that (i) A reversal in the synthetic pattern occurs in the Y from an early replication in somatic cells to late replication in spermatogonia, and (ii) The short arm of the Y is homologous with one of the arms of the X.

MOLECULAR AND STRUCTURAL ASPECTS OF THE ENDOMITOTIC CHROMOSOME CYCLE IN ANGIOSPERMS

WALTER NAGL
Institute of Botany, The University, Rennweg 14, Vienna 1030, Austria

Microscopic preparations will demonstrate: (1) the chromatin structure of nuclei from roots of *Allium flavum* during G_1, G_2, and endomitosis, (2) the polytene chromosomes of highly endopolyploid suspensor nuclei of *Phaseolus coccineus*, (3) incorporation of ^{3}H-thymidine into heterochromatin during the condensed (chromocentre) stage, (4) incorporation of ^{3}H-uridine into dispersed chromatin during endomitosis.

Diagrams will illustrate: (1) the position of endomitosis in comparison with the mitotic cycle, (2) the effect of the endomitotic short-cut on chromatin structure (polytene chromosomes), (3) the different effect of inhibition of RNA synthesis by actinomycin D on the mitotic and endomitotic indices, (4) the different effect of lysine-rich histones on the mitotic and endomitotic cycle.

THE RELATIONSHIP BETWEEN NUCLEAR DNA AMOUNT AND THE DURATION OF THE MITOTIC CYCLE

G. M. EVANS, H. REES, C. L. SNELL and S. SUN
Department of Agricultural Botany, University College of Wales, Penglais, Aberystwyth

The duration of the mitotic cycle in root meristems of diploid species increases with increasing nuclear *DNA* content. The rate of increase varies as a result of polyploid and the addition of B chromosomes. For comparable amounts of *DNA* the duration of the cycle differs between monocotyledons and dicotyledons.

DNA CONTENT OF RELATED SPECIES

D. P. FOX
Department of Genetics, Marischal College, Aberdeen, AB9 1AS

Previous work with *Dermestes* (Fox, *Chromosoma 27*, 130-144 (1969)) has shown that in spite of karyotype uniformity, the spermatid DNA value may be markedly different in related species. Further, in some somatic tissues, these differences are maintained since the nuclei have DNA contents which are members of a doubling series (Fox, *Chromosoma 28*, 455-456 (1969)). However, in other tissues, many nuclei occur which are not members of a doubling series (Fox, in press.)

This situation has been analysed further by making detailed density maps of Feulgen-stained nuclei in order to assess the proportion of the DNA which is heterochromatic in different tissues and species and the status of replicating of the hetero- and eu-chromatin within nuclei of the same tissue.

The data will be discussed in relation to the process of tissue differentiation and chromosome evolution in the genus.

THE MOLECULAR STRUCTURE OF NUCLEOHISTONE

J. F. PARDON and B. M. RICHARDS
Searle Research Laboratories, High Wycombe, Bucks., U.K.

Results obtained from X-ray diffraction studies of both intact nuclei and isolated nucleohistone are very similar and, consequently, nucleohistone structure can be interpreted in relation to the structure of interphase chromosomes. These results indicate that a regular supercoil structure is very likely to occur in nucleohistone molecules and work is in progress to ascertain which of the histone types is responsible for constraining the DNA double-helices into this regular tertiary configuration.

In order to examine the interaction of histone proteins with DNA we have built molecular models of histone $F2a_1$. The possible arrangements of this histone relative to the DNA double helix and its ability to induce supercoil formation will be discussed.

CHROMOSOME STRUCTURE STUDIED BY NUCLEIC ACID HYBRIDISATION IN CYTOLOGICAL PREPARATIONS

MARY LOU PARDUE and JOSEPH G. GALL

M.R.C. Epigenetics Research Group, Department of Genetics, University of Edinburgh and Department of Biology, Yale University

A technique is described for the formation of molecular hybrids between either DNA or RNA in solution and the DNA of cytological preparations. Specific DNA sequences can be localised at the chromosome level by this means following the autoradiographic detection of radioactive hybrid molecules.

In *Xenopus* oocytes a mass of extra DNA forms a cap over the chromosomes at pachytene. Ribosomal RNA binds strongly to this mass which shows that it contains many extra copies of genes which code for this type of RNA.

In the salivary glands of *Drosophila* the ribosomal cistrons are located within the nucleolus. In those of sciarid flies, on the other hand, they are present both in the nucleolar organsing regions of the chromosomes and in the numerous micronucleoli which are scattered throughout the nucleus. The extra DNA in the DNA puffs of sciarid flies does not hybridise with ribosomal RNA which shows that genes other than those coding for ribosomal RNA can undergo amplification.

Satellite DNA sequences of the mouse are localised in the centromeric heterochromatin of all the chromosomes except, possibly, the Y chromosome. In this species, therefore, centromere associations in various types of interphase cell can be studied from the pattern of binding of satellite DNA in them.

REGULATION OF RNA SYNTHESIS IN THE EVALUATION OF BREAD WHEAT

H. K. JAIN and R. N. RAUT

Division of Genetics, Indian Agricultural Research Institute, New Delhi 12, India

The nucleolus organising locus is an active site of RNA synthesis, accounting for the bulk of cellular RNA. We have studied the incorporation of tritium labelled uridine in the root tip cells of (i) common bread wheat, (ii) its three diploid progenitors, which contribute the A, B and D genomes, (iii) two of the tetraploid wheat species and (iv) the different ditelosomic and monotelosomic lines of bread wheat, in which the chromosome arm carrying the organising locus of one of the four such pairs has been deleted in a homozygous or hemizygous condition.

The single organising locus of the A genome synthesises more RNA than the combined turnover of the two loci of the B genome and the locus present in the D genome is the least active. These conclusions are supported by observations on all four types of materials listed above.

The synthetic activity of the organisers in polyploid nuclei is not additive. Thus, the synthesis in the hexaploid and the tetraploid nuclei does not add up to the turnover of the respective diploid parental species. An interaction between the different organising loci is also indicated by the observation that in hexaploid nuclei the loss of the organiser present on chromosome 6B or 5D does not significantly reduce the turnover of RNA. In other words, an increased compensatory activity is shown by other organisers in such nuclei. There is also evidence from monotelosomic lines that an organising locus can be as effective in the hemizygous as in the homozygous condition.

ACIDIC PROTEIN AND RNA IN THE INITIATION OF PUFF FORMATION

THOMAS K. H. HOLT
Cell Biology Section, Department of Zoology, University of Nijmegen, The Netherlands

In the salivary gland chromosomes of *D. hydei*, specific puffs can be induced by temperature treatment. These puffs show a high affinity for dyes which can be bound electrostatically to proteins. Quantitative determinations of the binding capacity of puffs for naphthol yellow S at various times after their induction reveals a considerable increase in protein staining during puff formation. Specific amino acid staining of these regions is only found for tryptophane. Autoradiographic experiments with labelled amino acids and alkyl-groups did not reveal any puff-specific incorporation. Measurements with the interference microscope indicated a considerable increase in dry matter even after extraction of the RNA.

These results strongly support the idea that proteins participate in the activation of genes.

II. CHROMOSOME MECHANICS AND MUTATION

INFLUENCE OF UV MICROBEAM ON SPINDLE FINE STRUCTURE AND ANAPHASE CHROMOSOME MOVEMENTS

ANDREW BAJER
Department of Biology, University of Oregon, Eugene, Ore. 97403, U.S.A.

In the endosperm of *Haemanthus katherinae*, mid-anaphase chromosome movements in one of the half-spindles can be retarded or stopped by microslit UV irradiation of either the kinetochores or adjacent regions of the half-spindle. Interzonal irradiation affects movement in both anaphase groups. Early and mid-anaphase are very sensitive but, as a rule, late anaphase is not affected. Fragmentation or reduction in the number of microtubules can be found 30-60 seconds after irradiation.

ANAPHASE BEHAVIOUR OF INVERSIONS IN LIVING CRANE-FLY SPERMATOCYTES

ROLAND DIETZ
Max-Planck-Institut für Zellbiologie, 74 Tübingen, Germany

Chromosome movement has been studied in 32 spermatocytes of an X-ray induced mutant of *Pales ferruginea* in which two pairs of autosomes detach regularly when the chiasmata open at the beginning of anaphase while a third pair remains connected by an inversion bridge.

The free dyads approach their poles with nearly constant velocity until they have reached kinetochore-to-pole distances of 4 to 1 μ. The distances at which the bridge kinetochores stop approaching their poles, or at which they retard considerably, are generally much larger. Before the pole approach is retarded, the velocity of the bridge kinetochores is positively correlated with the velocity of the free dyads. The length increment of chromatid bridges during anaphase is larger, when (*a*) the interkinetochore ditsance of the bridge bivalent at metaphase is greater, *i.e.* the less the chromosomes are contracted, (*b*) the larger the length increment of the spindle, and (*c*) the faster the free dyads approach their poles. The polar approach of the bridge kinetochores stops when the bridges have reached the length expected on the basis of the correlations quoted, irrespective of the kinetochore-to-pole distance at this time.

The residual distances differ considerably, depending on spindle length at the beginning of anaphase. Except for distances smaller than 4 μ, the strength of the anaphase force is independent of the kinetochore-to-pole distance. In the case of crane-fly spermatocytes, at least, this means that within the range mentioned the anaphase force is independent of the length of the chromosomal fibre. This finding argues strongly against certain hypotheses of chromosome movement, and is conceivable on the basis of others.

MODE OF ACTION OF THE PAIRING-LIMITATION GENE IN WHEAT (*TRITICUM AESTIVUM*, 6x)

C. J. DRISCOLL, N. L. DARVEY and H. NEWTON BARBER
Botany School, University of N.S.W., Australia

It has been suggested that the gene(s) on chromosome $5B^L$, which restrict pairing to true homologues, operate by leading to a closer somatic pairing of true homologues during the pre-meiotic mitoses. We find there is no difference in the mean distance apart at metaphase in root-tips of telocentrics, whether they be homologous or non-homologous.

Again, a study of the frequency of nucleolar fusion has shown that homologous nucleolar organisers are no closer to one another than are non-homologous organisers.

Pairing at meiosis may involve three phases: (i) association of homologues and homoeologues during the last pre-meiotic mitoses or the last interphase. This process is inhibited by colchicine; (ii) at the commencement of zygotene pairing, $5B^L$ enables the *recognition* of true homologues and (iii) completion of pairing.

GENETIC CONTROL OF THE POST-SYNAPTIC PHASE OF MEIOSIS IN RYEGRASS

B. S. AHLOOWALIA
Plant Sciences Division, Agricultural Institute, Oak Park, Carlow, Ireland

In a desynaptic mutant of perennial ryegrass, *Lolium perenne* L., homologous chromosomes are paired at diplotene but separate before metaphase I. An increase in temperature during early meiosis increases the degree of desynapsis. All the plants in the selfed progeny were desynaptic and the F_2 of desynaptic × normal segregated to show that desynapsis was determined by a single recessive gene, *ds*, with or without modifiers. The control mechanisms of meiotic chromosomal behaviour will be discussed in the light of the data on chiasma number and univalent frequency in diploid, hypertriploid and tetraploid desynaptics.

LOCALISATION OF INTRASPECIFIC VARIATION IN MEIOTIC CHROMOSOME PAIRING

J. SYBENGA
Department of Genetics, Gen. Foulkesweg 53, Wageningen, The Netherlands

When three marked homologous chromosomes, derived from different inbred lines of *Secale cereale* are brought together, the association in twos is non-random: some chromosomes are more efficient in attracting a partner than others. The relative frequencies of MI configurations in interchange heterozygotes (break point in main region of pairing initiation) with an extra telocentric chromosome, are used to localise the difference between specific chromosomes with respect to pairing efficiency. In one case, the greater part of this difference resides in only a few of the sites (zygomeres) normally effecting pairing initiation in rye.

