chromosome marker

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Chromosome Marker

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With 120 Illustrations

J. & A. Churchill, Ltd.
104 GLOUCESTER PLACE, LONDON, W.1.
1963

Printed in Northern Ireland at
The Universities Press, Belfast

CHROMOSOME MARKER

Frontispiece. The living chromosomes during meiosis in the male of *Chorthippus brunneus* (2n = 16 + X) as seen with the phase contrast microscope (ca. ×1,500).

1. Early pachytene showing paired chromosomes and their chromomeric structure. Heteropycnotic X at 6 o'clock.
2. Late pachytene showing more contracted chromosomes and small, detached nucleolus at 10 o'clock.
3. Early diplotene with homologues falling apart and nucleolus breaking up.
4. Diplotene showing chiasmate bivalents. X chromosome at 10 o'clock still heteropycnotic and small centrally placed nucleolus.

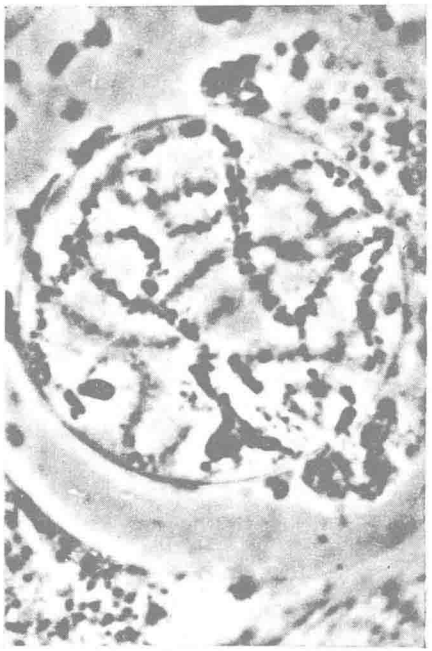
To

C. D. Darlington F.R.S.,

whose industry, insight and imagination transformed the study of chromosomes from a crude science to a fine art.

'Trace Science, then, with modesty thy guide;
First strip off all her equipage of pride;
Deduct what is but vanity or dress,
Or learning's luxury or idleness;
Or tricks to show the stretch of human brain,
Mere curious pleasure, or ingenious pain;
Expunge the whole, or lop th'excrescent parts
Of all our vices have created arts;
Then see how little the remaining sum,
Which served the past, and must the times to come'.

ALEXANDER POPE.



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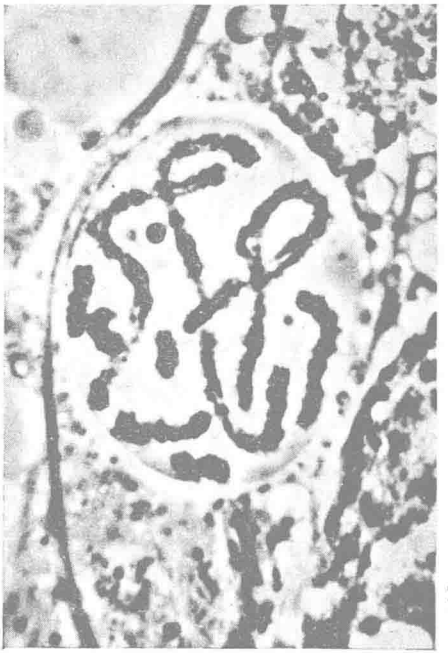

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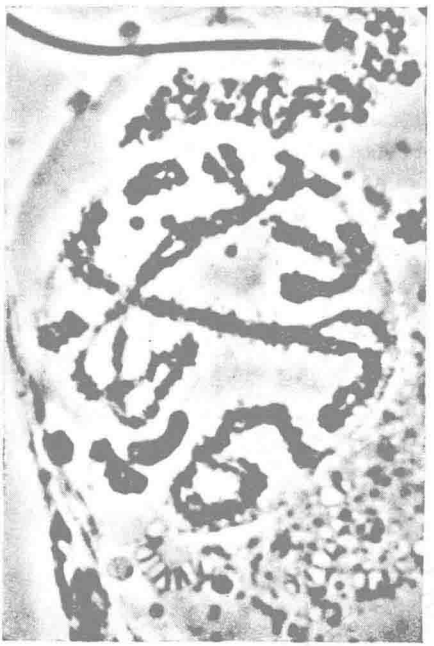

