EVIDENCE ON SPIRAL STRUCTURE AND CHROMOSOME PAIRING IN *OSMUNDA REGALIS* L.

By IRENE MANTON, B.A., Ph.D.
Lecturer in Botany, The University of Manchester

(*Communicated by W. H. Lang, F.R.S.—Received* 8 *December* 1938)

[Plates 16–19]

CONTENTS

	PAGE
Introduction	179
Morphology of the chromosomes of *Osmunda regalis*	182
Notes on some physical properties of spirals	184
The spiral in the mitotic cycle	185
The chromosome spiral not a state of strain	188
Note on the geometry of chromatid separation at meiosis	189
The early meiotic prophases	190
Chromosome elasticity as evidence of submicroscopic convolutions	195
Interpretation of leptotene in terms of the known structure of organic fibres	197
The phenomenon of supercontraction	199
Discussion	200
Summary	207
Appendices	208

Introduction

The present work is in the nature of an essay on certain aspects of chromosome structure which have been impressed upon me by prolonged study, with a variety of technical methods, of chromosome behaviour in one plant, *Osmunda regalis*. The tissue to be examined has been principally that of the developing sporangium, supplemented by observations on roots and prothalli, which, however, will not be referred to on this occasion. The sporangium itself offers ample material for the study of both mitosis and meiosis since the development from the young archesporium to the ripe spore is a rapid and continuous one. The technical methods employed are principally three, that of sections, that of the aceto-carmine squash (Manton 1937), and Sax and Humphrey's modification of the ammonia method for spiral structure. Each of these methods if used by itself has limitations which necessarily result in an incomplete picture; each, however, has certain powers peculiar to it, and, as will be shown, the dovetailing together of information from all three provides a body of evidence which to me personally has been most illuminating. I am, however, fully conscious of the innumerable pitfalls with which the subject abounds and am prepared to find that some of my observations and deductions may need modification. Nevertheless, since

The Royal Society is collaborating with JSTOR to digitize, preserve, and extend access to
Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences. STOR®
www.jstor.org

a comparable body of evidence does not appear to exist for any other cytologically worked organism the risk of error is perhaps worth taking.

No disparagement of other investigators is intended by this last remark, since our knowledge of the best-known cytological types such as *Zea*, *Tradescantia*, *Fritillaria*, *Stenobothrus*, *Osmunda*, is in each case a composite one to which each generation of workers has contributed. Certain facts about all these types are by now sufficiently clear, in a descriptive if not in a causal sense. In each case, however, the sum of published descriptions is deficient, either in details of spiral structure, or in knowledge of chromosome lengths at crucial stages such as leptotene, to name only two of the most important omissions. Yet the discovery of spiral structure alone, or rather its rediscovery* in recent years, is probably one of the most far-reaching facts with which modern cytology has to deal. The realization that the apparent shape of the chromosome is merely the external form of a spiral coil must obviously entail very considerable reorientation of old ideas. The rewriting of descriptive cytology in conformity with this new knowledge is, however, impossible unless and until the facts regarding it are fully known. The extreme confusion which now reigns in the literature on several aspects of spiral structure would therefore seem to be primarily the expression of the difficulty of obtaining these facts. A contributory cause is unfortunately also the desire to reach a rapid theoretical explanation of exceedingly complex phenomena, a weakness to which cytologists have always been particularly prone.

With these difficulties in mind a deliberate choice of method has been carried out. In the first place, the initial collection of data, which has necessitated work over a number of years, has as far as possible been carried out for its own sake with no ulterior motive of a theoretical kind to influence the choice of material. Throughout the work all observations have been recorded in the most objective possible way, namely, by photography. Selection of specimens to be photographed has been made on the sole criterion of clarity, and in no case have observations depended on details of fixation so minute that their existence could be open to question. With the knowledge that application of statistical methods is impossible in material of this kind it has been felt that a few well-documented observations are more likely to be reliable than many

* The literature on spiral structure, as is well known, dates back to the very beautiful observations of Baranetzky on living mother cells of *Zebrina pendula* (= "*Tradescantia zebrina*") in 1880, but for the next forty odd years the subject is only referred to incidentally, e.g. Janssens 1924; Kuwada 1921; etc. In 1926 Kuwada followed up his earlier work and made a serious attempt at analysis of the spiral at anaphase of the mitotic chromosomes of *Vicia faba* in sectioned roots, and in the same year Fujii in Japanese and Kuwada and Sugimoto in English published preliminary accounts of the meiotic spirals in living cells of "*Tradescantia virginica*" (subsequently reidentified as *Tradescantia reflexa*). 1927 marks the beginning of special techniques by the publication of the hot-water treatment, accompanied by some excellent photographs by Sakamura. Kuwada (1927), Maeda (1928), Shinke (1930) and Sax (1930) extended the study of meiotic spirals in this and other plants by smear methods using aceto-carmine and diluted stains. 1932 marks the introduction by Kuwada of the ammonia vapour technique which has since been extensively used both in Japan and elsewhere, the methods of Sax and Humphrey (1930) and La Cour (1935) being convenient modifications of it.

indefinite ones. For this reason all measurements have been carried out on the photographed specimens and only these are quoted. This does not mean that observations have been made singly. Every measurement, with one exception, has been verified more than once, and in most cases confirmatory evidence is provided by the different techniques themselves. The one exception for which confirmatory evidence has not so far been obtained is the leptotene measurement which is discussed in detail in the text. The limitation of numerical observations to the photographed specimens means that very special importance must be attached to the Plates. These should not be regarded merely as illustrations to the text but as the data on which the paper is based, and it is most desirable that they should be referred to by the reader wherever quoted.

In the presentation of the subject-matter it has been found convenient to relegate the details of measurements and calculations to appendices in order to avoid encumbering the text and for ease of reference. For Appendix I, which involves the mathematical treatment of spirals, I am indebted to Mr Dearden, M.Sc., of the Physics Department of this University who has carried out the calculations on measurements provided by me. For the other three appendices dealing with estimations of elasticity and supercontraction and with direct measurements of length I alone am responsible.

The main body of the paper has been developed as a continuous narrative subdivided into sections according to types of evidence. In order to do this it has been necessary to curtail the chronological description of events and some familiarity with well-known cytological appearances and terminology has had to be assumed on the part of the reader. Supplementary description will, however, be found in the legends to the plates, and a preliminary perusal of Plate I will be found helpful to the less experienced. Simplification of the nomenclature has, however, been introduced wherever possible, particularly in relation to spiral structure, and many terms used by some other authors (such as major and minor spiral, relational coiling, super-spiral, relic spiral, etc.) are here omitted for the sake of clarity and for reasons which will appear. This does not mean that the use of common words such as convolution, spiral, twist and torsional stress or strain is in any sense haphazard. It is, on the contrary, particularly important that the sense in which these words are used should be clearly apprehended. Every effort will be made to use them consistently and to explain them clearly where explanation is necessary, for confusion between them, especially between the words twist and torsional strain, will certainly lead to misunderstanding and possibly to unprofitable controversy.

In order to avoid controversy, discussion of literature is as far as possible relegated to footnotes where it will chiefly take the form of acknowledgements of previous observations on the type of facts to be used. Certain subjects for which this method would be quite inappropriate, owing to the extent of their literature, will be omitted altogether. Foremost among these is the question of the time of chromosome

splitting and the general problem of the origin and nature of chiasmata. These are admittedly important topics, but consideration of them here would overcrowd the work and obscure the outlines of the main results.

Finally, a word may perhaps be given on the species chosen and the reasons for its selection. *Osmunda regalis* is a classic cytological object which has already, in the hands of the earlier investigators,* given results which are landmarks in the history of the science. Its systematic position as a relatively primitive fern gives it a comparative value which more than compensates for a rather higher chromosome number and a somewhat smaller chromosome size than in the more frequently studied flowering plants. All the available members of the Osmundaceae have been found to be readily amenable to a variety of technical treatments, and for *O. regalis* itself invaluable additional evidence has been supplied by the existence of polyploid material both triploid and tetraploid (cf. Manton 1932; Lang 1924). Much information regarding both polyploids and other species is withheld and will be published elsewhere, but two relatively unimportant figures in the aceto-carmine series (figs. 55, 59, Plate 18) refer to *O. gracilis*. With these exceptions, all other figures refer to *O. regalis* itself or to its auto-polyploids.