NON-DIVIDING CELLS IN MERISTEMS

F. A. L. CLOWES
Botany School, University of Oxford, England

Analysis of pulse-labelled roots shows that there is a proportion of non-dividing cells in most parts of the meristem. In the quiescent centre in *Zea* more than half the cells do not divide and about 17 per cent. have a " fast " mitotic cycle of 40 hours compared with a cell-doubling time of 230 hours for the region. In the cap initials with a cell-doubling time of 15 hours, 65 per cent. of the cells have a mitotic cycle of 10 hours and 15 per cent. of the cells do not divide. In the stelar meristem the proportion of non-dividing cells falls from 20 per cent. to none in moving 200 μm from the quiescent centre.

THE ACTION OF 2-THIOCYTOSINE ON THE MITOSIS OF HUMAN LYMPHOCYTES IN CULTURE

J. TIMSON and D. J. PRICE
University Department of Medical Genetics, The Royal Infirmary, Manchester, M13 9WL, United Kingdom

The effect of 2-thiocytosine on the rate of mitosis of phytohaemagglutinin-stimulated human lymphocytes *in vitro* has been studied. At concentrations of 10^{-2}, 10^{-4} and 10^{-6}M inhibition of mitosis occurs while at 10^{-3} and 10^{-5}M the mitotic rate is increased. There is, therefore, an oscillation of the effect on mitosis with concentration. Cytosine administered at equimolecular concentrations simultaneously removes this oscillation, the mitotic rate being effectively the same as the control between 10^{-2} and 10^{-6}M. Thymine and uracil similarly remove most of the effect of 2-thiocytosine.

ENDOSPERM FAILURE AND INCOMPATIBILITY IN CROSSES BETWEEN *TRITICUM* AND *SECALE*

J. P. MOSS
Department of Agricultural Botany, University of Reading

When *Triticum* species are pollinated by *Secale cereale* a hybrid embryo is usually produced but it rarely survives unless it is cultured. Embryo death is always accompanied, and usually preceded, by failure of the endosperm.

The paper outlines the normal development of embryo, endosperm and antipodals in wheat and rye and discusses the abnormalities that occur in hybrid embryos and endosperms produced by pollinating the hexaploid, tetraploid and diploid wheats with diploid and tetraploid rye. A comparison is made between crosses which produce hybrid embryos which can be cultured, and those which produce embryos which have not yet been cultured successfully.

CHROMOSOMALLY MARKED POPULATIONS OF CELLS IN HUMAN BONE MARROW

SYLVIA D. LAWLER
Department of Clinical Research, Royal Marsden Hospital and Institute of Cancer Research, Fulham Road, London S.W.3

In the chronic myeloproliferative diseases of man chromosomally marked populations of bone marrow cells may be observed. In some untreated patients a major or minor population of cells may show aneuploidy. If treatment has been by irradiation, stable clones of cells with radiation induced structural chromosomal

abnormalities may arise. The frequency of both such abnormal clones can be studied in relation to the fluctuating levels of the formed elements of the blood. At all stages of chronic myeloid leukaemia, almost all the cells carry a specific chromosomal abnormality, the Philadelphia chromosome (Gp−). The postulate that all the bone marrow cells in chronic myeloid leukaemia originate from a single cell has been exploited to examine the validity of the Lyon hypothesis as applied to the sex-linked Xg blood-group.

INSTABILITY OF THE PERICENTRIC REGION AND SELECTIVE REDUPLICATION OF CHROMOSOME 2 IN MAN

M. FRACCARO, J. LINDSTEN, L. TIEPOLO and N. RICCI

Gruppo Euratom, Via Forlanini, 14, 27100. Pavia, Italy. Dept. Endokrinilogisk, Audelningen, Karolinska Sjukhuset, Stockholm 60, Sweden

Apparent discontinuity of the long arm region adjacent to the centromere of chromosome No. 2 was found in a proportion of mitoses from blood cultures of three individuals, without relation to the phenotype. The discontinuity was in most instances heterozygous and variable in extension, with occasional total dislocation of the two chromosome arms. In all cases there were a few cells with total reduplication of the two chromatids distal to the discontinuity resulting in a chromosome arm composed of four chromatids. Autoradiography with tritiated thymidine and electron microscopy failed to reveal DNA continuity at the sites of the discontinuity. Experiments with internal radiation from excess of tritium did not increase the prevalence of the abnormality.

ON THE ORIGIN AND NATURE OF ACHROMATIC LESIONS

JYOTI P. CHAUDHURI

Department of Clinical Physiology, Cytogenetics Section, University of Ulm, D-79 Ulm, Germany

For about ten years, achromatic lesions have been distinguished from chromatid and chromosome breaks and considered as a distinct type of damage to the chromosomes. Ionising radiations and radioimetic chemicals induce achromatic lesions, viral damage to chromosomes is reported to be mainly of this type and they appear to predominate among spontaneous aberrations. They are said to arise during the S or G_2 phase of the cell cycle and restitution, as a rule, takes place in the subsequent cell cycle (Scott and Evans, *Mutation Res.*, 4 (1967) 579). Thus, like chromatid type aberrations, they may provide a good indication of the time of occurrence of an induced chromosomal lesion. But as the definition of achromatic lesions is somewhat vague and their identification often difficult, a detailed re-examination of their real status is necessary.

On the basis of microscopic observations on human and other vertebrate chromosomes, and after a critical comparison with chromatid breaks, centromeric constrictions and secondary constrictions, it appears very likely that the achromatic lesion represents a kind of tertiary constriction arising from some disturbance in the spiralisation of a chromosome.

THE PARTICIPATION OF CHROMOSOME ARMS IN RADIATION INDUCED CHROMOSOME TYPE EXCHANGE

JOHN R. K. SAVAGE

M.R.C. Radiobiology Unit, Harwell, Berkshire

In contrast to *chromatid* exchanges, participation of arms in *chromosome* exchanges can only be investigated by indirect means. In cells with few chromosomes, such

studies can only be performed at low radiation doses, when the observed exchanges are unmodified by undetectable secondary events.

Using *Campelia zanonia* microspores and doses below 150 rad X-rays, exchange between and within arms has been studied.

Probability of exchange between arms is not a simple function of length, and size distribution patterns of acentric fragments suggest that preferential exchange occurs at certain loci.

THE INDUCTION OF CHROMOSOMAL ABERRATIONS BY SULPHUR MUSTARD IN MARSUPIAL LYMPHOCYTES

DAVID SCOTT and T. R. L. BIGGER
M.R.C. Radiobiology Unit, Harwell, Didcot, Berkshire

Lesions, from which chromatid aberrations are derived, can be induced in cells in all stages of the mitotic cycle after treatment with alkylating agents. The aberrations are not formed, however, until the time of DNA synthesis (S-phase) and are presumably the result of replication errors. But lesions can be repaired before the damage is " fixed " at S.

III. CHROMOSOMES IN NATURAL POPULATIONS

CYTOGENETICS OF A GROUP OF MORABINE GRASSHOPPERS WITH XY AND X_1X_2Y MALES *

M. J. D. WHITE and J. CHENEY
Department of Genetics, University of Melbourne, Australia

The " P52 " group (Orthoptera, Eumastacidae, Morabinae) includes four undescribed species of grasshoppers which live on shrubs in central Queensland. These are referred to as P52a, P52b, P85, and P53. P52a, P85 and P53 are neo-XY species with $2n♂ = 16$, in which the original X has become fused to one of the six small autosomes (fusion F14). They differ slightly in the relative size of the neo-X chromosome and especially in the shape and size of the neo-Y; in addition P52a shows four different types of Ys, each apparently characteristic of a particular geographic area. There are also differences between the small autosomes of these species which indicate that numerous structural changes must have occurred in their phylogeny. P52b is an X_1X_2Y species with $2n♂ = 15$, $2n♀ = 16$, in which an additional Y-autosome fusion (F15) has occurred. One individual of P52a was heterozygous for a newly arisen 3-break chromosomal rearrangement involving a pericentric inversion and a mutual translocation.

The crosses P53♂ × P85♀ and P85♂ × P53♀ gave offspring of both sexes, but no offspring were obtained from the crosses P52a♂ × P53♀ and P53♂ × P85♀. Male hybrids between P53 and P85 had underdeveloped testes. Some of these testes were inhibited in development to such an extent that no meiotic divisions occurred; others showed highly abnormal spermatogenesis with many univalents. Female hybrids had abnormal ovaries with few oocytes; but those oocytes that had undergone development showed normal first meiotic divisions with complete synapsis of the chromosomes of the parental species.

* Supported by Public Health Service Grant No. GM-07212 from the United States National Institutes of Health and by a grant from the Australian Research Grants Committee.

THE CHROMOSOMES OF SOME CHRYSOMELID COLEOPTERA : DIABROTICITES

S. G. SMITH
Insect Pathology Research Institute, Department of Fisheries and Forestry, Sault Ste Marie, Ontario

A survey is reported of New World Diabroticites covering 55 species and subspecies in eight genera. With one exception, all have an XX : XO sex-determining system. B chromosomes occur in 15 taxa in five of the genera but ignoring these, male diploid numbers range between 13 and *ca.* 37, with interspecific differences present in six genera. All 29 *Diabrotica* taxa, the genus most widely investigated, have 19 chromosomes in males. *Acalymma* ($2n$ = 19-*ca.* 37) includes a Mexican species, near *A. trivittatum*, with presumed XXY males but, because B chromosomes are present, uncertainty exists as to the precise autosomal number and, due to quarantine regulations, no females have been available for study. *A. trivittatum* itself has $2n$ = 19 and lacks B chromosomes. The possibility that polyploidy occurs is discussed.

THE STRUCTURE AND ROLE OF B CHROMOSOMES IN THE MOTTLED GRASSHOPPER

GODFREY HEWITT
School of Biological Sciences, University of East Anglia, Norwich, U.K.

The elucidation of the role of B chromosomes has remained a somewhat intractable problem. The B chromosome system of *Myrmeleotettix maculatus* has been approached from a variety of angles to increase our understanding of it. Two areas particularly have yielded interesting results. It has been possible to isolate and characterise the DNA of these B chromosomes from several populations and the significance of these results to the function and evolution of this element will be discussed. At the population level detailed analysis of a few locations, in zones of transition between B-containing and non-B areas, provide data for an understanding of the evolutionary forces at work in this system.

POPULATION CYTOGENETICS OF THE MALAYAN HOUSE RAT, *RATTUS RATTUS DIARDII*

HOI SEN YONG
Division of Genetics, School of Biological Sciences, University of Malaya, Kuala Lumpur

Owing to pericentric inversion, the longest pair of autosomes in *Rattus rattus diardii* is heteromorphic, and the individuals are of three types, homomorphic acrocentric (A/A), homomorphic subterminal (S/S) and heteromorphic (A/S).

The frequencies of the three karyotypic classes conform with the Hardy-Weinberg distribution but differ in different populations.

Variation in chromosome number, mainly trisomy, was observed only in homomorphic subterminal and heteromorphic individuals. A case of Robertsonian translocation with $2n$ = 41 was also found.

The results are compared with the findings reported for other forms of *Rattus rattus*.

HYBRIDISATION IN NATURE BETWEEN TWO CHROMOSOME FORMS OF SPINY MICE

J. WAHRMAN
Laboratory of Genetics, The Hebrew University, Jerusalem, Israel

Many closely related mammals differ in their karyotype and, if their distribution is contiguous, hybrids between them may be formed in nature; alternatively hybrids can often be produced in the laboratory. An analysis of chromosome pairing in those hybrids where meiosis occurs may provide clues to the evolution of the forms involved. Studies of the mitotic chromosomes alone, on the other hand, are of limited value.