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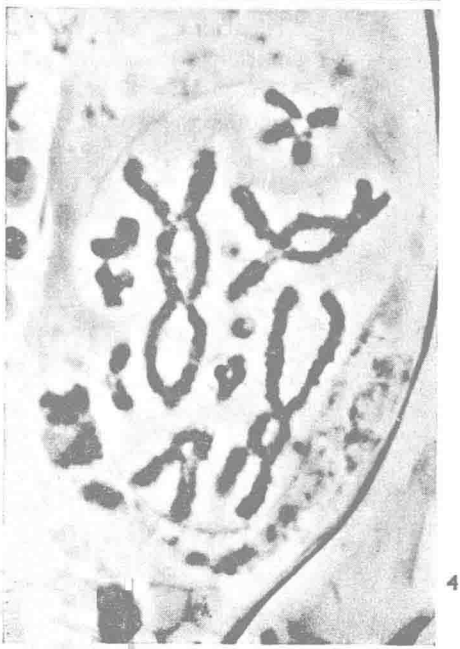

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PROLOGUE

'It is when we attempt to reveal and explain ourselves to others that we realise our ignorance on the subject, and find that we must build our house room by room, while we take visitors through it'.

André Maurois.

Biology has many techniques at its disposal. Some of these are of recent origin, others have undergone conspicuous advances in recent years. In this book we are concerned with a particular technique in biology, namely, that of looking at chromosomes. Technical advances have, of course, been made in the handling of these cell organelles (Darlington and La Cour 1960), but it cannot be described as a new technique. On the contrary, as a general method it is about a hundred years old. Nor, unlike many modern techniques, is it difficult or expensive to practise.

Certain techniques, like certain ideas, are designed to replace others but some can only be complementary to those already in existence or, indeed, to those which have yet to be discovered. We offer no apology, therefore, for considering this established method for, while other methods of studying heredity and variation form necessary complements, no method can replace it. In fact, there is much that remains to be learned by using this technique and much that can be discovered in no other way. And in our opinion this technique is simpler, more useful and more widely applicable than any other single biological method.

In essence, we have considered here how those biologists who look at chromosomes spend their working hours. It is then, a 'What Katy did' type of book. We have also discussed the results of their labours in connection with particular problems. Others will see these same investigations from different points of view and see in them things we have missed or the importance of which we have not recognised. Indeed, many of the investigations were undertaken in contexts other than those we have given them. Since we were not content with description, it was inevitable that our own attitude and approach to biology should colour (or 'taint') our consideration of the facts. This too was intentional for we decided to write with hypothesis as our guide. These hypotheses will not be to everyone's taste and we hope that the reader will not slight the original investigations because, in his view, we have misused them.

The book is in four sections. The first of these is introductory and deals with the nature of the materials, the mechanics and the mutation of the chromosomes. The second section relates the chromosome theory to the laws of classical and contemporary genetics. One topic which would find a

place here is conspicuous by its absence, namely, the mechanism of crossing-over. Our reasons for not considering it are various. In the first place, very little additional chromosome evidence has been added to the body of fact discussed by Darlington (1931, 1937) in connection with his hypothesis of partial chiasmatypy. Secondly, the additional evidence that there is comes mainly from the study of recombination in micro-organisms using the technique of experimental breeding and partly from chemical studies on the synthesis of DNA and other compounds during the meiotic cycle; and a detailed consideration of this kind of evidence is outside the scope of this book. However, in this connection, we would like to elaborate a point we made earlier. Whatever new facts emerge, the nature of the recombination process must not be discussed in terms of them alone. New observations do not necessarily invalidate the old; the chromosome evidence stands, and it is certainly not 'too coarse' (contra Pontecorvo 1959). Only rarely does scientific knowledge improve by substitution; generally, it increases by accretion. Thus, just as the later facts concerning extra-chromosomal inheritance, carry-over effects and the nature and behaviour of natural populations had to be fitted into the principles of classical genetics so new information regarding recombination must be added to that already adduced.

Cell genetics and development are considered in the third section. There are many approaches to these problems and many techniques available for their study. We have considered in detail only the chromosome evidence. In the fourth section, however, we move from controlled experiment to natural situations. And here it was necessary, for obvious reasons, to give more attention to other lines of evidence as well. This, as one might expect, is the largest of the sections, since much of the information touched on in the other sections finds its full meaning only in relation to time and change.