Morphology of the chromosomes of *Osmunda regalis*

A glance at fig. 58, Plate 18, will show the general appearance of the haploid chromosome complement of *O. regalis* at anaphase of the second meiotic division. The chromosomes appear rod-shaped, are closely similar in size, and their number, as is well known (Guignard 1898; Strasburger 1900; Digby 1919; Manton 1932), is 22. It is not practicable to distinguish individuals, though at some stages it might be possible. This disadvantage, however, is off-set by the general uniformity and, for the present purpose, any chromosome which can be completely seen at any stage, will be used as a basis of comparison. An element of uncertainty is admittedly introduced by such interchange, but for the moment this is immaterial, since many of the more difficult measurements only admit of approximate evaluation in any case.

* Without quoting the literature fully, the most important papers on the cytology of *Osmunda* may be enumerated as: 1894, Strasburger's demonstration of different chromosome numbers in sporophyte and gametophyte and the fact of numerical reduction occurring in the spore mother cells; 1900, Strasburger's first figures of the reduction process including various stages from diplotene to the second division in *Osmunda*; 1904, some very excellent figures of prophase stages in *Osmunda* by Farmer and Moore, but misinterpretation of the "early spireme" led to the unfortunate conception of telosynapsis; 1907 and 1910, some very good figures by Grégoire, correctly interpreted, of zygotene and pachytene in this plant with consequent re-emphasis of idea of parasynapsis; 1919, paper by Digby giving detailed interpretation of *Osmunda* in terms of telosynapsis; 1927, paper by Szakien repeating Grégoire's interpretation in terms of parasynapsis; 1932, announcement (Manton) of chromosome numbers in polyploid material derived from aposporously produced prothalli (Lang 1924); 1936 (Manton), demonstration of spiral structure.

A morphological detail, the effect of which should be understood, is the position of the attachment constriction. This is in no case median but is terminal or subterminal. The chromosomes therefore appear rod-shaped at a normal metaphase or anaphase (figs. 26, 46, Plate 17), but at the end of the first meiotic division they appear V-shaped (cf. fig. 54, Plate 18). This V is not caused by a bend in the middle of each chromosome but is due to the fact that each is already split in preparation for the next division, and the split halves diverge from the terminal attachment constriction. Were the attachment constriction median, the split halves would diverge from both sides of it to produce an X shape. This relation between the position of the spindle attachment and the shape of the split chromosomes at anaphase of the first meiotic division is well known, having been explained first by Grégoire (1910) and in greater detail by Janssens (1924). Further consideration of the lateral movement involved will be found on p. 190 below.

The gross morphology of the paired chromosomes at diakinesis is shown in fig. 52, Plate 18. Points to notice are the relatively few chiasmata and the marked absence of terminalization.

The gross morphology of the spirals is shown on Plate 19 at a magnification of $\times 3000$. The first meiotic division is represented by figs. 64–68. The first of these shows chromosomes in position on the spindle, the remainder are isolated individuals with various numbers and positions of chiasmata, all orientated as they would be on the equator. In fig. 65 is a pair of chromosomes united by a single chiasma at the end away from the spindle attachment, in fig. 66 the single chiasma is at the same end as the spindle attachment, fig. 67 shows two chiasmata, one at each end, and fig. 68 is of an unpaired chromosome from a triploid. Neither number nor position of chiasmata appears to affect the coils appreciably, their number in all these figures being the same, namely, 4.*

In all these figures it will be noticed that only one large spiral, not two parallel ones, is visible in each chromosome, although it is well known from other evidence† that a chromosome thread at metaphase is already double. The two chromatids are however still in close physical contact and share a common path. They do not separate, in *Osmunda*, until the onset of anaphase, but the time of separation appears to be a variable one with different organisms. Thus in *Rhoeo* (Sax 1935) it occurs at late metaphase, but it has been recorded as early as diakinesis in *Trillium* (Huskins and Smith 1935). The significance of this time difference is unknown.

Determination of the number of visible coils at the second meiotic division is more

* In the preliminary note published in *Nature*, 1936, figs. 65, 67, Plate 19, were reproduced on a larger scale, and the number of coils was then estimated as "about 5". This appears to have been an over-estimate owing to the difficulty of evaluating fractions of a coil. In unpaired chromosomes, a considerable number of which have since been seen, the number seems to vary from 4 incomplete coils to somewhat less than 5 coils. This no doubt reflects the range of diversity in length between different chromosomes and 4 seems to be the average.

† See general text-books: White (1937), Sharp (1934), Darlington (1937).

difficult owing to their smaller diameter. In fig. 69, Plate 19, only an approximate count can be made of the spirals at this stage, but uncertainty is removed by the very clear single chromosome shown in fig. 70. This is a lost chromosome from the second division in a triploid and it has 8 small coils.

Corresponding details for the chromosomes of an ordinary mitosis are even more difficult to obtain, owing to the fact that the technique cannot be applied to a tissue, but only to isolated cells. Spore mother cells can readily be isolated by smearing, but those of the tissue of the sporangium in the premeiotic division are too compact to be separated. The same difficulty applies to other growing parts such as roots, and only quite exceptional mitotic cells such as pollen grains (*Allium*, Geitler 1938; *Tradescantia*, Sax and Sax 1935) or pollen tubes (*Tulipa*, Upcott 1935) have so far been worked at all with the spiral structure technique. Fortunately, in *Osmunda*, mitotic figures may still be found in the tapetum till well on into meiosis, and, though infrequent, it does sometimes happen that somatic chromosomes from this source can be obtained in a smear of mother cells. Observation of the spirals is even more difficult in these than at the second division, owing to their greater length, but the chromosome marked with an arrow in fig. 71, Plate 19, has certainly in the region of 14 coils. It is possible that in this chromosome the number 14 is an under-estimate and the real number of coils is perhaps 16. It is also probable that individual differences exist owing to real differences of length, since other chromosomes are suggestive of having 12. The number 14 is thus only an approximate value for the average. It was determined for its own sake before any of the subsequent calculations were made, and though any element of uncertainty here is much to be regretted, even an approximate value is an indispensable piece of information. Pending greater exactitude therefore, the number 14 will be provisionally used.

Notes on some physical properties of spirals

Before proceeding from chromosome morphology to chromosome behaviour, it is desirable to make clear some very simple geometrical facts relating to spiral objects in general. This will explain to some extent the nature of the verbal distinctions mentioned in the introduction. Other explanations will be found on pp. 187 and 202. No great originality is claimed for these remarks nor are they exhaustive, but they represent several different points of view all of which are in various ways applicable to chromosomes.*

* The need for clarity about the properties of spirals has of course been felt by more than one writer on spiral structure. Kuwada and his colleagues, in particular (1927, 1933, etc.), have dealt very lucidly with a number of most pertinent geometrical considerations, some of which are included above, while other writers, e.g. Koshy (1937), Darlington (1935), Upcott (1937), Nebel (1932), etc., have stressed individual points. Clarity on single points is, however, insufficient to meet the problem, and failure to appreciate its diversity is probably in part responsible for discordance in the literature.

In the first place, a visible spiral may, but need not, involve a twist of the whole thread. This will be readily understood if a flattened object, such as a piece of tape, be wound spirally upon a stick. If the tape be wound in such a way that it lies flat upon the stick throughout, a spiral will be formed which will, naturally, be much shorter than the actual length of the tape. On removal of the stick, the tape can at once be pulled out to its true length, but it will be found to be twisted upon itself as many times as there were coils in the spiral. If, however, the tape is wound in such a way that the ends are held firmly in the hands and not allowed to rotate, a "non-torsional" spiral will be formed in which the tape does not lie flat at every point but bends completely upon itself once for every coil made.* On removing the stick and re-extending the tape it will this time show no twist. This difference, due here to the manner of winding, is extremely clear if tape is used; if, however, the experiment is repeated with a radially symmetrical object such as a piece of string it may be quite impossible to determine, by inspection of the spiral, which type is involved.