In the genus *Acomys* a hybrid zone between two sibling forms that differ by a single Robertsonian change was recently discovered in Sinai and two chromosomally similar but geographically isolated forms were experimentally crossed. A complex history of chromosome rearrangements was revealed by the formation of several chromosome chains, one of which included about 28 chromosomes. In this case the occurrence of independent series of centric fusions, rather than fissions, is inferred.

CYTOLOGY OF SOME INDIAN ASCLEPIADACEAE

MIRA JASH and ARCHANA SHARMA
Department of Botany, University of Calcutta, Calcutta 19, India

Detailed cytological studies have been carried out on 17 species under 14 genera of the family Asclepiadaceae. All of them show a basic set of $n = 11$ chromosomes. Cytologically, they form a homogenous assemblage and only changes at the gene level and minor chromosome alterations were important in their evolution. The inclusion of *Cryptolepis*, *Cryptostegia* and *Hemidesmus* under a separate group by certain taxonomists cannot be supported from the observations presented here. The position of this family under the order Apocyanales, showing affinities with the Apocyanaceae, has been justified.

POLYPLOIDY AND CHROMOSOME SIZE

A. K. SHARMA
Department of Botany, University of Calcutta, Calcutta 19, India

Chromosome size and polyploidy vary within different groups of plants. In certain orders of algae, an increase in chromosome size has been noted along with increase in chromosome number. In a majority of the pteridophytes, chromosome size does not show any significant variation in relation to polyploidy. In angiosperms, on the other hand, diminution in chromosome size is mainly associated with polyploidy, especially in induced autopolyploids.

MEIOSIS AND FERTILITY IN APOGAMOUSLY PRODUCED DIPLOID PLANTS OF *ASPLENIUM TRICHOMANES*

J. BOUHARMONT
Institut Carnoy, 24, Vaartstraat B-3000 Louvain, Belgium

In order to verify the autoploid origin of some tetraploid Aspleniums ($2n = 144$), apogamous diploid sporophytes have been induced by culture of gametophytes on synthetic media and mature plants have been obtained for *A. trichomanes*. The pairing of the chromosomes was rather regular; the frequency of univalents was low

but variable depending on the stage and the sampling. Nevertheless, some chromosomes were lost at anaphase and the spores were smaller than in the natural diploids. Eighty per cent. of these spores were well formed but their germination was very poor. Thus the genomes of the tetraploid plants were only partly homologous.

CYTOGENETIC PATTERNS IN *AMARANTHUS*

T. N. KHOSHOO and M. PAL
National Botanic Gardens, Lucknow, India

Species differentiation in the generally cross pollinated Section *Amaranthus* Sauer involves both genic and cryptic structural differences. Bivalent pairing and various degrees of sterility are found in diploid interspecific hybrids, the polyploid forms of which show autopiold or segmental alloploid characters.

On the other hand, in the generally self-pollinated Section *Blitopsis* Dumort., interspecific hybrids show interchange heterozygosity involving long segments of 4 to 14 chromosomes and total segregational sterility. Complete fertility is restored in amphidiploids. The naturalness of the two Sections is evident from these patterns together with the lack of hybrids between them.

INTERCHANGES ON INTERPOPULATIONAL GENE EXCHANGE IN *CLARKIA SPECIOSA*

WILLIAM BLOOM and HARLAN LEWIS
Division of Life Sciences, University of California, Los Angeles, California 90024, U.S.A.

Clarkia speciosa ($2n = 18$) comprises two chromosomal races which come into contact in one area. Hybrids between them form a ring of 16 chromosomes and one pair and have low fertility because of a high frequency of nondisjunction. Thirteen populations along a twenty-mile transect through the boundary area were examined and with one exception were found to include only structurally homozygous individuals or those with rings of 4 or 6. The exceptional population included a few individuals with rings of 8 and one with a ring of 10. From these populations, ten arrangements have been identified, all of them different from those characteristic of the two chromosome races. The arrangements are distributed in such a fashion that crosses within and between populations rarely form individuals heterozygous for more than two interchanges. Since small rings of 4 or 6 have a very high frequency of regular alternate segregation, they have little if any effect on fertility. Thus a system of arrangements has evolved within the boundary area which effectively eliminates what would otherwise be a major chromosomal barrier to gene exchange between the two races.

IV. PAPERS IN ABSTRACT ONLY

CHROMOSOMES AND THE PRODUCTION OF ISO-ENZYMES

H. NEWTON BARBER, C. J. DRISCOLL and R. S. VICKERY
Botany School, University of N.S.W., Australia

Attempts to locate genes producing enzymes in wheat and rye have been successful for two different esterases. The genes producing the electrophoretically fast-moving set are on the homoeologous arms of all chromosomes of group 3, including 3R of rye. The slower ones are on the other arm of chromosomes 3A and 3D. 3B is inactive.

All homoeologous polypeptides of the fast group can interhybridise, whilst the slow group cannot.

In addition, *Triticale* shows a new exterase enzyme which is not present in rye or wheat. For its production, chromosome 6R of rye is necessary. It is not known which, if any, wheat chromosome is involved.

Preliminary work has also shown that following infection of wheat of *Puccinia graminis* new iso-enzymes not present in the uredospore or the plant can appear.

CYTOGENETIC AND BIOCHEMICAL STUDIES OF MAMMALIAN X CHROMOSOME INACTIVATION

MAIMON M. COHEN and MARIO C. RATTAZZI

Division of Human Genetics, Department of Pediatrics, State University of New York at Buffalo, and Buffalo Children's Hospital, Buffalo, New York, U.S.A.

Supportive evidence bearing on the single active X hypothesis has been derived from diverse experimental systems, each yielding only partial proof, and the validity of the hypothesis has been challenged recently. By utilising hybrid animals, with suitable markers, several tenets of the Lyon hypothesis have been investigated within a single set of experimental conditions which might simultaneously elucidate some of the biochemical and cytological aspects of mammalian X chromosome inactivation. Fibroblast cultures of 3 female mules, whose maternal and parental X chromosomes are distinguishable, have been examined by autoradiography. Significant deviations from the expected 1 : 1 ratios (horse : donkey) of late X chromosome replication were obtained in all cases. A similar finding was observed in a female hybrid of two gerbil species. Clones of cultured fibroblasts from these animals have been established and will be investigated autoradiographically to ascertain the pattern of X chromosome inactivation in the progeny of a single cell. Biochemical determinations, both quantitative and qualitative, of glucose-6-phosphate dehydrogenase (G-6-PD) will be correlated with the cytological studies.

CHROMOSOME ACTIVITIES AND DIFFERENTIATION *

A. B. DA CUNHA

Universidade de São Paulo, Departmento de Biologia, Caixa Postal 8105, São Paulo, S.P., Brasil

In *Bradysia elegans* and *B. mogiensis* the salivary glands have sharply differentiated segments, the cytoplasmatic products of which are different. The polytene chromosomes are also sharply differentiated. They differ mainly in size and in the timing of DNA and RNA synthesis. Other species of Sciaridae such as *Rhynchosciara angelae*, *R. bachanti*, *R. milleri* and *Sciara ocellaris*, do not have salivary glands with sharply differentiated segments. Their glands present gradients in the secretion of protein and mucopolysaccharides and there is no sharp chromosome differentiation.

RNA puffs were found in all the species. The gland cells of *Bradysia* secrete throughout the larval period, but well developed RNA puffs appear only at the end of the larval period when the cytoplasmatic products are not elaborated but excreted.

DNA amplification was also found in all the species studied but different forms of DNA amplification were found in *Hybosciara fragilis*, *Tricchosia pubescens* and *Plecia sp.* Numerous micronucleoli are produced and released at several regions of the polytene chromosomes in the salivary glands of *H. fragilis* and *T. pubescens*. The micronucleoli consist of a DNA core surrounded by material rich in RNA. In *Plecia sp.*, however,

* This research is being done by A. B. da Cunha and J. S. Morgante (São Paulo, Brasil) in collaboration with C. Paxan and J. J. Biesele (Austin, U.S.A.) with the technical aid of M. C. Garrido, J. Marques, T. Picard, I. Romeo (São Paulo, Brasil) and R. Riess (Austin, U.S.A.) and financial aid from FAPESP and CNP_q (Brasil) and NIH (U.S.A.).

lateral loops with granules of DNA are produced in the polytene chromosomes of salivary glands and, especially, Malpighian tubules. The loops break and segments are released into the nucleoplasm.

Chromosomal differentiation was always found with cytoplasmatic differentiation but the pattern of chromosomal changes varied from species to species.

Gregarine and virus induced differentiation were also studied in the intestinal caeca cells of *Trichosia pubescens* and in *Rhynchosciara angelae* respectively.

The gregarine studied develops in the cytoplasm but attached to the nuclear membrane of the host cell. Cell growth to giant size, chromosome and nucleolar enlargement, chromosome constrictions and fibrillation are induced by the growing gregarine.

The DNA virus studied in *R. angelae* multiples within the nuclei of the infected cells. It induces chromosome and nucleolar enlargement as well as breaks and constrictions. The latter appear at specific regions but their distribution depends on the virus strain. Electron microscopy reveals the phases of virus development and multiplication as well as the relations between the virus and the host cell chromatin which is used by the virus.

Cells infected by gregarine or virus are really unicellular tumours induced by the parasite.

CHROMOSOME ULTRASTRUCTURE

M. B. E. GODWARD

Department of Plant Biology and Microbiology, Queen Mary College, Mile End Road, London, E.1

Pretreatment of nuclei leads to " unpacking " of the chromosome fibrils whose relationship to the complex structure is clarified.

DNA IN ANTS

ELIZABETH HAUSCHTECK-JUNGEN

Zoologisches Museum der Universität Zürich, 8006 Zürich Künstlergasse, Schweiz

DNA, measured by Feulgen microspectrophotometry, and nuclear volume were investigated in two specimens of *Lasius* (*Dendrolasius*) *fuliginosus* (Formicidae). One was kept in the laboratory for about two years, the other was collected from a natural habitat and analysed immediately.

Extinction and volume of the first sample varied around a mean value while in the second sample values decreased continuously from May to October. UV measurements and first autoradiographical results lacked evidence for additional DNA synthesis.

CYTOGENETIC POLYMORPHISM AND EVOLUTION IN MAMMALIAN SOMATIC CELL POPULATIONS *IN VIVO* AND *IN VITRO*

D. T. HUGHES

Institute of Child Health, University of London, W.C.1

Studies on chromosomal variation in relation to age and cytogenetical instability apparently demonstrated a state of balanced chromosomal polymorphism in certain somatic cell populations. This has been interpreted to be the result of co-operative as well as competitive interactions between cells. Further details of work reported earlier (*Nature, 217*, 518-523) will be presented and discussed in relation to subsequent studies on mosaics and the possible utilisation of cultured cells as a model system for studies on evolution.

MALE MEIOSIS IN PTINID BEETLES

EUGENIO ORTIZ
Instituto de Genética y Antropología, Centro de Investigaciones Biológicas, C.S.I.C., Madrid

Eleven species belonging to eight different genera of ptinids have $2n = 18$ chromosomes ($16+Xy_p$), the X and autosomes being either metacentric or submetacentric. The non-specific association of the parachute type between the sex chromosomes is maintained by nucleolar material. A nucleolus associated with autosomes may also be present.

In a number of cases variations were observed in chromosome size, extent of autosomal heterochromatin, chiasma localisation and failure of pairing.

The differences found in meiosis among several families and genera of beetles are connected with their phylogeny.