In choosing our examples we have tried not to discriminate between plants and animals. But from the experimental point of view a difference becomes apparent. Nearly all the detailed studies on chromosome variation during development that we describe come from animals. But plants figure far more prominently in our last section on evolution. To some extent this may be an artefact with a historical basis. For most botanists cytology means chromosomes but for many zoologists it means cytoplasm. Further, botany seems to have assimilated much more nuclear cytology than has zoology—a difference commonly reflected in the courses of university departments.

Even so the plant cytologist has been concerned almost exclusively with the chromosomes of the meristem and the germ line. The structure of the cell-surface and the pattern of development in animals, on the other hand, are such that the embryo and mature tissues are more amenable to investigation. Thus even though cytological studies of different kinds have predominated in the two kingdoms, there can be little doubt that chromosome changes play a greater part in evolution of plants and a more prominent role during development in animals. So, although we have attempted to be impartial, nature has not.

This is not a text-book nor is it directed towards a particular audience but we hope it will find a place wherever chromosomes are looked at and talked about. It is perhaps, too vain to hope that it might penetrate even those places where the chromosomes are paid only lip-service. Paradoxically, these places are of two kinds—those which ignore heredity and those which are so pre-occupied with particular aspects of it that they cannot see beyond the limits of a molecule, a mould, a mouse or a mathematical matrix.

We have tried to build a house. It is well founded, for the foundation stones were laid by abler hands than ours. Some of the rooms will require redecorating before others and, while some of them may be without doors, we trust that the stairway continues to the roof. The basement is let to the chemist and physicist, the attic to the experimental breeder. Rooms in the intervening floors are occupied by morphologists, physiologists, ecologists, taxonomists, physicians and many others. And we have spent most of our time on the stairs. This is the place where disciples meet and mingle and, until such time as the internal walls are torn down, it is the means of connection and communication.

There is one confession we have to make. In the tables given in section 4 we have not always consulted the original references, but the source of these is given. We realise that substituting belief for labour cannot be justified and we can only hope that our confession will partly atone for our sin.

We would like to thank all those who have helped us by digging-up references, carrying books, reading proofs and putting up with long periods of silence. In particular we would like to mention Mrs. Ann Freeman and Mrs. E. C. John. Finally, we ask the readers indulgence for inevitable errors and commend to him E. J. Phelp's contention that 'the man who makes no mistakes does not usually make anything'.

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ACKNOWLEDGEMENTS

Some of the illustrations in this book have been modified from previously published pictures and thanks are due to editors of journals and to publishers of books for permission to reproduce in an altered form the illustrations specified.

Advances in Genetics, Figures 81 and 101; *Biblioteca Genetica*, Figure 46; *American Journal of Botany*, Figures 106 and 108; *The American Naturalist*, Figure 22; *Chromosoma*, Figures 2b, 5a and c, 8, 13, 57, 74, 93, 94, 95, 98, 99 and 100; *Experimental Cell Research*, Figure 75; *Heredity*, 2a, 4, 27, 53, 105b, 111; *International Review of Cytology*, Figure 40.

Cold Spring Harbor Symposia in Quantitative Biology, Figure 25; A Century of Darwin, Heinemann, Figure 66; Patterson and Stone, Evolution in the genus Drosophilla, New York, The Macmillan Co., Figure 38; Stebbins, Variation and Evolution in Plants, Columbia University Press, Figure 17; Proceedings International Genetics Symposium 1956. Science Council of Japan, Figure 107.

CONTENTS

Section 1—The Chromosomes in Cell Division

'Cell division is always a link between past and future'.

C. D. DARLINGTON.

'A body continues in its state of rest or of motion with uniform velocity unless acted upon by force'.

ISAAC NEWTON.

'Mutations are accidents and accidents happen'.

A. H. STURTEVANT.

Chapter 1

New cells arise only by the division of pre-existing cells. The process of cell division therefore, necessarily underlies development, growth and regeneration; it is also common to asexual and sexual reproduction since both of these processes involve the production of new cells. The details of cell division may, indeed usually do, differ in somatic and germinal cells; they may also differ in different kinds of somatic cells. But underlying these differences is an essential uniformity of mechanism. Most of what we know about this mechanism is based on the intelligent interpretation of observations made with the use of the light microscope. And it is with these observations that we need to start.