Another, more fundamental, distinction between spiral objects lies in their relation to external forces. Both the spirals described in the previous paragraph were deformations of an originally straight object which, so long as it was contorted, was in a state of strain. All such spirals are inherently unstable since the contorted object will tend to return to its original shape when the stress is removed, as long as its elasticity is unimpaired. Objects of exactly the same shape as either one of those referred to may, however, be perfectly stable, if their shape is constructional and not due to deformation. A spiral staircase has the shape of a twisted spiral but is not experiencing torsional stress. Its shape is constructional and energy would have to be exerted in order to straighten it. In doing so two different processes would be involved, the large coils would have to be removed by pulling and the torsion by untwisting. These two processes might, but need not, occur together.

It is thus clear that straightening is not synonymous with untwisting, nor "spiral" with "twist", and the spiral shape may cover a variety of states. It is the business of the cytologist first to determine which of the several alternatives is represented by the spirally contorted chromosome.

The spiral in the mitotic cycle

All cytologists would probably agree in regarding the *shape* of the spiral in the mitotic chromosome as that of the twisted spiral described in the previous section. This follows from the well-known spatial relation of the split halves at prophase. These are twisted about each other in chromosomes large enough for the split to be observed. *Osmunda* is inconveniently small for this purpose though the split can just be seen (fig. 46, Plate 17). One photograph of the larger chromosomes of *Allium*

* This bending is no doubt another expression of the "compensating spiral" of some other writers.

is therefore appended in illustration (fig. 81, Plate 19). Now it is clear that if a twisted structure with the form of a spiral staircase were to split longitudinally along a line passing through the middle of each step, the split halves would be in exactly the same spatial relation to each other as are the halves of the prophase chromosome. The situation may be optically demonstrated by a very simple experiment. If two pieces of wire are wound on a stick so that they are parallel to each other, i.e. as if one wire or the tape previously used had split longitudinally, it will be found that when the stick is removed the two wires will be twisted together in such a way that it will be impossible to pull them apart sideways for they will interlock at every turn. They can only come apart if the coils are first unwound. This unwinding is a well-known feature of prophase and has often been described (e.g. Koshy 1933; Sax and Sax 1935; Darlington 1935; etc.). In *Osmunda*, as in other plants, it is almost though not quite complete by metaphase; the lowest chromosome in fig. 46, Plate 17, still shows one turn. The fact that untwisting is geometrically necessary before the half-chromosomes can separate is clear evidence that the spiral shape of the unsplit chromosome must be of the torsional type. The occurrence of untwisting in prophase does not, however, in itself justify any deduction further than this, and the nature of the twist in the definitive chromosome, i.e. whether constructional or unstable, can only be inferred from other evidence.

Some other evidence is supplied by the behaviour of the spiral through the mitotic cycle. The general features of the principal stages of mitosis are shown in figs. 1–9, Plate 16, and details of the most important of them in figs. 23–28, Plate 17. At early prophase the nucleus is still of the "solid" type (cf. Manton 1935), i.e. it is an oval or pear-shaped mass of ill-defined chromosome bodies, in which are embedded several nucleoli (cf. fig. 2 for general view, fig. 23 for detail of chromosomes). At late prophase (fig. 4) the nucleus is spherical, being dominated by the liquid of the nuclear sap in which the chromosomes now float (fig. 25). Change of shape of the nucleus does not involve mutual displacement of the chromosomes, for even at late prophase the polarized arrangement of the previous telophase is preserved (compare upper nucleus in figs. 1 and 4).

From the point of view of spiral structure, the detailed views of middle prophase (fig. 24, Plate 17) and late telophase (figs. 27, 28) are the most instructive, for in them the spirals can be clearly perceived, even in sections of the ordinary kind. This is certainly owing to the fact that the coils, at these stages, are less closely packed than at metaphase and anaphase. Quantitative estimate of the number of coils is not practicable at telophase, owing to the flattened state of the nucleus and to the impossibility of determining where one chromosome ends and another begins. This difficulty is not present at prophase, and at the stage shown in figs. 3 and 24 whole chromosomes can be followed throughout their length with complete certainty. Figs. 80*a–d*, Plate 19, give several focal levels and a drawing of one chromosome from the nucleus of fig. 24, Plate 17. The whole thread is distorted into an irregular zigzag by having

expanded its coils in a confined space. The coils themselves, however, though pushed apart, are still present.* At least 10 are still intact, and complete straightening has only been achieved near the ends of the chromosome. Since this stage, though early, is not the beginning of prophase, it is highly probable that the untwisting, which certainly occurs in prophase, is confined to this stage. The *essential* feature of telophase cannot therefore be the "uncoiling and repacking of the chromosome thread" as has sometimes been thought (Darlington 1937, p. 34),† since in *Osmunda* very little, if any, uncoiling occurs till after rest.

Another very important fact emerges from a measurement of the prophase chromosome figured (fig. 80, Plate 19). At this magnification ($\times 2000$) the length of the figured specimen is 40 mm., as nearly as can be measured (see Appendix IV). Now if the apparent diameter of a chromosome at metaphase or anaphase is measured, it is possible to calculate the real length of the thread at these stages from the previous data on the number of coils. The diameter at this magnification, in both a tapetal chromosome (fig. 46, Plate 17) and an archesporial chromosome (fig. 26, Plate 17), is of the order of 1 mm. The length of the thread is then of the order of $1 \times 14 \times \pi = 43$ mm. if a spiral is treated as a pile of circles. This value is only a rough estimate and a more refined method is worked out in Appendix I. According to this the length of the tapetal chromosome at metaphase (fig. 46) works out as 42·7 mm. and the archesporial anaphase (fig. 26) as 41·1 mm. The tapetal anaphase of fig. 47 is under suspicion of being swollen by the fixative, for its result is unexpectedly high (49·1 mm.). Every method of estimation, however, contains elements of uncertainty most of which tend to make the result too big (cf. p. 203 below). The general agreement between the results of calculation and measurement is therefore rather striking.

The obvious conclusions arising from these facts are: (1) That the length of the chromosome thread is probably constant throughout mitosis though the closeness of the coils can change.‡ (2) That the thread is twisted at all times except at prophase, when one twist gives place to another (cf. Koshy 1933; Darlington 1935; etc.). (3) That except at prophase the twist is perfectly stable and is therefore probably constructional.

* The presence of two kinds of contortions in the prophase chromosome was first, and correctly, distinguished by Darlington (1935), who gave them the names of "super-spiral" and "relic spiral" respectively. These names will not be used here from the general desire to simplify terminology. "Super-spiral", for the zigzag seen in *Osmunda* is also of doubtful validity. "Super", in the sense of "additional" it certainly is, but whether it is a "spiral" and not merely a fortuitous bending is not evident.

† In justice to this writer it should be pointed out that his own description of telophase behaviour in *Fritillaria* (Darlington 1935) does not exactly accord with his general statement (1937) quoted above. He is careful to explain in the former (*loc. cit.* p. 47) that expansion (or "relaxation" to use his own word) does not necessarily mean untwisting. It is also perfectly possible that different species may differ in the precise point at which uncoiling begins, though evidence for this is lacking. The essential point that telophase involves something other than and additional to such coiling or uncoiling as may occur seems however, clear from the facts for *Osmunda*.

‡ These results are in general agreement with those of Sax and Sax (1935) in *Tradescantia* and with the expressions of opinion by several other writers.

THE CHROMOSOME SPIRAL NOT A STATE OF STRAIN

One of the most important of the conclusions to be presented here is implicit in the last paragraph and will now be further substantiated. It is that spiral structure is not in itself the expression of a torsional strain in the chromosome thread, but is an equilibrium condition from which the thread has no tendency to depart except under special circumstances. In other words, a chromosome is by nature a contorted structure which will only become straight under compulsion and is not, as has often been supposed, a potentially straight thread, forcibly twisted and constantly trying to recover from that twist.