CYTOLOGIC DEMONSTRATION OF MITOTIC CROSSING-OVER IN MAN

K. PATAU and E. THERMAN
Genetics Building, University of Wisconsin, Madison, Wis., 53706, U.S.A.

Chromatid interchange figures (quadriradials—QRs) were studied in different samples (lymphocytecultures): X-ray induced, Fanconi's anaemia, " spontaneous ", Bloom's syndrome. The frequency of " mitotic chiasmata " (homologous, isolocal, alternate) among all QRs ranges from about 6 per cent. in the first to 89 per cent. in the last sample. The occurrence of mitotic chiasmata in different chromosomes is strikingly nonrandom, that of other QRs random. Whereas the latter are as often symmetrical as asymmetrical, asymmetrical counterparts to mitotic chiasmata are very rare. Evidently, mitotic chiasmata do not result from ordinary translocation but from crossing-over based on some kind of homologous pairing.

THE EVOLUTION OF SEX CHROMOSOMES AND THE FORMATION OF W CHROMATIN IN SNAKES

S. P. RAYCHAUDHURI, L. SINGH and T. SHARMA
Cytogenetics Laboratory, Department of Zoology, Banaras Hindu University, Varanasi, 5, India

The sex chromosomes of snakes have differentiated according to the evolutionary status of the families. At the chromosome level, the primitive family Boidae has homogametic males and females and the highly evolved families Elapidae and Crotalidae have a mixed situation. The W in various species varies considerably in size and in some species it is much larger than the conservative Z.

The heterogametic females with a differentiated W chromosome have a distinct chromocentre in somatic interphase nuclei of different tissues which is comparable to the mammalian sex chromatin. Such a prominent heterochromatic body is absent in similar tissues of homogametic (ZZ) males. There is a positive correlation between the size of the chromocentre (W chromatin) and that of the W chromosome which it represents. The W chromatin does not incorporate ^{3}H-uridine which indicates an inactivity at the transcriptional level similar to that of the mammalian sex chromatin.

The DNA replication pattern of the W differs from that of the Z and in different species the W shows a different timing of DNA replication. In the krait, *Bungarus caeruleus*, a large portion of the giant W completes DNA synthesis very late whereas in the cobra, *Naja n. naja*, the W finishes DNA synthesis much earlier.

ABSTRACTS OF DEMONSTRATIONS

MITOTIC CYCLES IN MAIZE AND MOUSE

P. W. BARLOW

Pediatric Research Unit, Cameron House, Guy's Hospital Medical School, London, S.E.1

Maize: Pulse labelled mitosis data from root tips of *Zea mays* have been computer-analysed using Macdonald's technique (*Biometrika*, in press). A map will be presented that gives phase lengths for two regions of cap initials, quiescent centre as well as for epidermis, cortex and stele in positions throughout the dividing region of the apex.

Mouse: Advantage has been taken of the visibility and near-synchronism of early mouse cleavage divisions *in vitro* to examine the first six to seven divisions. By using autoradiographic and microdensitometric techniques it is possible to estimate changes in the duration of G_1, S and G_2 during this early phase of embryogenesis.

THE EFFECT OF ADDITIONAL CHROMOSOMES ON CELL DIVISION

P. W. BARLOW

Pedriatric Research Unit, Cameron House, Guy's Hospital Medical School, London, S.E.1

Meiosis: The partly heterochromatic supernumerary (B) chromosome of *Puschkinia libanotica* increases the A chromosome chiasma freqency in *p.m.c.*'s. Each additional B adds an extra chiasma in the A complement. In *Listera ovata* additional euchromatic B chromosomes raise the chiasma frequency in the A complement but in a zig-zag fashion that is parallel in both *p.m.c.*'s and *e.m.c.*'s.

Mitosis: The effect of B chromosomes on the mitotic cycle of *Puschkinia* root tips has been investigated using the ^{3}H-TDr pulse labelling technique. Continuous ^{3}H-TDr labelling and colcemid metaphase collection has been used to determine the effect of additional X chromosomes in human cells from individuals who are mosaics with respect to X chromosome aneuploidy (45,XO/46,XX and 45,XO/47,XXX). In both the plant and animal material the additional heterochromatic chromosomes prolong the cell cycle.

RELATIONSHIPS BETWEEN THE DURATION OF MEIOSIS AND SOME OTHER NUCLEAR CHARACTERS

M. D. BENNETT

Plant Breeding Institute, Cambridge

A survey shows that in diploid plant species the duration of meiosis is linearly correlated with the amount of DNA per cell and with mitotic cycle time. Increased meiotic duration is usually composed of proportional increases in all the stages of meiosis. Meiosis is much faster in three polyploid plant species than would be expected for diploid species having equal DNA amounts, while it is much slower in three animal species.

THE DURATION OF MEIOTIC STAGES IN CEREALS

M. D. BENNETT, M. W. BAYLISS and RALPH RILEY
Plant Breeding Institute, Cambridge

The duration of meiosis and its stages in *Triticum aestivum* has been determined by sampling procedures and by labelling the premeiotic S phase with ^3H thymidine. Results from the two methods are in reasonable agreement. A genotype which is asynaptic at low, and synaptic at higher, temperatures has been subjected to temperature changes to determine the temperature-sensitive stage. The procedures used and the results obtained will be demonstrated. The duration of meiosis and its stages was also determined in *Secale cereale* and in *Triticale*.

MITOTIC DISJUNCTION OF A HUMAN RING CHROMOSOME

MARTIN BOBROW
Population Genetics Unit, Headington, Oxford

Studies have been carried out on peripheral blood cultures from a child with a ring chromosome.

Despite the apparent stability of the ring, 103/210 nuclei studied at anaphase and telophase show double chromatin bridges. Some are clearly of the interlocking type while others are dicentric. In the majority, the bridge is too attenuated for this distinction to be made. The bridges usually break although examples of rings being excluded from the daughter nucleus and of chromatin bridges persisting into interphase have been seen.

The latter may be the reason for the high proportion of tetraploid cells seen in these cultures. Such cells show a dicentric double-sized ring or two monocentric rings. Interlocked rings have not been seen in tetraploid cells, which cannot yet be explained.

CORRELATIONS BETWEEN CHROMATID AND SUB-CHROMATID ERRORS DURING MEIOSIS

P. E. BRANDHAM
Royal Botanic Gardens, Kew, Richmond, Surrey, U.K.

Sub-chromatid bridges with double side-arms are found at AI of meiosis in a number of plant genera. In a large sample of species in the tribe Aloineae (Liliaceae) they are always accompanied by U-type chromatid bridges and acentric fragments.

The distribution of the sites of the two types of bridge corresponds with that of chiasmata; it is suggested that sub-chromatid and chromatid bridges are both results of different patterns of reunion of sub-chromatid units following iso-locus breakage of non-sister chromatids during prophase.

WHEAT CHROMOSOMES

VICTOR CHAPMAN and RALPH RILEY
Plant Breeding Institute, Cambridge

This demonstration illustrates the evolutionary history and cytogenetic structure of bread wheat (*Triticum aestivum* $2n = 6x = 42$). It also shows how chromosome markers are used in genetic studies and how alien genetic variation can be transferred to wheat by chromosome manipulation and by interference with the genetic regulation of meiotic chromosome pairing.

ANALYSIS OF MEIOTIC EXCHANGES

T. A. CRAIG-CAMERON and G. H. JONES
Genetics Department, University of Birmingham

Meiotic exchanges can be analysed cytologically in two ways. One involves scoring chiasma frequencies in diplotene bivalents, while the other employs tritium autoradiography to trace exchanges between labelled and unlabelled anaphase I chromatids. Comparisons between these two methods applied to *Schistocera gregaria* spermatocytes have clearly and directly established that diplotene chiasmata correspond to points of crossing over by breakage-reunion of homologous chromatids. Further, it appears that some patterns of label exchange can only be explained by assuming meiotic sister chromatid exchange, albeit at a low frequency.

A NEW MECHANICAL MODEL ACCOUNTING FOR GEOMETRIC SHAPES OF LATE PROPHASE BIVALENTS

LEE T. DOUGLAS
Department of Genetics, University of Nijmegen, Nijmegen, The Netherlands

According to the model, crossing over together with stiff binding between sisters produces a twist in each chromatid before the beginning of diplotene. Stiffening of the bivalent during late meiotic prophase causes each of the chromatids to untwist, thus doing away with torsion imposed by chiasma formation earlier. If sister pairing at late prophase is sufficient to prevent detorsion of the respective chromatids about their long axes, this untwisting could be achieved only in conjunction with a bending of each chromatid. Such " detorsive bending " would be expected to give opening out similar to that observed in many species, especially in *Locusta migratoria*.

In opened-out bivalents with two or more chiasmata, a hypothetical basis for the cytological detection of chromatid interference is shown to be a consequence of the model.

A NEW DEVICE FOR MAKING SQUASH PREPARATIONS OF CHROMOSOMES

GILLIAN M. FEARN
Botany School, University of Oxford, England

A hand press has been devised for making squash preparations of chromosomes. The main advantages of this apparatus over squashing by hand are as follows:

1. Much greater pressure is available. This is particularly advantageous for material with large numbers of chromosomes.

2. Pressure is evenly distributed over the whole of the area under the coverslip.

Photographs and slides which illustrate these points are demonstrated.

TISSUE ORGANISATION OF LOCUSTS AND DERMESTID BEETLES IN RELATION TO GERM-LINE DNA CONTENT

D. P. FOX
Department of Genetics, Marischal College, Aberdeen

Many of the nuclei which populate somatic tissues of insects have DNA contents greater than the 2C amount and are usually described as being endopolyploid. The DNA classes present in a tissue are not always geometric multiples of the 2C value and under-replication of the genome is frequent. Further, the types and proportions

of nuclei in a tissue are characteristic of that tissue. In one case—the *Dermestes* malpighian tubule—it has been possible to give a more detailed description of under-replication by making density maps of Feulgen stained nuclei. Here under-replication seems to be confined to the DNA present in heterochromatin.

CHROMOSOME STRUCTURE ELECTRON-MICROGRAPHS

M. B. E. GODWARD
Department of Plant Biology and Microbiology, Queen Mary College, Mile End Road, London, E.1

Pre-treatment of nuclei leads to " unpacking " of the chromosome fibrils whose relationship to the complex structure is clarified.

CHROMOSOMAL CHANGES IN TUMOURS

INGER-LISE HANSTEEN
Institute for General and Experimental Pathology, University of Oslo, Norway

Changes in chromosome numbers are shown for six 20-Methyl-cholanthrene induced sarcomas in mice. One or two stem-lines were found in each tumour, with euploid numbers in two of them. A different marker chromosome was found in two of the tumours.

The stem-lines of tumours which appeared soon after the application of carcinogen were near-diploid while those of tumours appearing later were $3x$-$4x$.

The growth rate (GR), increase in weight of a tumour, can be expressed as the net result of the rate of cell proliferation (RCP) and any concomitant cell loss (CL) going on in the tumour

$$GR = RCP - CL.$$

The growth rate and the rate of cell proliferation are, therefore, different parameters which do not necessarily give identical information about the behaviour of the tumour. A tumour can have a slow GR but a high RCP. This is true for many human tumours.

The mice were killed when the tumours had reached a diameter of *ca.* 1·5 cm and the RCP was estimated by means of a stathmokinetic method (Colcemid). The cell loss cannot be measured directly.

The RCP in early-appearing tumours was higher than that in late-appearing tumours which indicates a possible relationship between the RCP and the chromosome number.

MULTIPLE SYNAPTINEMAL COMPLEXES IN THE REGION OF GENE AMPLIFICATION IN *ACHETA*

HALINA JAWORSKA and A. LIMA-DE-FARIA
Institute of Molecular Cytogenetics, University of Lund, Sweden

Ovaries of *Acheta domesticus* (house cricket) containing oocytes mainly in (*a*) interphase and early meiotic prophase, and (*b*) pachytene and diplotene, were studied by electron and light microscopy.