MITOSIS AND MEIOSIS

'The nucleus does not divide; it is divided'.

Theodore Boveri.

All cells capable of division are organised into two principal areas, the nucleus and the cytoplasm. Both of these are involved in cell division though the division of the nucleus necessarily precedes that of the cytoplasm. When cells are not in the process of dividing, their nuclei are referred to as interphase nuclei. There is great variation in the appearance of such nuclei, particularly in animals, but all include up to three components. These are:

(i) One or more usually spherical bodies, the nucleoli,
(ii) One or more pieces of deeply staining or heteropycnotic material, and
(iii) A series of fine threads, the chromonemata.

Nucleoli and heteropycnotic material can be seen with an ordinary light microscope following fixation and staining. They can also be seen in the living cell with the use of phase-contrast microscopy. This is not usually true of the chromonemata, but they have been seen with the interference microscope (Ambrose 1957). When somatic cells embark upon the process of division, however, all three components become readily visible and undergo a remarkable series of changes (Fig. 1). First the chromonemata become fixable and appear as a series of basophilic threads called chromosomes ('coloured bodies', Waldeyer 1888). It was, indeed, this striking change in the appearance of the nucleus that led Flemming to call the process of division 'mitosis' (mitos = thread). In suitable material these chromosomes can be seen to be double along the greater part of their length from their first appearance. Each chromosome is thus composed of two half-chromosomes or chromatids which are usually held together at or near a region of the chromosome, the centromere, which regulates its movement later in the sequence. These centromeric regions are in some cases grouped or polarised to one side of the nucleus.

The heteropycnotic material of the interphase nucleus can now be clearly identified either as parts of chromosomes or even whole chromosomes. Such chromosomes or chromosome regions are often called heterochromatic to distinguish them from the euchromatic ones. Similarly nucleoli can be seen to be attached to specific regions of particular chromosomes.

Following their appearance the chromosomes shorten and thicken by a process of spiralisation, each chromatid behaving quite independently of its sister. The centromeric region, however, does not coil and neither, in some complements, do certain other chromosome segments. Such regions appear