This conclusion is so fundamental and so much at variance with what has generally been tacitly assumed by most cytologists,* including myself until quite recently, that the quotation of further direct evidence is desirable.

A clear piece of direct evidence is presented by the normal behaviour of separating chromosomes at the anaphase of the first meiotic division. The assumption of a V shape by the diverging chromatids has already been commented upon (see p. 183) and stages can be seen in figs. 41–44, Plate 17. Fig. 72, Plate 19, on the other hand, shows early anaphase when treated for spiral structure. Every chromosome in this cell is of interest, and another focal level is given, at a lower magnification, in fig. 72*a*. If now the chromosome on the extreme left of fig. 72 is examined, it will be seen that traces of the four coils are still distinctly present in each arm of the V, but they have been somewhat disarranged by the mechanical stresses of movement and separation. The chromosome marked with a short arrow in fig. 72*a* shows the same thing at an earlier stage, the straightening being even more complete. This disarrangement is, however, purely temporary, for by the time the poles are reached four compact coils are again displayed in each arm (fig. 75, Plate 19). This type of behaviour was first observed by Sax in *Lilium* (1930), and in all cases its interpretation is the same. It indicates that the meiotic spiral is an equilibrium position of the chromosome thread to which it will spontaneously return even if forcibly straightened.

Direct evidence of the converse of this is provided by lost chromosomes. In both polyploids, but especially in triploids, chromosomes both paired and unpaired may be lost at either meiotic division through delaying too long on the spindle. Such lost chromosomes do *not* uncoil though they may remain undecomposed in the cytoplasm till the tetrad stage, when they fragment. An example of a chromosome lost at the

* It is interesting in this connexion to compare the various views which have been advanced to account for the spiral configuration. They include: rotation of the spindle attachment; very rapid elongation (Newton 1925), elongation of the chromonema within an inextensible pellicle (Bělař 1928), shrinkage of a "matrix" without change of length of the chromatic thread (Kuwada 1926). All these suggestions presuppose some form of torsional stress as a cause of the spiral and regard the unstressed condition of the thread as straight. This objection cannot so easily be raised against the rather tentative molecular suggestions of Darlington (1935) and Naithani (1937), but neither of these has any experimental evidence to clarify it. The only serious attempt at experimental work on this very obscure subject appears to be in the most recent publications of Kuwada which cannot yet be fully quoted.

second division has already been quoted as the best evidence obtainable for the number of coils at that stage (fig. 70, Plate 19). Chromosomes lost at the first division are readily distinguished by their large coils which are visible even in aceto-carmine (fig. 60, Plate 18). In neither case has straightening occurred.

Finally, the evidence already given on the constancy of the coiling at all stages of mitosis may be amplified by observation of interkinesis. This is shown in fig. 20, Plate 16, figs. 55, 56, Plate 18, and fig. 77, Plate 19. In fig. 55 the V-shaped chromosomes can be seen at the beginning of the optically diffuse stage, while figs. 77 and 56 are successive prophases of the second division. Fig. 77 is particularly striking. It is from an ordinary section (the same which was used for Plates 16 and 17), but the coils displayed agree exactly with those brought out by the ammonia treatment at the preceding anaphase. The exceptional clarity of the spirals at the prophase of the second meiotic division is a well-known fact, and they were several times observed at this stage by writers (e.g. Newton 1925) before spiral structure was recognized as a general phenomenon. The resting stage of interkinesis is therefore an episode which has extraordinarily little effect on the real configuration of the chromosome thread, in spite of great changes in its optical properties.

All this evidence confirms completely the previous conclusion. Spiralization, in meiosis as in mitosis, cannot be regarded as an innovation at any stage since it is characteristic of all, with the possible exception of early meiotic prophase, details of which will be given later. The chromosome, like the spiral staircase, must therefore be a constructionally coiled object, and the immediate question which arises is not so much why this should be so, an inscrutable problem at present, but why the mitotic prophase chromosome untwists the old spiral in forming the new. It is the *un*twisting here which is the exceptional phenomenon, and the only probable explanation of this would appear to be a change in internal symmetry in each chromatid, since operation of external forces seems excluded. Further speculation on these lines is at the moment unnecessary, however, for it is sufficient to have shown, in *Osmunda*, that, except at prophase, the spiral configuration of the chromosome thread, both at mitosis and at meiosis, is not fundamentally altered through metaphase, anaphase, telophase and rest.

Note on the geometry of chromatid separation at meiosis

A very marked difference in the manner of separation of chromatids (half-chromosomes) after splitting has already been referred to in describing mitosis and meiosis. Analysis of this difference is not an essential part of the present argument, but it may perhaps avoid misconstruction if the matter is briefly referred to.*

* The reader is again referred to Kuwada as the pioneer in working out the geometry of these relations. Since the above was written, however, a very excellent summary of the position has been given by Geitler in his book on chromosome structure (1938). The position will be abundantly clear if Geitler's figs. 27 and 38 are compared with his fig. 32.

It has been shown that an essential part of a mitotic prophase is the mutual unwinding of the split halves of the original spiral chromosome. At meiosis, on the other hand, though unwinding of "relational coiling" between the paired chromosomes certainly occurs at strepsitene (see p. 193 below), this is not immediately followed by separation of chromatids, for only one large spiral (not two parallel ones) is manifested in each chromosome before the onset of anaphase. At anaphase, however, as has already been shown (figs. 42, 43, Plate 17), the two chromatids in each chromosome come apart laterally without any further unwinding and without disturbance of the spiral. The momentary straightening described in a previous paragraph (p. 188) is due to the pull of the moving spindle attachments on the chiasmata as they unloosen, it has nothing to do with the lateral separation of the chromatids (comparison of metaphase and anaphase in an unpaired chromosome is instructive in this connection, cf. figs. 68, 73, Plate 19).

This manner of separation which is characteristic of meiosis may, at first, seem a contradiction in terms, but it will be made clear by another very simple experiment.* If two pieces of wire are tied together at one end to represent the spindle attachment constriction and wound upon a stick in such a way that the free ends are held together firmly and not allowed to rotate, a non-torsional spiral will result (cf. p. 185), which will behave exactly like the meiotic anaphase chromosome. When the stick is removed the two wires will at first share a common spiral, but their coils do not interlock at any point and they can separate laterally without any impediment. Two spirals, each with the amplitude of the original one, will then diverge from the tied end.

It seems highly probable that the manner of separation of chromatids at meiosis is an indication that the meiotic spiral is of a different geometrical type from that exemplified in mitosis. Since both are constructional it seems probable that chromosome structure after pachytene must in some way be different from chromosome structure at mitosis. The words "after pachytene" are used intentionally rather than "after pairing", for these geometrical considerations of the spiral apply to all chromosomes at the first meiotic division, whether paired or unpaired (e.g. as in figs. 68, 73 from the triploid). The physical basis of this difference cannot at the moment be fully specified, but one fact regarding it will be described on p. 199.

The early meiotic prophases

The way is now clear for examination of the early meiotic prophases. The general appearances of these are shown in figs. 10 *et seq.*, Plate 16, and the details in figs. 29–45, Plate 17. The technique is the same as for the preceding stages of mitosis, all of which are from one pinna. All the early meiotic figures up to zygotene (i.e. figs. 10–14) are from another pinna of the same frond fixed two days later. The remaining stages,

* An inexperienced reader is strongly advised to perform this experiment or alternatively to refer to Geitler's figs. 27, 38 and 32 (1938) previously quoted.

from pachytene to the tetrad (figs. 15–22), are, with one exception (fig. 21), from a third pinna. The greatest possible uniformity of treatment is thus assured.