Every oocyte contains a DNA body which appears as a " puff " at pachytene and diplotene. In the light microscope, an inner core of DNA and an outer shell of RNA can be distinguished in the body. In the E.M. the core is seen to consist of fibrillar material while the shell includes areas of high electron opacity composed mainly of tightly packed particles and fibrils. Synaptinemal complexes, present as

single units, are found in relation to paired chromosomes throughout the nucleus but they are seldom associated with the DNA body at these stages. During interphase and early prophase, the body is active in DNA synthesis and appears as a Feulgen positive sphere with Feulgen negative areas under light microscopy. Corresponding E.M. observations of the body reveal :

(*a*) the two components found at pachytene,
(*b*) a third electron dense component which is more homogeneous than the others, and
(*c*) large assemblies of synaptinemal complexes arising from several centres in a part of the DNA body only. The complexes are circularly packed and they occur in packages of 300 to 400 units.

The amplification of ribosomal cistrons in the DNA body appears to be correlated with the formation of multiple axial complexes. These may form either between the extra gene copies of the two homologues, or here and between the extra copies of each chromosome.

CHROMOSOMES IN ANTIPODAL CELLS OF *AEGILOPS* SPECIES AND OF *HORDEUM VULGARE*

J. K. JONES and M. O. OMIDIJI
Department of Agricultural Botany, University of Reading

Antipodal cells dissected from ovaries of *Aegilops* species and *Hordeum vulgare* can be identified by their size and shape, and in some cases, by their position. The nucleus is large and stains intensely. In a small number of antipodals there is a distinct number of chromatin bodies, or of aggregations of chromosomes or of giant chromosomes. The chromonemata, chromatids and chromosomes are visible in some cells.

The maximum number of chromatin bodies or giant chromosomes per cell, and the number apparent in most cells, equals the reduced gametic number of chromosomes for each species. Apparently in some, and possibly in most, of the antipodal cells each chromosome is multi-replicated, and the multi-chromosomes become separate in some cells. The considerable replication of genetic material is presumably associated with intense metabolic activity comparable with that in glandular and nurse cells.

There is a typical range of numbers of antipodals in each species. The number is not necessarily higher in either natural or induced polyploids. In each species the number is approximately doubled after pollination, probably after fertilisation. The nuclear division may not be a normal mitosis. The size of the nuclei increases for 2 to 3 days, but degeneration becomes apparent 3 to 4 days after pollination. Cell remnants only are visible 7 days after pollination. Antipodal development after intergeneric pollination is the same as after self-pollination, except that degeneration may be delayed in some ovaries.

NEOCENTRIC ACTIVITY IN RYE MEIOSIS

G. H. JONES
Genetics Department, University of Birmingham

Inbred lines of rye derived from an interspecific cross between *Secale dighoricum* and *S. turkestanicum* show pronounced and localised neocentric activity during anaphase of both meiotic divisions. The structure and behaviour of this neocentric region is described. The localisation of the neocentric region in these lines permits its use as a marker to plot the distribution of normal and abnormal meiotic exchanges. Parallel studies have shown that the neocentromere acts independently of physical continuity with the centromere and also that the neocentromeres have a pronounced dosage dependent effect on exchange frequencies.

THE SYNAPTINEMAL COMPLEX

L. F. LA COUR and B. WELLS
John Innes Institute, Colney Lane, Norwich

Some aspects of chromosome structure and behaviour relevant to the synaptinemal complex which is formed at zygotene/pachytene are illustrated in microphotographs and electron micrographs taken from pollen mother cells of *Vagaria parriflora*, *Fritillaria lanceolata* and an asynaptic mutant of *Triticum durum*. Electron micrographs of leptotene chromosomes and synaptinemal complexes from pollen mother cells of some other plants are also demonstrated.

CHROMOSOMAL ELEMENTS IN ELONGATING SPERMATID NUCLEI OF GRASSHOPPERS

A. G. J. M. VAN DER LINDEN
Department of Genetics, University of Nijmegen, The Netherlands

Elongating spermatid nuclei of *Chorthippus brunneus*, *Ch. parallelus* and *Schistocerca gregaria* are characterised by many DNA-fibrils, roughly parallel to the long axis of the nucleus. Some of these nuclei show a transverse banding pattern which strongly resembles that of polytene chromosomes. The morphological similarities between banded spermatid nuclei and individual polytene chromosomes suggest a parallel structure of identical sub-units in the spermatid nucleus. A similar bonding is induced in fibrillar nuclei after treatment of testes in $ZnSO_4$.

Some other types of nuclear structure are described. All these types of elongating spermatid nuclei were found in natural populations of the three species.

ENDOSPERM DEVELOPMENT IN CEREALS AND CEREAL HYBRIDS

J. P. MOSS
Department of Agricultural Botany, University of Reading

After self-fertilisation, the endosperm develops rapidly by synchronous divisions and no cell walls are formed. The synchrony breaks down at about four days and cell walls are formed six days after pollination.

In hybrids, there are fewer divisions, and these are accompanied by chromosome breakage, bridges and other abnormalities. The older hybrid endosperm thus contains fewer cells, many of which are highly polyploid. The majority of cells cease dividing, form irregular groups and degenerate. The premature death of the endosperm produces shrivelled seed.

CHROMOSOME VARIATION IN *ALLIUM SCHOENOPRASUM*

JOHN PARKER
Department of Plant Biology and Microbiology, Queen Mary College, London

Chromosome variation has been found in two populations of *Allium schoenoprasum* from western Britain. In one, plants with up to 8 B-chromosomes occur. The B's are of two types, telocentric and sub-metacentric. The relationships of the B's and their influence on recombination in the A chromosomes will be illustrated.

The other population included plants which were heterozygous for an interchange and two with a highly irregular meiosis.

LOCALISATION OF CHIASMATA IN MAN

PETER PEARSON
Population Genetics Unit, Headington, Oxford

Data derived from normal males shows that chiasmata are not randomly distributed along the chromosome at diakinesis, but that there is a preponderance of chiasmata at the ends of the chromosomes. Evidence is presented for the non-random behaviour of chiasmata in particular bivalents identified by having a distinctive quinacrine fluorescence.

POLYPLOID GERM CELLS IN THE HUMAN MALE

PETER PEARSON
Population Genetics Unit, Headington, Oxford

In normal males polyploid germ cells are found at every stage of meiosis at a frequency of between 1 per cent. and 5 per cent. Most of these are tetraploid but higher levels of ploidy are sometimes found. Normally at diakinesis only bivalents are present. It has been suggested that such cells are formed by cytoplasmic fusion after pairing has occurred. Rarely, diplotene cells are found with multivalents. These cells are likely to have been derived from polyploid spermatogonia. However, they appear to have degenerate chromatin and to be selected against. Such cell selection would account for their low frequency.

FLUORESCENCE IN MAMMALIAN CHROMOSOMES

PETER PEARSON, MARTIN BOBROW, CANIO VOSA and PETER BARLOW
Population Genetics Unit, Headington, Oxford, Botany School Oxford, and Paediatrics Research Unit, London

Quinacrine fluorescence in man has shown that intense fluorescence is to be found in the distal half of the long arms of the Y chromosome, the satellites of some acrocentrics and the centromere region of chromosome 3.

DESYNAPSIS IN MAN AND MOUSE

PETER PEARSON and DAVE PARNELL
Population Genetics Unit, Headington, Oxford and Genetics Department, Cambridge

A desynaptic man and mouse have been detected by virtue of their infertility. In both meiosis appears to be normal up to and including the pachytene stage. Diakinesis is aberrant with a gross reduction in chiasma frequency.

CHROMOSOMAL INSTABILITY OF *ASPERGILLUS NIDULANS*

J. A. ROPER
Department of Genetics, The University, Sheffield

In *A. nidulans*, chromosome imbalance provokes instability at mitosis and strains with a duplicate chromosome segment produce frequent variants. One class of variants has deletions, of variable size and at least sometimes interstitial, in either duplicate segment. Another class, including some with enhanced instability, may be explained by new tandem duplications which arise on either duplicate segment and subsequently transpose to an non-duplicated region.

Genetic and other factors affect the degree and pattern of instability. A central question is whether the phenomena reflect only disordered mitosis or whether they have relevance to normal processes of higher organisms.

VARIATION IN HETEROCHROMATIN ASSOCIATED WITH INDUCED HERITABLE CHANGE IN *LINUM*

J. N. TIMMIS
Department of Agricultural Botany, University College of Wales, Penglais, Aberystwyth, Cardiganshire

Heritable changes in plant weight have been induced in *Linum* by treatment with specific combinations of fertiliser (Durrant 1962). The induced changes are accompanied by changes in the amount of nuclear *DNA*. The evidence indicates that the *DNA* variation is located in heterochromatic segments.

B CHROMOSOME FREQUENCY AND ECOLOGY IN *LISTERIA OVATA*

C. G. VOSA
Botany School, Oxford University, England

The frequency of B chromosomes in populations of *Listeria ovata* increases with latitude and varies from about 20 per cent. in the southernmost Italian populations to about 90 per cent. in the British Islands. Within Britain there is a positive correlation between high frequency of B chromosomes and marshy habitats.

HETEROCHROMATIN AND DNA-BINDING FLUOROCHROMES

C. G. VOSA
Botany School, University of Oxford, England

Certain types of acridine derivatives may be used as fluorochromes to stain differentially various kinds of heterochromatin in plant and animal chromosomes. Four or more types of heterochromatin are present in plants showing all the possible combinations of cold starvation effect and positive and negative fluorescence.

ACHIASMATIC MEIOSIS IN AN AFRICAN THERICLEINE GRASSHOPPER

M. J. D. WHITE
Department of Genetics, University of Melbourne, Parkville, Victoria 3052, *Australia*

The bivalents of *Thericles whitei* Dirsh (northern Transvaal) are totally achiasmatic in the male. In the female 1-2 chiasmata are formed per bivalent.

ASYMMETRICAL HETEROPYCNOSIS IN TETRAPLOID CELLS OF A MORABINE GRASSHOPPER

M. J. D. WHITE
Department of Genetics, University of Melbourne, Parkville, Victoria 3052, *Australia*

In tetraploid spermatogonia of the neo-XY species " P52a " there are two X chromosomes, each consisting of an XL limb which represents the original X chromosome and a shorter XR limb which was originally autosomal. The two XL limbs behave differently. One of them shows negative heteropycnosis (the normal condition in diploid cells with a single X), the other is condensed to the same extent as the autosomes. This apparently inherent cellular mechanism may be compared with the heteropycnotic inactivation of one of the two X's in somatic cells of female mammals.

LABELLING PATTERN OF THE PARTHENOGENETIC GRASSHOPPER *MORABA VIRGO*

M. J. D. WHITE and G. C. WEBB
Department of Genetics, University of Melbourne, Parkville, Victoria 3052, *Australia*

Moraba virgo is a parthenogenetic species in which males have never been found. $2n♀ = 15$. The chromosome number is doubled prior to meiosis in the oocyte. Synapsis is then restricted to sister chromosomes, so that although 15 chiasmate bivalents are formed there can be no genetic recombination. In somatic cells the two large chromosome pairs are both significantly heterozygous for labelling pattern, following injection of the insects with tritiated thymidine.

SEX CHROMOSOMES OF AN AUSTRALIAN MANTID *RHODOMANTIS PULCHELLA*

M. J. D. WHITE
Department of Genetics, University of Melbourne, Parkville, Victoria 3052, *Australia*

Two species of the genus *Rhodomantis* (sub-family Mantinae) have an X_1X_2X (♂): $X_1X_1X_2X_2$ (♀) sex chromosome mechanism. They differ from all other Mantinae known to possess a sex chromosome mechanism of this type in that X_1 and X_2 are synapsed with the *same* limb of the Y at first metaphase in the male, leaving the other arm of the Y free. Presumably two chiasmata are formed in a triple pairing segment.