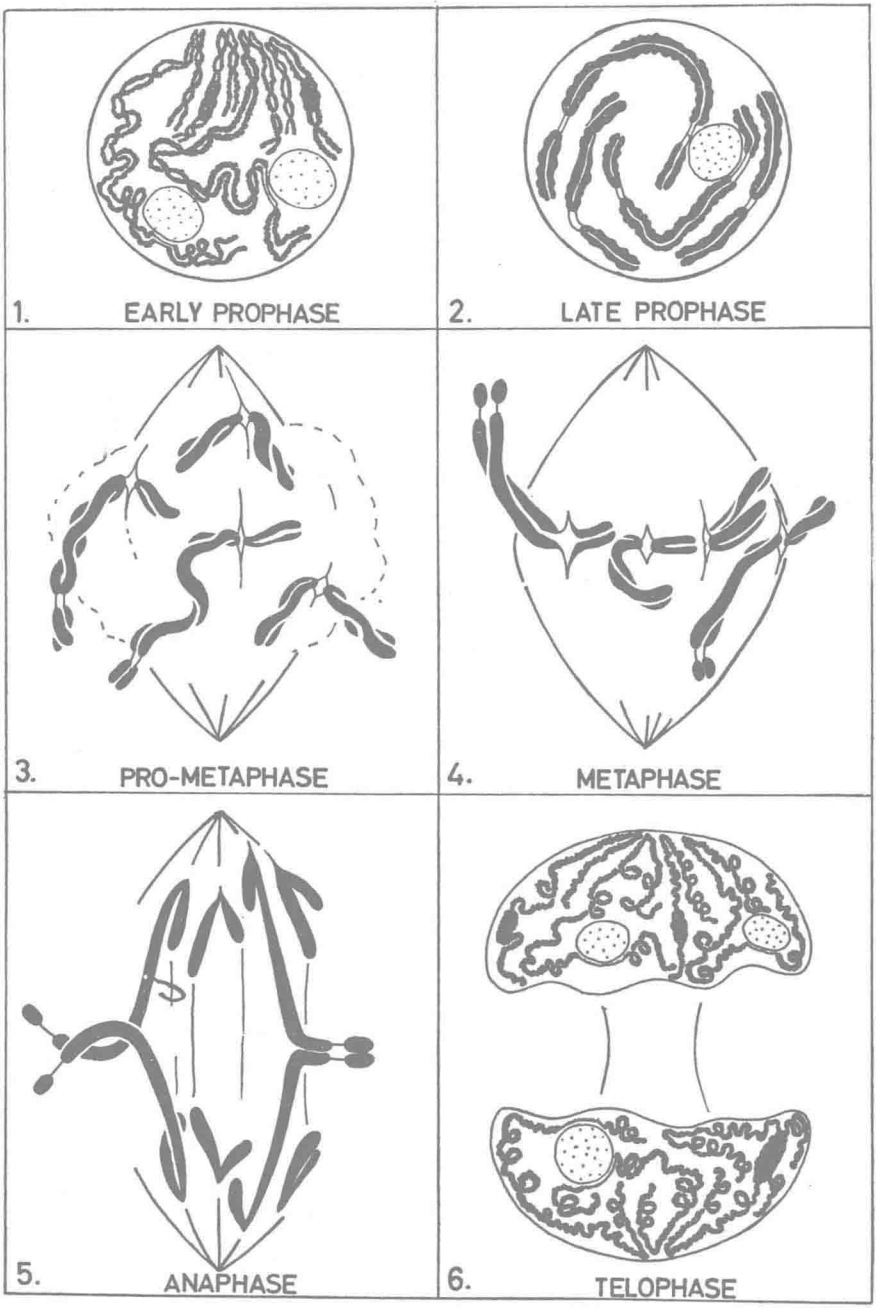

Fig. 1. Mitosis in a diploid cell. Four chromosomes are illustrated; two of these are small metacentrics with a subterminal heterochromatic segment. The other two are large acrocentrics with a nucleolar constriction in the long arm.

as constrictions. The centromeric constriction is known as the primary constriction, the others as secondary constrictions. Many such secondary constrictions have attached nucleoli. In conjunction with the process of spiralisation the nucleolus gradually disappears, as does the visible difference between the heterochromatic and euchromatic material.

These events are followed by the breakdown of the membrane which normally separates the nucleus and the cytoplasm. This is accompanied by the development of a fibrous system which, because of its shape, is referred to as the spindle, for in many cases it has a broad central equator and two pointed extremities or poles. Because their refractive index is so little different from their surroundings the spindle fibres cannot normally be seen in living cells with either ordinary light or the phase-contrast system. But the spindle can be seen in a wide variety of living cells with polarised light and with such light it appears birefringent. Indeed, shortly before the nuclear membrane breaks down, a narrow birefringent zone appears outside the nuclear membrane (Inoué and Bajer 1961); this appears to constitute spindle-percursor material.

In most animals and in those plants possessing cilia or flagella at some stage in their life cycle a pair of centrioles are present. At the onset of division these lie latent immediately outside the nuclear membrane and it is to them that the centromeres are often polarised. They separate before the nuclear membrane disrupts and, subsequently, come to lie one at either pole of the spindle. In some cells each centriole is responsible for organising a radiating system of fibres termed the aster which subsequently cap the spindle poles. These centrioles and the aster systems to which they may give rise presumably play no more than a subsidiary role in localising spindle poles since they are absent in most plants and some animals. In such cases the spindle tends to be barrel-shaped with broader truncated ends. In the ciliate Protozoa the centriolar system is intranuclear, the spindle forms, and indeed the whole subsequent division proceeds, within the intact nuclear membrane (Belar 1926).

With the appearance of the spindle the chromosomes become attached to it by their centromeres. It was formerly believed that the centromere contributed new chromosomal fibres, sometimes called half-spindle fibres, to the spindle by virtue of its own synthetic activity. In these terms the spindle was considered to consist of continuous spindle fibres of extra-chromosomal origin, which extended uninterrupted from pole to pole, and half-spindle fibres of chromosomal origin which connected the centromere to the poles. However, it seems more probable that the function of the centromere is to convert spindle material into half spindle fibres with an increased orientation. Significantly, birefringence is always strongest adjacent to the centromere.

The attachment of the centromeres to the spindle apparatus may occur at any point. Once attachment is achieved, however, the chromosomes undergo a complex series of oscillations which culminate in all of them lying