"Rest", after completion of the premeiotic division, is shown in fig. 10. It is a state in which staining is very faint, and only the suggestion of chromosome threads can be made out here and there (fig. 29). The nucleus is definitely of the "solid" type with several nucleoli embedded in the mass of transformed chromosomes. Prophase is heralded by the appearance, in this mass, of sharply defined, closely coiled threads (fig. 30, Plate 17). At first the outline of the whole nucleus is unchanged, being often either oval or pear-shaped according to the space available in the cell (fig. 11, Plate 16). It soon becomes spherical (fig. 12) as at mitosis. This change of shape is not, however, accompanied by an apparent shortening and thickening of the chromosomes but by the exact opposite. The coils enlarge and spread out (fig. 31, Plate 17) and are finally effaced (fig. 32) as the thread elongates. Whether the thread also untwists as it elongates cannot be determined by observation at this stage, but subsequent behaviour, e.g. the presence of relational coiling at pachytene, suggests that it may not.

So little attention has been paid to these early stages in recent years that it is not easy to decide which of them should be covered by the name "leptotene". For the present it is perhaps clearest if leptotene is confined to the last only, i.e. the stage, just before pairing begins, at which elongation and straightening of the smaller convolutions is complete. This stage is shown in fig. 13, Plate 16, and fig. 32, Plate 17, the previous figures (figs. 11, 30; 12, 31) can then be described as early and late preleptotene.* At leptotene, "chromomeres" are visible in sectioned material (fig. 32) and free liquid has begun to appear in the nuclear area. It is an open question how much the gross morphology of the nucleus is an artefact at this and the succeeding stages. The "synaptic knot" of the older writers has often been waived aside as "artificial shrinkage", although the observations of Ethel Sargant (1897) on the living cells of *Lilium* still seem to be valid evidence to the contrary.† Be that as it may, two observations on the condition in *Osmunda* cannot be illusory. One is the very considerable increase in the total volume of nuclear sap, which began at late preleptotene (fig. 12) and continues until zygotene (fig. 14), as was accurately described by Lawson (1911). The other is that at leptotene, unlike preleptotene, mutual displacement of parts of chromosomes certainly occurs. The fact that the chromosomes not only open out their loops but slip over one another in doing so is clearly reflected in the behaviour of the nucleoli. These tend to be pushed out of place; they often come in contact with each other and coalesce, so that whereas at

* The need for separating leptotene from preleptotene for descriptive purposes was also felt by Janssens (1924).

† It is doubtful whether the situation regarding this much-debated appearance is materially different now from what it was in 1910 when Grégoire made a very just and able résumé of the evidence. His view was that the contraction is certainly often enhanced by reagents and may sometimes, especially in animals, be absent. In other cases he was satisfied that it was not entirely artificial for it could be observed equally in reagents which swell or contract chromatin and in living cells.

rest and preleptotene several nucleoli are generally visible, after leptotene there is usually only one. Further displacement may cause the single nucleolus to be pushed to the surface of the chromosome mass or even out into the liquid. The latter is particularly common at zygotene. It may perhaps profitably be recalled that in the early prophase of mitosis the chromosomes seem to have been unable to push past one another since the expanded thread is distorted into a large zigzag (fig. 24). This difference between the two states must have some physical explanation, but it is at present unexplored.

Leptotene is followed by zygotene, the stage at which pairing is taking place. As shown in fig. 14, Plate 16, the nuclear sap is still more abundant and the mass of chromosomes looser. Paired and unpaired threads can both be detected, often side by side (fig. 33, Plate 17), and the paired pieces always give the suggestion of being coiled together. I am unable to determine at what part of the thread pairing begins, but it is always incomplete since unpaired regions can still be detected at all stages of pachytene. These unpaired regions (text-figs. 3, 7; fig. 35, Plate 17) may be either near one end of a chromosome or in the middle of it.

The general views of early and late pachytene appear in figs. 15, 16, Plate 16. It is perhaps unfortunate that the change from one day's fixing to another should be at this point for the comparability of the nuclear sizes is open to suspicion. As shown in fig. 15, there is a definite suggestion of a temporary diminution of size associated with the disappearance of free liquid outside the chromosome mass. This diminution is followed by a further increase* as the paired chromosomes become evenly spread through the nucleus (fig. 16) and the nuclear size is then constant till the end of diakinesis.

Detailed views of early and late pachytene appear in figs. 34, 35, Plate 17. At early pachytene (fig. 34) the texture is very different from that of zygotene (fig. 33), and it is probable that all movements of pairing have ceased. This is certainly the case at late pachytene (fig. 35). The dividing line between the end of pachytene and the beginning of the following stage of strepsitene is arbitrary, and it is possible that this last figure is really early strepsitene. The clear distinction between paired and unpaired regions and the presence of a mutual coiling in the paired regions are, however, in themselves characteristic of late pachytene. This mutual coiling† in the paired regions is shown more clearly in another nucleus from the same sporangium in fig. 36.

Strepsitene is the stage at which pachytene coiling unwinds. The name "strepsitène"‡

* That these size relations are nevertheless real is suggested by comparison of published figures of other organisms. Thus Janssens (1924) on *Stenobothrus* shows exactly the same thing in his figs. 223, 226, 232, 233.

† Very little will be said about the coiling of paired chromosomes round each other at zygotene and pachytene though mention of the matter in passing cannot be avoided. Detailed consideration would, however, encroach on the excluded subject of chiasmata.

‡ The name "strepsitène" was coined by Grégoire on the basis of the older term "strepsinema" which was introduced by Dixon (1900) in connexion with a view of chromosome pairing which has

was introduced by Grégoire (1907), primarily with reference to *Osmunda*, in which it is a very distinct stage preceding diplotene. For this material, therefore, it is useful to retain the term although it has sometimes been deleted by more recent authors. Good general views of it are shown in the aceto-carmine series of Plate 18, figs. 50*a*, *b* being two focal levels of one nucleus. A single chromosome from the sectioned series of Plate 17 appears in fig. 37. Characteristics of the stage are the incomplete effacement of the pachytene coiling and the diminished chromosome length. It is no longer possible to distinguish the limits of paired and unpaired regions with certainty, but chiasmata cannot yet be separated, optically, from twists. This difficulty ceases at diplotene (figs. 38, 38*a*, Plate 17; fig. 51, Plate 18). From diplotene to diakinesis (fig. 52, Plate 18; fig. 39, Plate 17; and fig. 18, Plate 16) appearances are too well known to need description.

In sectioned material the earliest stage at which a whole chromosome can be traced throughout its length is pachytene. Free ends can be detected even as far back as preleptotene, but the mass is then too closely tangled for any one thread to be followed very far. At early pachytene (fig. 34) this can just be done, and at late pachytene (fig. 35) it is comparatively easy owing to the fact that the chromosome threads are not only better spaced but shorter. Now a very interesting fact emerges from a measurement of the chromosome of fig. 35. Allowing for the two ends being slightly out of focus the length is 36 mm. (see Appendix IV). But this figure is of the same order as the values previously obtained for the length of a somatic chromosome (the measured prophase specimen came to 40 mm.). This means that in meiosis the chromosome at pachytene, after the movements of pairing have ended, is roughly comparable in length to the somatic chromosome at its fullest extension.

It is, however, important to notice that in comparison with earlier stages of meiosis the chromosome at late pachytene is *not* at its fullest extension. If whole chromosomes at early and at late pachytene are compared the former are invariably the longer. This is at once apparent if the outlines of the photographed specimens are drawn side by side (see text-figs. 3, 4, p. 204). Allowing the smallest amount possible for foreshortening, the length of the early pachytene specimen at the previous magnification of $\times 2000$ comes to 57 mm.

Very valuable additional information is supplied by the aceto-carmine series of Plate 18. Aceto-carmine does several things which are peculiar to it. It enlarges the chromosomes at every stage, a feature which will be discussed later, but it can also be used to flatten out the whole contents of a cell on to the surface of the slide or cover-slip.* This makes it possible to see in one focal field every chromosome in a com-

since been found to be quite untenable. It is possibly for this reason that the strepsitène stage has sometimes been merged with diplotene.