HETEROMORPHISM IN THE LONGEST AUTOSOME OF *RATTUS RATTUS DIARDII*

HOI SEN YONG
Division of Genetics, School of Biological Sciences, University of Malaya, Kuala Lumpur

Three karyotypic classes of the Malayan house rat, *Rattus rattus diardii* (Jentinck) are demonstrated. These are acrocentric homomorphic (AA), subterminal homomorphic (SS) and acrocentric-subterminal (AS) heteromorphic. Pericentric inversion is the mechanism involved.

ROBERTSONIAN POLYMORPHISM IN THE MALAYAN HOUSE SHREW, *SUNCUS MURINUS*

HOI SEN YONG
Division of Genetics, School of Biological Sciences, University of Malaya, Kuala Lumpur

This shrew shows Robertsonian polymorphism. The three karyotypic classes have diploid numbers of 38, 39 and 40. They differ in the number of metacentric and acrocentric autosomes but have the same fundamental number (N.F. = 56).

ROBERTSONIAN TRANSLOCATION IN *RATTUS RATTUS DIARDII*

HOI SEN YONG
Division of Genetics, School of Biological Sciences, University of Malaya, Kuala Lumpur

Reduction of the chromosome number by one (i.e. $2n = 41$) and formation of a new bi-armed chromosome in the Malayan house rat, *Rattus rattus diardii* (Jentinck) is depicted. This involves Robertsonian translocation between heterologous chromosomes.

AUTHOR AND REFERENCE INDEX

Authors of papers contributed to this volume are indicated by an asterisk. Page numbers in italics refer to the extent of the author's contribution. The other entries refer to the reference lists given at the ends of papers.

Abdel-Hameed, F., 221
Abonyi, D., 146
Abraham, A., 251
Aellen, P., 266
*Ahloowalia, B. S., *93-103*, *289*, 101, 102
Aiyangar, H. R., 252
Alexander, M., 22
Allfrey, V. G., 23
Altmann, H. W., 22
Amaldi, F., 51
André, M., 196
Ansley, H. R., 102
Arora, C. M., 246
Atallah, S. I., 236, 237
Avanzi, S., 23, 117
Avery, A. G., 266
Ayonoadu, U. A. U., 31, 221

Backhouse, W. O., 131
Badr, F. M., 226
Badr, R. S., 226
Bain, J. A., 123
*Bajer, A., *63-69*, *288*, 68, 85
Baker, T. G., 150
Barber, H. N., 91, 176
*Barigozzi, C., *8-11*, *285*, 11, 146
*Barlow, P. W., *299*, *305*
Barnicot, N. A., 145
Bassel, A., 150
Bauer, H., 206
Bauer, W., 45
*Bayliss, M. W., *300*
Beadle, G. W., 102
Beaudry, J. R., 131
Beck, S., 176
Beerman, W., 22
Bell, G. D. H., 55
Bender, M. A., 175
Benjamin, W. B., 62
Bennett, H. D., 251
*Bennett, M. D., *299*, *300*, 3
Bentham, G., 245
Berenedes, H. D., 62
Berger, R., 146
Berry, C. A., 150
Berry, E. W., 137
Bessey, C. E., 245
Bezbaruah, H. P., 246
Bianchi, N. O., 15, 226
Bier, K., 22
*Bigger, T. R. L., *162-176*, *292*, 92, 161
Birnstiel, M. L., 51, 52
Biswas, I., 246
Blackith, R. E., 196
Blackith, R. M., 196
*Bloom, W., *268-284*, *295*, 69, 284
*Bobrow, M., *300*, *305*
Böcher, T. W., 246
Bodmer, W. F., 3
Bonner, J., 22, 46, 62
Börner, R., 22
Bösemark, N. O., 221
Boublick, M., 46
*Bouharmont, J., *253-258*, *294-295*, 258
Bowden, W. M., 246
Boyes, J. W., 131
Bozzini, A., 102
Bracq, C., 137
Bradbury, E. M., 46
*Brandham, P. E., *300*
Brecher, G., 123
Brent, T. P., 176
Breuer, M. E., 51
Brink, R. A., 131
Britten, R. J., 37, 51, 62, 221
Brock, R. D., 131
Brøgger, A., 145
Brown, D. D., 51, 52
Brown, F. M., 221
Brown, S. W., 11, 37
Bruni, L., 146
Bühler, E. M., 145
Bühler, U. K., 145
Buongiorno-Nardelli, M., 51
Burdick, C. J., 23
Burnham, C. R., 2266
Burt, B., 16
Busch, H., 46

Cairn, S. A., 246
Cairns, J., 31
Callan, H. G., 196
Callender, S. T., 137
Calvin, M., 6
Cameron, C. B., 137
Cameron, D. R., 132
Capanna, E., 226
Carlson, W. R., 221
Carson, H. L., 196
Caspersson, T., 3
Cattanach, B. W., 145
Chao, C. Y., 102
*Chapman, V., *300*, 52, 92, 132
Chasen, F. N., 227
Chatterjee, P., 251
*Chaudhuri, J. P., *147-151*, *291*, 150
Chen, T. R., 52
*Cheney, J., *177-196*, *292*, 196
Chipcase, M., 221
Chopde, V. P., 246
Citoler, P., 22
Civitelli, M. V., 226
Cleaver, J. E., 175
Clein, G. P., 137
Cleland, R. E., 266
Cleland, S., 85
Clever, U., 62
Clifford, H. T., 266
*Clowes, F. A. L., *110-117*, *290*, 117
Cohen, M. M., 296
Comings, D. E., 22, 102
Commoner, B., 123
Comorosan, S., 123
Conen, P. E., 145
Cooper, D. C., 131
Cooper, J. P., 102
Cooper, K. W., 11
Corlette, S. L., 52
Covas, G., 246, 247
*Craig-Cameron, T. A., *301*
Crane-Robinson, C., 46
Crathorn, A. R., 176
Crouse, H. V., 51
Crowson, R. A., 206

*Da Cunha, A. B., *296-297*, 3
Dahmus, M. E., 62
D'Amato, F., 22, 23, 117
Dancholt, B., 62
*Darlington, C. D., *1-3*, 3, 206, 251
*Darvey, N. L., *86-92*, *289*, 91
Datta, N., 246
Davidson, E. H., 62, 151, 221
Davidson, W. M., 145
Davies, H. G., 46
Dawid, I. B., 51
Day, E. J., 145
De Bianchi, M. S. A., 15
Deitch, A. D., 62
DeLange, D. J., 46
De Mesa, R. S., 196
Deprey, C., 123
Deshpande, B. D., 246
De Vidal Tioja, L. A., 226
Dhaliwal, S. S., 227
Dhillon, T. A., 266
Dickinson, F. G., 102
Diers, L., 246
*Dietz, R., *70-85*, *288-289*, 85
Dingman, R. E., 237
Dolfini, S., 11
Donnelly, A. J., 176
Doty, P., 46, 51
*Douglas, L. T., *301*
*Driscoll, C. J., *86-92*, *289*, *295-296*, 91
Dupraw, E. J., 46, 150
Durdick, C. J., 151
Dutrillaux, B., 146
Dyer, A. F., 131

Echalier, G., 11
Eckhardt, R. A., 52
Edström, J. E., 62
Egyházi, E., 62
Eigner, J., 51
Ellerman, J. R., 237
Ellgaard, E. G., 62
Engler, A., 246
Ennis, T. J., 206
Enns, H., 102
Enzenberg, U., 131
Ephrussi, B., 3
Erbrich, P., 22
Erkman, B., 145
Evans, D., 51
*Evans, G. M., *24-31*, *286*
Evans, H., 31, 92
Evans, H. J., 160, 161, 175, 176

Fambrough, D. M., 46
*Fearn, G. M., *301*
Feldman, M., 92
Ferrante, E., 146
Fialkow, P. J., 137
Fischer, C. L., 150
Fischer, P., 146

Fitzgerald, P. H., 137
Flamm, W. G., 51, 222
Flemans, R. J., 137
Fliedner, T. M., 150
Flinn, R. R., 221
Forer, A., 69
Fox, A. S., 11
*Fox, D. P., *32-37*, *286*, *301-302*, 37, 161
*Fraccaro, M., *138-146*, *291*, 11, 15, 146
Franzke, C. J., 102
Frenster, J. H., 62
Frost, H. B., 102
Fukaya, T., 227
Furth, J. J., 22

*Gall, J. G., *47-52*, *287*, 52
Galton, M., 16
Garber, E. D., 266
Gardner, L. I., 123
Gartler, S. M., 16, 137
Geitler, L., 22
Georgiev, G. P., 22
Gerbi, S. A., 52
Gerstel, D. U., 266
Ghosal, S. K., 16
Giblett, E. R., 137
Gibson, I., 3, 221
Gifford, E. M., Jnr., 22
Gill, K. S., 266
*Godward, M. B. E., *297*, *302*, 251
*Goitein, R., *228-237*, 237
Goldschmidt, E., 206
Goldschmidt, R., 6
Golob, E., 146
Gooch, P. C., 150
Goodman, R. M., 62
Gorovsky, M. A., 62
Gottschalk, W., 102
Grant, V., 266
Grant, W. F., 266
Grimaldi, S., 146
Gropp, A., 22
Gunz, F. W., 137
Gustavsson, I., 15

Hägele, K., 22
Hahn, G. M., 176
Haim, A., 237
*Halfer, C., *8-11*, *285*, 11, 146
Halfor, C., 3
Hall, B., 137
Hall, B. D., 52
Hallier, H., 246
Hancock, R., 150
*Hansteen, I-L., *302*
Harris, H., 3, 37
Harrison, D. L., 236
Harvey, M. J., 246
Hasitschka, 23
*Hauschteck-Jungen, E., *297*
Hayashi, H., 46
Hazarika, M. H., 37
Heimberger, M., 37, 252
Heitz, E., 22
Heller, R., 258
Helwig, E. R., 196
Hennig, W., 221
Hepler, P. K., 85
Heslop-Harrison, J., 102
*Hewitt, G., *208-222*, *293*, 3
Hewitt, G. M., 196, 221
Heyes, J. K., 22
Hinton, H. E., 37
Holden, J. H. W., 266
Holt, S. F., 16
*Holt, Th. K. H., *56-62*, *288*, 62
Hooker, J. D., 245, 246
Horikawa, M., 11
Hotta, Y., 102, 103, 150
Hsu, T. C., 16, 22
Hu, W. L., 102
Huang, C. C., 62
Huang, R. C., 22
Huberman, J. A., 31
*Hughes, D. T., *297*
Hughes-Schrader, S., 37
Huguley, C. M., 123
Hulten, M., 15
Hungerford, D. A., 176
Hurwitz, J., 22
Husted, L., 247
Hutchinson, J., 246
Huxley, H. E., 145
Huynh, K. L., 246

Imai, H., 227
Inoué, S., 69, 85
Isaacson, J. H., 145
Ishikawa, K., 46
Ito, M., 102
Iwai, K., 46
Izutsu, K., 69

Jacobs, P. A., 196
Jagger, J., 69
*Jain, H. K., *53-55*, *287*, 23, 55
Jans, D. E., 37
*Jash, M., *238-247*, *294*

Jauhar, P. P., 102
*Jaworska, H., *302-303*, 246
Jermany, D. A., 31
John, B., 37, 196, 206, 221, 266, 267
John, H. A., 52
Johns, E. W., 46
*Jones, G. H., *301*, *303*
*Jones, J. K., *303*, 131
Jones, K., 251
Jones, K. W., 52
Jones, R. N., 3, 37, 221, 251
Jordan, J., 46

Kabat, D., 150
Kang, Y. S., 227
Kay, H. E. M., 137
Kaye, J. S., 46
Kempana, C., 92
Key, K. H. L., 196
Keyl, H.-G., 51
*Khoshoo, T. N., *259-267*, *295*, 266, 267
Khush, G. S., 267
Kihara, H., 132
Kihlman, B. A., 176
Kimball, R. F., 176
Kit, S., 52
Klebe, R. J., 52
Klingstedt, H., 196
Knörr-Gärtner, H., 150
Knudson, L., 258
Kodani, M., 6
Kohne, D. E., 37, 51, 220
Krause, M. O., 37
Krooth, R. S., 176
Kumar, S., 161
Kunze-Mühl E., 146

*La Cour, L. F., *304*, 251
Lafourcade, J., 146
Lambert, B., 62
Lampert, F., 151
Lane, D., 52
Lange, W., 132
Larson, K., 246
Larter, E. N., 102
Law, C. N., 102
*Lawler, S. D., *133-137*, *290-291*, 146, 137
Lawrence, J. F., 206
Lay, D. M., 237
Lea, D. E., 176
Leao, J. C., 123
Lebowitz, J., 46
Lee, S. S, Y., 103
Lein, A., 132
Lejeune, J., 146
Levan, A., 137
*Lewis, H., *268-284*, *295*, 267, 284
Lewis, J. H., 237
Lewis, K. R., 196, 206, 266, 267
Lewis, M. E., 284
Lewis, R. E., 237
Lewis, W. H., 246
Li, C. H., 102
Li, H. W., 102, 103
*Lima-de-Faria, A., *302-303*, 23
Lindhorst, T. E., 123
*Lindsten, J., *138-146*, *291*, 15
Linskens, H. F., 102
Lisker, R., 137
Litardière, R. D., 252
Littau, V. C., 23, 151
Lloyd, P. H., 151
Lopane, F., 246
Löve, A., 246
Löve, D., 246
Lovis, J. D., 258
Low, B., 137
Lubs, H. A., 146
Luchsinger, U., 145
Luykx, P., 69

McCallum, M., 51
McCarthy, B. J., 221
McClintock, B., 132
MacDonald, P. A. C., 145
MacGregor, H. C., 52
McIntosh, A. J., 176
McIntosh, J. R., 85
MacKenzie, A., 102
MacKinney, A. A., 123
McLaren, A., 222
McLeish, J., 31, 123
McMaster-Kaye, R., 46
Maio, J. J., 52
Malamy, M., 22
Man, N. T., 221
Mangenot, G., 246
Mangenot, S., 246
Mann-Lesley, M., 102
Mannoni, P. P., 137
Manton, I., 258
Marmur, J., 51, 52
Marshall, R., 145
Martin, P. G., 46
Martini, G., 102
Mateyko, G. M., 62
Mathew, P. M., 251
Matthey, R., 237

Medway, Lord, 227
Mehes, K., 145
Mello-Sampayo, T., 92
Mercer, F. L., 123
Mesa, A., 196
Mesa, R., 196
Michaelis, A., 161
Miege, J., 246
Millard, R. E., 137
Miller, O. L., 102
Milošević, M., 237
Mirskey, A. E., 23, 151
Misra, R. N., 267
Mitra, J. N., 246
Mitra, K., 246
Mitui, K., 258
Moens, P., 102
Molè-Bajer, J., 68, 69, 85
Monesi, V., 16
Mookerjea, A., 247
Moore, R. J., 246
Moorhead, P. S., 22
Morel, G., 258
Morgan, J. F., 123
Moriguchi, Y., 227
Moriwaki, K., 227
Morris, R., 55
Morrison-Scott, T. C. S., 237
Morton, H. J., 123
Mosquin, T., 267, 284
Moss, G. I., 102
*Moss, J. P., *124-132*, *290*, *304*, 132
Moyer, L. S., 246
Mukhergee, B. B., 16
Mukhergee, I., 266
Mulay, B. N., 246
Müller, H. A., 23
Muntzing, A., 252
Murray, K., 46
Murray, M. J., 267

*Nagl, W., *17-23*,*-286*, 3, 23, 132
Nakamura, A., 227
Nasjleti, C. E., 23
Natarajan, A. T., 161
Nelson-Rees, W. A., 37
Nerwal, S. K., 55
Neu, R. L., 123
Nevo, E., 237
*Newton-Barber, H.. *86-92*, *289*, *295-296*
Nezer, R., 226
Nichols, W. W., 137
Nicklas, R. B., 85, 69
Nilsson, S.-B., 137
Nirula, R. L., 246
Nishiyama, I., 132
Nitsch, C., 3
Nitsch, J. P., 3
Noda, S., 3
Norden, A., 137
Norman, A., 161, 176
Nowell, P. C., 176
Nur, U., 221
Nuti Ronchi, V., 23

Odenbach, W., 132
Ogawa, Y., 46
Ohanessian, A., 11
Ohnuki, Y., 151
Okada, T. A., 102
Okamoto, M., 92
*Omidiji, M. O., 131, *303*
O'Riordan, M. L., 137
*Ortiz, E., *298*, 206
Östergren, G., 85
Özarslan, S., 69

Painter, R. B., 31, 176
Painter, T. S., 7
*Pal, M., *259-267*, *295*, 267
Pao, W. K., 102
Papworth, D. G., 161
Parchman, L. G., 102
Pardi, P. N., 246
*Pardon, J. F., *38-46*, *286-287*, 46
*Pardue, M. L., 47-52, 287, 52
*Parker, J., *304*
Parker, R. C., 123
Parker, V., 176
*Parnell, D., *305*
*Patau, K., *298*
Patton, J. L., 237
Paul, J. S., 62
Paulette-Vanrell, J., 226
Paunovic, D., 222
Pavan, C., 3, 51
Peacock, A. R., 151
*Pearson, P., *305*, 3
Pelling, C., 62
Pera, F., 23
Phillips, D. M. P., 46
Pierce, W. P., 252
Pisciotta, A. V., 123
Prakken, R., 102
Prescott, D. M., 175
*Price, D. J., *118-123*, *290*, 123
Pritchard, A. J., 102
Pritchard, W. A., 161
Puska, K., 7

Quagliarotti, G., 46
Quastler, H., 31

Race, R. R., 137
Raghavan, R. S., 246
Raina, S. N., 267
Rajhathy, T., 103
Ramshorn, K., 22
Rao, C. R., 176
*Rao, S. G. A., *12-16*, *285*
*Rao, S. R. V., *12-16*, *285*, 16
Rasch, E. M., 117
Rasch, R. W., 117
Rasmussen, R. E., 31, 176
Rastrick, J. M., 137
*Rattazzi, M. C., *296*
Rattenbury, J. A., 92
*Raut, R. N., *53-55*, *287*, 55
Raven, P. H., 267
*Raychaudhuri, S. P., *298*
Reeder, R. H., 52
*Rees, H., *24-31*, *286*, 3, 31, 37, 221, 251, 267
Reeves, B. R., 146
Reiger, R., 161
Research Bulletins I & II 1968, 1970, 252
Rethoré, M-O., 146
Rezzonico, R. G., 11
*Ricci, N., *138-146*, *291*
*Richards, B. M., *38-46*, *286-287*, 46
Rietsema, J., 266
Riggs, A. D., 31
Riley, H. P., 246
*Riley, R., *300*, 55, 92, 102, 132, 267
Ringborg, U., 62
Ris, H., 46
Ritossa, E. M., 52
Rivers, S. L., 123
Roberts, J. J., 176
Robertson, F. W., 221
Robinson, M. G., 151
Romball, C. G., 62
Rootham, D. L., 221
*Roper, J. A., *305*
Ross, J. G., 102
Ross, K. F. A., 37, 62
Roth, T. F., 102
Rothfels, K., 37, 252
Ruddle, F. W., 52
Rudkin, G. T., 52, 62
Ruscoe, C. N. E., 222
Rutishauser, A., 132

Salmon, C. H., 137
Samuelsom, J., 146
Sanders, M. E., 102
Sandler, L., 4
Sanger, R., 137
Sarkar, S. K., 247
Sasaki, M. S., 161, 176
Satina, S., 266
Sato, H., 69, 85
Sauer, J. D., 267
Sauter, J. J., 23
*Savage, J. R. K., *152-161*, *291-292*, 161
Savić, I., 237
Sax, K., 161, 247
Schaefer, A. W., 31
Schildkraut, C. L., 51, 52
Schinz, H., 267
Schmid, W., 16
Schnack, B., 246, 247
Schrader, F., 37
Schwarzacher, H. G., 23
Scoggins, R. B., 123
*Scott, D., *162-176*, *292*, 31, 175, 176
Sears, E. R., 55, 92
Secker-Walker, L., 137
Sens, S., 252
Seshadri, C., 16
Setzer, H. W., 237
Sexsmith, E., 37
*Shah, V. C., *12-16*, *285*, 16
*Sharma, Archana, *238-247*, *294*
*Sharma, A. K., *248-252*, *294*, 247, 252
*Sharma, T., *298*
Sharma, V. B., 132
*Sharma, V. K., *12-16*, *285*, 16
Sharman, G. B., 176
Shastry, S. V. S., 267
Shaw, D. D., 37, 206
Shaw, M. W., 146, 176
Shebaski, L. H., 55
Sheridan, W. F., 102
Sherman, F. G., 31
Shimakura, K., 227
Shkolnik, A., 237
Sieger, M., 23
Sinclair, J. H. 52
*Singh, L., *298*
Singh, U., 23, 102
Sinkus, A. H., 146
Small, J. V., 46
Smith, E. L., 46
Smith, R. F., 206
*Smith, S. G., *197-207*, *293*, 206
*Snell, C. L., *24-31*, *286*
Sokolovskya, A. P., 247
Soldatović, B., 237
Soost, R. K., 103

Sorsa, M., 7
*Sorsa, V., *5-7*, *285*, 4, 7
Southern, E. M., 222
Spencer, H. H., 23
Spiegelman, S., 52
Staehly, C. A., 69, 85
Stalder, G. R., 145
Stanbuck, W. C., 46
Stebbins, G. L., 267
Stebbins, G. L. (Jnr.), 252
Steeves, T. A., 258
Steffenson, D. M., 151
Stephens, R. E., 85
Stern, H., 102, 103
Stevens, B. J., 62
Stohlman, F., 123
Street, H. E., 23
Stubblefield, E., 16
Stützer, H. R., 151
Subirana, J. A., 85
Sun, S., *24-31*, *286*
Sunderland, N., 4, 31
Swift, H., 52, 62
*Sybenga, J., *104-109*, *289*, 267

Takenouchi, Y., 206
Tanaka, R., 4
Taylor, C. W., 46
Taylor, J. H., 31
Tchernov, E., 237
Tepper, H. B., 22
*Therman, E., *298*
Thomas, H., 103
Thomas, P. T., 102
Thompson, W. P., 131, 132
*Tiepolo, L., *138-146*, *291*, 11, 15, 146
*Timmis, J. N., *306*
*Timson, J., *118-123*, *290*, 123
Tobgy, H. A., 252
Tolani, U., 246
Torrey, J. G., 23
Tough, I. M., 137
Tschermak-Woess, E., 23
Tsuchiya, K., 227

Ullerich, F-H., 37
Utakoji, T., 16

Van Breugal, F. M. A., 62
*Van der Linden, A. G. J. M., *304*
Van Heemert, C., 132
Van't Hof, J., 31, 246
*Vickery, R. S., *295-296*
Villalobos-Pietrini, R., 102
Vinograd, J., 45, 46
Virkki, N., 206
Virrankosky, V., 7
Von Lehmann, E., 237
*Vosa, C. G., *305*, *306*, 4
Voss, G., 69

*Wahrman, J. *228-237*, *294*, 237
Walker, P. M. B., 51, 221, 222
Walker, S., 258
Wallace, H., 52
Walp, R. L., 258
Wang, S., 103
Wardlaw, C. W., 23
Watkins, A. E., 132
Watson, M. L. 146
Watson, R., 46
*Webb, G. C., *307*, 196
Weiss, J. J., 151
*Wells, B., *304*
Wheeler, C. M., 151
*White, M. J. D., *177-196*, *292*, *306*, *307*, 196, 206, 222, 267
Whittier, D. P., 258
Wicks, F. M., 4
Wie, D. G. Van, 85
Wimber, D. E., 31
Wolf, U., 23
Wolfe, S. G., 46
Wolfe, S. L., 46
Woodard, J., 62, 117