* This property of acetic acid was first used by Belling (1928) and later, in a most spectacular manner, by McClintock (1930, 1931). The method of obtaining it here is not quite the same but the foundation of the technique is McClintock (1929; see Manton 1937).

plete cell at late stages such as diakinesis, metaphase or anaphase (figs. 52–54). At other stages the same process enables one to observe single chromosomes in a way which no other technique can equal. An example of the effect at early pachytene is shown in fig. 49. A pair of chromosomes showing incomplete lateral pairing has become separated from the rest and lies flat upon the slide free from foreshortening. Another, more important, example is contained in fig. 48. This represents leptotene in a triploid with one complete chromosome extruded in the loosened mass to the right. This specimen is of such exceptional interest, being so far unique not only among my own slides but so far as I am aware in the whole of the botanical literature, that it is shown again at a higher magnification in fig. 61 *a*, *b* with the pachytene chromosome on the same scale in fig. 62. The best possible optical apparatus has been used for these last photographs, the lens being a Zeiss apochromat. 1·4 as opposed to the Zeiss apochromat. 1·3 previously used. The depth of focus is thereby diminished, hence the need for two exposures to contain all parts of the chromosome thread. The increased resolving power has not revealed any further interpretable details of structure, but as a demonstration of length these photographs are very striking. The pachytene chromosome at this magnification measures 102 mm., while the leptotene specimen measures 155 mm. This is a ratio of approximately 100 : 150.*

Whilst all allowance must be made for the undesirability of using a unique specimen as evidence, it is still difficult to resist the conclusion that the testimony of these photographs is trustworthy. All obvious objections have been examined carefully and will be considered in the discussion, but no facts have so far been found which could be interpreted as incompatible with the above results. Whilst recognizing that further confirmation is desirable the measurements will therefore be provisionally accepted.

But this result, if true, leads to some very important conclusions. It has already been shown that a somatic chromosome at its fullest extension is equivalent in length to a chromosome at pachytene. It would now appear that as a preliminary to pairing the meiotic chromosome, during preleptotene, not only stretches out the 14 coils retained from the previous division but also experiences a genuine increase in length of the order of 50%. It therefore follows, of necessity, that the act of chromosome pairing itself, the central fact of meiosis, must be carried out when the chromosomes are in quite an exceptional state. The length attained as a preliminary to pairing has no counterpart at any point in the mitotic cycle, and this statement may well express a very fundamental, if not the most fundamental, difference between the prophases of mitosis and meiosis.

In this connexion it is perhaps appropriate to notice two sentences written by Grégoire in 1907 (p. 391): "La différence entre la prophase somatique et celle-ci, c'est que, dans la première, les filaments à peine formés, subissent tout de suite une condensation rapide qui les transforme en rubans chromosomiques, tandis que, dans

* Only the *relative* dimensions are significant here. Absolute sizes between aceto-carmine preparations and others are not directly comparable.

la prophase hétérotypique, les filaments continuent à s'étirer et deviennent très longs et minces. Cette formation de filaments longs et minces (noyaux leptotènes) constitue la vraie caractéristique du début de la prophase hétérotypique." In the light of the facts presented above it would appear probable that this expression of opinion as to the essential difference between the early prophases of meiosis and mitosis is as authoritative as anything which has been written on this subject in the last thirty years.* Before attempting to interpret it further it is, however, necessary to examine another type of physical evidence, namely, the manifestations of chromosome elasticity.

Chromosome elasticity as evidence of submicroscopic convolutions

Chromosome elasticity can be observed in two ways, firstly, by actual pulling on the chromosomes by mechanical interference with the cell, and secondly, by observation of chromosome behaviour as they pull on each other. Some evidence from both these sources is available in *Osmunda*.

Evidence from direct interference can be obtained incidentally while making smears for spiral structure if, as often happens, a cell is damaged by the tool and its contents strewn upon the slide. If chromosomes are present they are not necessarily injured by this, and some of the clearest detail has been obtained from such isolated individuals (e.g. figs. 65–67, Plate 19). Sometimes, however, a chromosome may be so caught that it is actually in contact with the moving tool and then it will be rather violently distorted. An example of this is seen on the left of the general view of the second meiotic division (fig. 69, Plate 19), and fig. 78 shows the same effect in the tapetum. This figure is of a portion of the same tapetal cell which gave the number of coils in a somatic chromosome (fig. 71), though it is at a lower magnification (i.e. $\times 1500$). Some undamaged chromosomes are visible at the bottom, but those above are injured, and one in particular has been enormously elongated by stretching. This chromosome has not yet broken although, in the photograph, a piece of the middle is out of focus. Some of this elongation is accounted for by the forcible opening out of the 14 coils known to be there; the length that this would produce is indicated by ink lines. The additional extension, which is considerable (i.e. at least 115%, see Appendix II), must clearly have resulted from a deformation of some other type. The nature of this deformation cannot be directly observed, since it is certainly below the limit of visibility, but two alternatives suggest themselves. Molecular slipping may have occurred, in which case a chromosome so treated will have been rather fundamentally

* Recent cytological literature on this subject has shown so much preoccupation with chiasmata and chromosome splitting that this simple consideration of chromosome length has been largely lost sight of. "The chromosomes continue to lengthen during pachytene, reaching their maximum at the end of this stage when they divide" is a typical modern quotation (Darlington 1935, p. 89) yet no significant basis of new facts seems to underlie such a change of view. For further consideration of this see pp. 205–6 below.

disorganized internally, or (and the magnitude of the extension makes this inherently probable from the start) the stretching has opened out some inner convolution with elastic properties of the same order as those of the visible spiral. That this is the case appears to be demonstrated by the observable reactions of chromosomes when deformed by each other.

Mention has already been made (p. 188) of the elastic recovery shown at the first meiotic anaphase, of the coiled chromatids when momentarily distorted by the mechanical stresses of movement. This behaviour has been observed by Iwata (1935) and Sax (1930) in *Lilium*, and in all cases is indicative of the stability of the visible spiral. Recovery from this deformation is rapid and is generally completed during transit to the poles. If, however, for any reason, the separation of chromosomes at anaphase is impeded, other, and very characteristic, appearances ensue. Even in a diploid it often happens that an interstitial chiasma may unloosen with difficulty. This difficulty generally affects one pair of chromatids more than the other, and a very clear case is shown in fig. 44, Plate 17. The centres of figs. 72 and 72*a*, Plate 19, show parts of a similar chromosome when treated for spiral structure. In fig. 72*a* the chromatids marked with a long arrow have disjoined without difficulty, but the chromatids in the centre of fig. 72, which are the other halves of the same individuals, have not dissociated so easily from a chiasma placed near the spindle attachment. As a consequence, in these chromatids, that part of the thread lying between the chiasma and the moving spindle attachment, a distance of about one coil of the spiral, has been not only straightened but stretched. The extent of the stretching in this particular case can be at once apprehended by comparison with another chromosome in the same nucleus. Thus, in fig. 72, the stretched portion of the lower central chromatid measures about 12 mm., whilst the corresponding distance on the V-shaped chromosome to the left is only about 7 mm. A stretching of over 70% is thus demonstrated. The after-effects of such stretching are well seen on the left of fig. 54, Plate 18, in which a neck-like thin place is clearly visible in each affected chromatid. This appearance becomes exaggerated in polyploids, presumably owing to the enhanced difficulty of disjunction in multivalent groups. A very clear single chromosome from a triploid treated for spiral structure is given in fig. 76, Plate 19, and an extreme case of stretching appears in the tetraploid cell of fig. 79, Plate 19. Assuming that in this, as in the others, the affected regions represent about one coil, a stretching of the order of 200% is shown by this specimen (see Appendix II).

The fact that "necks" are still conspicuous objects at late anaphase, indicates that recovery from stretching is a slower and more difficult process than recovery from straightening. Recovery has certainly occurred in fig. 76 (see Appendix II), but it can never be completely followed owing to the onset of the diffuse stage of interkinesis. In a normal plant, however, no trace of the disturbance can be detected in the prophase of the next division, and though a case as extreme as that of fig. 79 may perhaps express permanent damage to the cell, such damage must be quite exceptional for reproduction

in the tetraploid is not impeded. Now recovery would not be expected to occur at all if the basis of mechanical extension of this order were molecular slipping. The only possible alternative would then seem to be that a *submicroscopic convolution* must exist, both in meiotic and probably also in mitotic chromosomes. The opening out of this would then explain the elastic behaviour.*

Interpretation of leptotene in terms of the known structure of organic fibres

The existence of a submicroscopic convolution in the chromosome thread, in addition to the visible spiral, at once appears to provide a clue to the true nature of leptotene. It has been shown that all the apparent changes of length in the *mitotic* cycle are probably explicable in terms of the visible spiral. In *meiosis*, on the other hand, it has been shown that a genuine size difference exists, and therefore the preleptotene changes must be of a different nature from those occurring in a somatic prophase. It would now appear likely that the essential feature of preleptotene is the opening out of the submicroscopic convolution.