Yang, S-J., 176
Yasmineh, W. G., 11, 52, 222
Yeh, P., 103
Yermanos, D. M., 266
*Yong, H. S., *223-227*, *293*, *307*, 227
Yoshida, A., 137
Yoshida, M. C., 4
Yosida, T. H., 227
Yunis, J. J., 11, 52, 222
Yvart, J., 137

Zadoo, S., 267
Zahavi, A., 237
Zavala, C., 137
Zecevic, L. J., 222
Zeven, A. C., 132
Zircle, R. E., 69
Živhović, S., 237
Zubay, G., 46

SPECIES INDEX

Acalymma, 197, 201, 203, 205, 293
  blandulum, 198, 201
  fairmairei, 198, 201
  goudi, 198, 201
  innubum, 198, 201
  pallipe, 198, 201
  quadrivittatum, 198, 201
  thiemei, 198, 201
  trivittatum, 198, 201, 202, 205, 206, 293
  vitttatum, 198, 201
Acheta domesticus, 302
Acomys, 294
  cahirinus, 228-232, 234-237
  dimidiatus, 234
  minous, 237
Adiantum pedatum, 258
Aegilops, 303
  speltoides, 53, 54
  squarrosa, 53, 54
Agropyron, 129
  repens, 131
Allium, 20, 30, 250, 251
  carinatum, 17, 23
  cepa, 25, 27, 30
  fistulosum, 27, 30
  flavum, 17, 23, 286
  schoenoprasum, 304
  stracheyii, 250-252
  tuberosum, 28
Amaranthus, 259, 263, 265, 266, 295
  bouchoni, 261
  caudatus, 259, 263
  caudatus var. atropurpureus, 260, 263
  dubius, 261, 267
  edulis, 259-263, 267
  edulis-caudatus, 261
  gracilis, 259, 260, 262
  graecizans var. graecizans, 262
  hybridus, 259, 260, 263
  hybridus-hypochondriacus, 261
  hypochondriacus, 259-263
  lividus lividus, 260, 262
  lividus-tricolor, 261, 263, 265
  spinosus, 261
  spinosus-dubius, 262, 264
  tricolor var salicifolius, 261
Amphelasma, 199, 205
  cavum, 198, 199
  cavum cavum, 198, 199
  cavum vicinum, 198, 199
Amphibia, 37
Anemone, 37
Anuren, 37
Apodemus, 217
Auragia, 244
Asclepias, 239, 243, 246
  curassavica, 239, 240, 246
Aspergillus nidulans, 305
Asplenium, 254, 257, 258
  ruta-muraria, 257
  septentrionale, 254, 257
  trichomanes, 253, 257, 258, 294
  trichomanes trichomanes, 253-255
  trichomanes quadrivalens, 253, 254, 257
Avena, 93, 129, 132, 266

Blitopsis, 259, 264-266, 295
Bradysia elegans, 296
  mogiensis, 296
Bungarus caeruleus, 298

Calotropis, 239, 243
  gigantia, 239, 240, 245
Camnula pellucida, 209
Campelia, 154, 156, 157, 160
  zanonia, 152, 153, 158-161, 292
Caralluma, 243
  fimbriata, 240, 242
Cassinia sp., 179
Centaurea scabiosa, 211
Ceropegia, 243
  woodii, 240, 242
  woodii debilis, 244
Cerotoma, 199, 205
  atrofasciata, 198, 199
  ruficornis, 198, 199
Chenopodium album, 22
Chironomous, 62
  tetans, 62
Chlorophytum, 249
Chorthippus, 192
  bicolor, 196
  bigattulus, 196
  brunneus, 304
  parallelus, 304
Circotettix verruculatus, 196

Clarkia, 266, 267, 284
nitens, 268
speciosa, 268, 280, 283-285
speciosa nitens, 269, 271
speciosa polyantha, 268-274, 277
Collinsia, 266
Commelina, 249
Crepis fuliginosa, 252
neglecta, 252
Cryptolepis, 242-245, 294
buchanani, 240, 242
Cryptostegia, 242-245, 294
grandiflora, 240, 242
Cultrata, 196
Cynanchum, 243
vincetoxicum, 244

Daemia, 243, 244
extensa, 240, 242
Datura, 266
Dermestes, 32, 33, 35-37, 286, 302
ater, 33, 35, 36
frischii, 33, 35
haemorrhoidalis, 32, 33, 35
lardarius, 33, 35
maculatus, 33-36
peruvianus, 32, 33, 35
Diabrotica, 197, 199, 203, 205, 293
adelpha, 198
balteata, 198
bioculata, 198
capitata, 198
circulata, 198
cortezi, 198
cristats, 198
dissimilis, 198
flaviventris, 198
graminea, 198
lemniscata, 198
limitata 15-punctata, 198
longicornis longicornis, 198
longicornis barberi, 198
longicornis nigricornis, 198
nummularis, 198
ochreata, 198
octoplagiata, 198
porracea, 198
scutellata, 198
sexmaculata, 198
tibialis, 198
trifasciata, 198
undecimpunctata 11-punctata, 198
undecimpunctata howardi, 198
undecimpunctata tenella, 198
undecimpunctata 12-notata, 198
"varibilis", 198
virgifera virgifera, 198
virgifera zeae, 198
viridula, 198
viridula rufibasis, 198
Dischidia, 243,
Dregea, 243
volubilis, 240, 242
Drosophila, 49, 51, 56, 58, 61, 62, 287
hydei, 49, 56, 61, 62, 288
melanogaster, 3, 4, 6-8, 10, 11, 52, 62, 144, 146, 285
Dryopteris, 253
dilatata, 257, 258
filix-mas, 257, 258

Elymus, 129
virginicus, 131
Epilobium, 266, 267
Escherichia coli, 48
Eumastacidae, 195
Eyprepocnemis, 192, 203
plorans, 196, 206

Festuca pratensis, 211
Frerea, 243
indica, 244
Fritillaria, 91, 249
amabilis, 3
lanceolata, 304
meleagris, 3, 87
Funambulus pennanti, 16

Gerbillus, 235
dasyurus, 228
Gilia, 266
Gomphocarpus, 243
Gossypium, 266
Gynandrobrotica, 200, 205
lepida, 198, 200, 201
nigrofasciata, 198, 200, 201, 206, 207
variabilis, 198, 200, 201

Haemanthus, 67
katherinae, 63, 68, 288
Helichrysum, sp., 179
Hemidesmus, 242-245, 294
indicus, 240, 242
Hoodia, 243
Hordeum, 93, 129, 160
bulbosum, 132
vulgare, 131, 132, 303

Hoya, 243, 245
parasitica, 240, 242, 245
sikkimensis, 240, 242, 245
Huernia, 243
macrocarpa, 240, 242, 244
Hyacinthus, 125
Hybosciara fragilis, 296

Isoetes, 249
coromandeliana, 248, 251

Keyacris scurra, 187, 194

Lasius fuliginosus, 297
Lathyrus, 37, 249
Lens, 249
Leptadenia, 243
Lilium, 130, 131
Linum, 266, 306
Listera ovata, 299, 306
Locusta migratoria, 209, 301
Lolium, 29, 102
perenne, 29, 30, 101, 289
Loxa flavicolis, 101, 102
Lycopersicon, 93
esculentum, 103

Mangenotia, 243
Mantis religiosa, 196
Marsilea strigosa, 117
Matthiola, 102
Melanoplus differentialis, 209, 221
Metaplexia, 243, 244
Microtus agrestis, 23
Moraba scurra, 196
viatica, 196
virgo, 194, 196, 307
Myrmeleotettix, 208, 209, 211, 218
maculatus, 208, 210, 218, 219, 221, 293

Naja n. naja, 298
Nephrotoma saturalis, 69
Nigella, 249
damascena, 24

Oenothera, 265
Ophioglossum, 248
Ophiopogon, 249
Orthoptera, 195
Oryza, 93

Pachycarpus, 243
Pales ferruginea, 70, 75, 77, 79, 84, 85, 288
Paramecium, 174
Paranapiacaba, 199, 205
connexa, 198, 199
tricincta, 198, 200
Paratriarius, 200, 205
curtisii, 198, 200
dorsata, 198, 200
Paris, 130, 131, 249
Pennisetum, 93
orientale, 102
Pentatropis, 243
Pergularia, 243
Periploca, 243, 244
Phaseolus, 3, 20, 23, 132
coccineus, 17, 23, 286
vulgaris, 17, 23
Phelum phleoides, 211, 221
Philibertia, 244
Phlomis, 125
Pinus silvestris, 23
Pissodes terminalis, 206
Pisum, 93, 102, 116
sativum, 102
Plecia sp., 296
Potorus tradactylus, 163
tridactylis apicalis, 176
Pteridium, 258
aquilinum, 253
Pteridophyta, 258
Puccinia graminis, 296
Puschkinia libanotica, 299
Pynorachis, 243
Pycnostelma, 243

Rattus rattus, 223, 225-227, 293
rattus diardii, 223-227, 293, 307
Rhodomantis pulchella, 307
Rhynchosciara, 3, 49, 50
angelae, 57, 296, 297
bachanti, 296
milleri, 296

Sarcostemma, 243
Schistocera gregaria, 23, 301, 304
Sciara, 49, 50
coprophila, 51, 52
ocellaris, 296

Scotussa daguerrei, 196
Scutigera forceps, 101, 102
Secale, 29, 124-126, 128, 129, 131, 266, 267
  ancestrale, 126, 128, 129
  cereale, 29, 30, 104, 127, 128, 129, 211, 289, 290, 300
  dighoricum, 303
  segetale, 124, 129, 130, 132
  turkestanicum, 303
Secamone, 243
Sinapis, 116
Sorghum, 93, 102
Spalax, 235
  leucodon, 237
Spiranthes, sinensis, 4
Spironema, 249
Stapelia, 243
  grandiflora, 240, 242
  reflexa, 240, 242, 244
  variegata, 244
Stephanotis, 243
  floribunda, 240, 242
Suncus murinus, 12, 15, 16, 226, 285, 307
Synbrotica, 200, 203, 205
  multipuncta, 198, 200
  tetraspilota, 198, 200

Tatera indica cuverii, 16
Thericles whitei, 306
Thomomys, 235
  umbrinus, 237
Thyanta, 37
Tradescantia, 160, 161
  paludosa, 31
Tricchosia pubescens, 296, 297
Trillium, 130, 131, 249, 251
  grandiflorum, 132
  tschonoskii, 251
Trimerotropis, 192
  suffusa, 196
Triticale, 296, 300
Triticum, 53, 93, 124-126, 130, 131, 290
  aestivum, 53, 54, 86, 92, 124, 126, 127, 130, 132, 289, 300
  durum, 53, 54, 304
  monococcum, 53, 54
  timopheevi, 128, 129
Tylophora, 243
  asthmatica, 240, 242, 244

Urginea, 250
  indica, 250, 251

Vagaria parriflora, 304
Viatica, 177, 192-196
Vicia, 46, 163, 168, 169, 175, 249
  faba, 3, 22, 31, 118, 160-162, 175, 176, 251
  sativa, 251
Vincetoxicum, 243

Xenopus, 47-49, 51, 287
  laevis, 47, 52

Zea, 29, 93, 115, 116
  mays, 29, 30, 102, 110, 115, 132, 299

IMPERIAL CANCER
LINCOLN'S INN
FIELDS
LIBRARY
RESEARCH FUND

WELLCOME
LIBRARY
INSTITUTE

6974-1