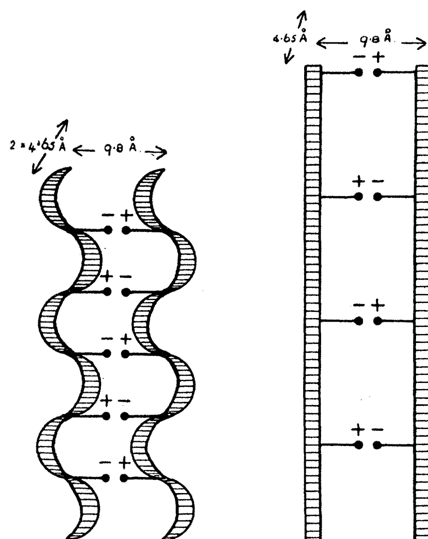
This is as far as the direct evidence in *Osmunda* can be taken, but analogy has so often been invoked to explain cytological phenomena, that it may perhaps be permitted here also, since a certain body of evidence has at least been assembled to indicate that the analogy chosen is the right one. There is no direct knowledge as to the size, or the nature, of the submicroscopic convolution in the chromosome thread, but the elastic behaviour shows many features in common with that of a number of other organic fibres of which the molecular structure is known. A number of these, e.g. wool and india-rubber, owe their elasticity to the possession of elongated molecules which, in a state of rest, are convoluted. Now if it is assumed that the chromosome molecules may be of the same general type, then a most instructive comparison is possible between certain cytological manifestations and the known behaviour of keratin, to take the most familiar protein.

The mechanism of molecular contortion visualized by Astbury† as a result of X-ray analysis of keratin and other substances is graphically indicated in the diagram reproduced here as text-fig. 1. Convolution of the molecular thread is represented as due to the mutual attraction, either electrical or chemical, of side-chains known to project

* It is important to notice that the conception of a submicroscopic convolution is introduced here to account for the facts of chromosome elasticity. It should *not* be thought of as an explanation of spiral structure, the physical basis of which is quite unknown.

† It should perhaps be pointed out that Astbury's work has already entered cytology in a paper by Naithani (1937) referred to in the footnote to p. 188 above. Naithani's suggestion was that a molecular change of the β-α keratin type might underlie the post-pachytene chromosome *shortening* recognized by Belling and Sax, but gave no facts of any kind to support it. In the light of what has been shown for *Osmunda* this suggestion would seem to be highly improbable and the post-pachytene shortening in *Osmunda* will be dealt with here under the heading of supercontraction.

out from the bodies of the long molecules.* This attraction is suggested on the diagram by the addition of + and − signs to the side-chains, though it is realized that such signs are primarily symbolic and do not imply precise knowledge of the physical basis of attraction. Now it is clear that, if such a convoluted molecule is straightened by mechanical or other means, the spacial proximity of the mutually attracted side-chains will be disturbed, and the chains themselves will project out from the straightened molecule with their attractive potencies incompletely satisfied. It seems highly probable that in this condition it would be possible for these attractive potencies to react externally to the molecule, if contact could be established with another molecular chain having the necessary order and arrangement of side-chains. Such external contacts would, however, not necessarily be permanent, for elastic recovery, if it



TEXT-FIG. 1. Reproduced (by kind permission of Dr Astbury and the Oxford Press) from Astbury (1933, fig. 62): "Illustrating part of the mechanism of contraction in the keratin chains. The positively charged centres in the basic side-chains approach as closely as possible to the negatively charged centres in the acidic side-chains."

occurred, would restore the original intramolecular equilibrium.* The applicability of this conception to the inscrutable mystery of chromosome pairing is very striking. By analogy with keratin the interpretation of chromosome structure would be that the physical basis of "homology" is the order of arrangement of mutually attractive

* It should perhaps be explained that in the case of the fibrous proteins for which the diagram was originally made, the units of construction are not single molecules but sheets of laterally connected polypeptide chains forming "polypeptide grids". An assemblage of polypeptide grids constitutes a micelle. Mechanical stretching of a grid will not disrupt all the side-chain connexions but the internal saturation of the structure will be disturbed and some attractive potencies will be unsatisfied. For a chromosome it seems unnecessary at the moment to use the full descriptive terms applicable to grids since nothing is actually known about the molecular construction. The expression of the above analogy in terms of single molecules is therefore perhaps a justifiable simplification.

potencies on a thread. That normally these potencies are satisfied internally to the chromosome in the production of the submicroscopic convolution. When this normal equilibrium is interrupted, presumably by chemical means, at leptotene, external reaction results in chromosome pairing (at zygotene). When elastic recovery has restored the original equilibrium (at pachytene) pairing ceases.

The phenomenon of supercontraction

Two further pieces of indirect evidence may be quoted in support of the interpretation of meiosis outlined in the previous section. The first is the well-known fact that the most striking other case of a chromosome in a state of exceptional elongation, that of the salivary glands of the Diptera, also shows chromosome pairing in an extreme degree. No explanation, either biological or physical, can generally be advanced for this behaviour in such a tissue. If, however, chromosome elongation and chromosome pairing are causally connected the matter becomes intelligible if not explained.

The second, perhaps more cogent, piece of indirect evidence is in the phenomenon of "supercontraction". This is exemplified by wool and other fibres as a normal after-effect of extreme stretching. If wool, for instance, is held under tension in steam for not more than two minutes certain intramolecular linkages are disrupted and 100% elongation of the visible thread may result. If, however, the thread is retained either in steam or cold alkalies (under conditions in which permanent recombinations are prevented) when the tension is released, elastic recovery will more than compensate for the stretching. The final length in each of the three substances, keratin, myosin and collagen, is only about one-quarter of the fully extended length. In the case of keratin and myosin this is equal to half the original length of the thread before the experiment began.*

Now it is either a most extraordinary coincidence or a startling demonstration of the correctness of the analogy of keratin with the chromosome that the chromosome in recovering from the exceptional state of leptotene also shows supercontraction. It has already been shown that the normal length characteristic of the chromosome before meiosis is re-established when pairing ends at pachytene, but this is *not* the definitive length of the meiotic chromosome. If the length at metaphase or anaphase of either of the meiotic divisions is calculated from the apparent dimensions and the known number of coils, the result is shorter than the calculated somatic length by an amount which varies from 33 to 50% according to the technique and stage employed. Details are worked out in Appendix III and discussed on p. 203 below. In all cases the calculated length of the finished meiotic chromosome is roughly equivalent to the

* I am indebted to a personal communication from Dr Astbury for these figures which are also to be found in his published works. The actual amount of supercontraction in any particular case depends to some extent on the treatment, though the maximum possible is a constant and the same for all three proteins. Collagen differs from the other two in being normally in the β or fully extended state.

visible length of the chromosome at strepsitene* (see Tables I and II, pp. 203, 205). When it is realized that there are reasons for believing that the highest value obtained (50 %, cf. p. 203) is the most reliable and that the maximum contraction of keratin and myosin is also 50 % of the normal (or α) length, the coincidence becomes very impressive. It is, in fact, so striking that it appears to be as near to proof of the correctness of the analogy made as can be expected to arise in the absence of direct molecular analysis.

Discussion

The principal conclusions which have emerged from this necessarily somewhat intricate mass of reasoning and data are the following. In the first place, it has been shown that a chromosome is fundamentally a contorted structure, which will neither become straight nor untwist except under special circumstances. Secondly, it has been shown that contortions of two orders of size are involved, the one a visible spiral of microscopic dimensions, the other a submicroscopic convolution,† possibly of molecular dimensions, since it admits of a very fruitful analogy with keratin. These are the essential conclusions, but on the assumption that a real analogy exists between the submicroscopic structure of the chromosome and the molecular structure of other organic fibres of which keratin is the best known, it is possible to provide a reasoned seriation of three otherwise disconnected cytological facts, namely, (1) that the chromosome in the prophase of meiosis differs from that at mitosis by its length, (2) that extreme elongation is associated with chromosome pairing wherever it occurs, i.e. in cells as different as the spore mother cells of a fern and the salivary gland cells of a fly, and (3) that extreme elongation is followed by supercontraction, in a definite sequence of cause and effect.

This by no means exhausts either the information which could be extracted or the range of problems which might be discussed on the basis of the photographs presented. It seems, however, suitable to close the argument at this point in order to examine as critically as possible the validity of the facts which have been used. This is particularly necessary in that some rather fundamental issues have been raised.

The conclusions rest primarily on the dovetailing together of three main topics, (1) determination of the numerical relations of the coils visible in chromosomes at different divisions, (2) observations on long-range elasticity, (3) determination of the actual lengths of the chromosome thread, whether spiral or otherwise, at a number

* The contraction of chromosomes after pachytene has been more effectively studied than has the extension before pachytene and these results agree in the main with the conclusions of Belling (1928) in *Lilium*, etc., and of Sax and Sax (1935) in *Tradescantia*, etc., to name only two papers dealing with this subject.

† It is essential here for the reader to bear in mind the caution as to nomenclature given on p. 181. "Convolution" and "spiral" are not interchangeable words in the above context and great confusion will result if the submicroscopic convolution is thought of as a "molecular spiral".

of significant stages. Each of these topics is a body of evidence of some importance even if the links between them have been misinterpreted. Each type of evidence, however, has not only inherent observational difficulties, but all are to some extent at the mercy of technical processes requiring considerable delicacy of manipulation. An element of uncertainty far in excess of what would be tolerated in a purely physical enquiry is thus inevitable, and only the broadest outlines can be expected to be discernible. The strength and weakness of the evidence on which these broad outlines rest will now be discussed for each topic in turn.

(1) In dealing with the number of coils at different divisions no doubt attaches to the main demonstration that the chromosome is coiled at all stages of mitosis and at all stages of both divisions of meiosis with the possible exception of early prophase (i.e. leptotene).

Further, no doubt attaches to the general demonstration that in *Osmunda* the chromosomes at different divisions have characteristic but different numbers of coils. The actual numbers 4 and 8 for the first and second meiotic divisions are well established, only the details of the somatic number are in slight uncertainty. That 14 is in the right region is, however, confirmed by some very simple calculations. If the lengths of chromosomes at anaphase in the tapetum and after the second meiotic division are compared (figs. 47, 45, Plate 17) they are found to be as 9 : 6. Since the shorter is known to have 8 coils the longer would appear to have 12 coils. Repeating the comparison with the archesporial anaphase chromosome (fig. 26) the ratio of lengths is as 11·5 : 6, suggesting 16 as the number of coils. Since only the lengths are taken into account in these comparisons it has to be assumed that the pitch of coil is a constant. This cannot be strictly true, and therefore these results are not fully significant. They do, however, provide a welcome confirmation of the validity of the number 14 determined from the more direct evidence of the ammonia treatment.

A third statement which admits of no doubt in the present material is that uncoiling occurs only at prophase both at mitosis and at meiosis, and in neither case is it a feature of telophase. This is contrary to what has sometimes been thought in other organisms.

(2) With regard to elasticity, the observations are beyond dispute, only their interpretation is open to discussion. Long-range elasticity of over 70% has been clearly demonstrated at the first meiotic division. By using polyploid material in aceto-carmine an extreme extensibility of at least 200% is indicated. For a somatic chromosome and for a chromosome at the second meiotic division the sole evidence is that of mechanical stretching. Recovery here has not been observed. The extent of the stretching obtained (at least 115%) is, however, unlikely to be due to a grossly different mechanism such as molecular slipping.

It must be clearly recognized that interpretation of the elasticity data in terms of convoluted *molecules* is pure hypothesis. The validity of the hypothesis can only be tested by its results, but in considering these it is important to avoid confusion between the sub-

microscopic convolution referred to above and another conception to be found in the literature, namely, the "minor spiral". The minor spiral* has been described, notably in *Tradescantia* by Kuwada (1932 *et seq.*), as a second visible spiral detectable after very special treatment along the gyres of the "major spiral" at the first meiotic division. It is very near the limit of visibility, and it is an open question (cf. Kuwada 1938) whether, in spite of its name, it is a true spiral or merely a corrugation. It is thought by Kuwada and Nakamura (1935), on evidence of double diffraction, to be present at the first meiotic division only, and not, in *Tradescantia*, either at the second division or in the stamen hairs. Now it is clear that Kuwada's minor spiral cannot be equated with the submicroscopic convolution postulated in the present paper from evidence of a very different kind. In the first place, a molecular configuration of the keratin type is *not* a spiral, and it may have been noticed that the use of the word has been most carefully avoided in describing it. Secondly, the size relations involved in the two conceptions are very different. Even the smallest visible structure would be measured in fractions of a micron, whereas a molecular convolution would be measured in Angstroms (one fold of keratin is of the order of 5 A), a unit which is a thousand times smaller. Thirdly, the elasticity data have indicated the existence of a submicroscopic convolution in all chromosomes (except possibly at leptotene) and not merely in those of the first meiotic division. There is evidence,† however, which suggests that in *Tradescantia* unlike *Osmunda*, *supercontraction* may actually be confined to the first meiotic division. It would therefore seem probable that the appearances noted by Kuwada are really other manifestations of the supercontracted state. The two conceptions of "minor spiral" and "submicroscopic convolution" are thus not necessarily incompatible although in the present state of knowledge it is probably desirable to keep them rather severely apart.

(3) By far the most difficult part of the work is the estimation of chromosome lengths at the various stages. All methods of calculating the true dimensions of a spiral thread are hampered by two serious difficulties. At these magnifications, even with the best lenses and successful photography, the apparent edge of a chromosome is an indefinite one and not a sharp line; the diameter can therefore only be approximately measured. Even were this not so, it is obvious that the apparent diameter of the coil will be greater than the mathematical diameter by an amount equivalent to the thickness of the

* The name "minor spiral" has also been applied to a great many other appearances, even in leptotene chromosomes, by different writers. Full discussion of these other meanings does not seem necessary at the moment.

† Even in *Tradescantia* there is no one species which has been fully worked out, but Sax and Humphrey (1930) figure 4–5 coils for the first meiotic division in *T. reflexa* and 20–25 for anaphase of the second division. For *T. paludosa* Sax and Sax (1935) report 5–6 coils for the first meiotic division and 20–25 for the division in the pollen grain. This looks as though recovery from supercontraction must occur in *Tradescantia* before anaphase of the second division. In *Rhoeo discolor* the same author (Sax 1935) quotes 4–5 and 12 for the coils of the two meiotic divisions, a ratio which is comparable to that in *Osmunda* rather than to that of *Tradescantia.*

coiled thread. Allowance for this thickness cannot be made without assumptions as to the shape of the chromosome thread in cross-section, about which there is no knowledge at all. The mathematical diameter of the chromosome is thus unattainable except where the centre of the spiral thread is actually visible (i.e. with the spiral structure technique). The estimations from Plate 19 are thus likely to be the most reliable.* It is, however, reassuring to find that the results obtained from sections (see Table I) do not differ from these fundamentally though all are somewhat larger, due no doubt to this source of error.

TABLE I. SUMMARY OF NUMERICAL DATA FROM PLATES 17 AND 19 (SEE APPENDICES I AND IV). IN CONVERTING THE MEASUREMENTS FROM MILLIMETRES AT THE STATED MAGNIFICATIONS TO MICRONS THE RESULTS ARE GIVEN TO THE NEAREST HALF-MICRON

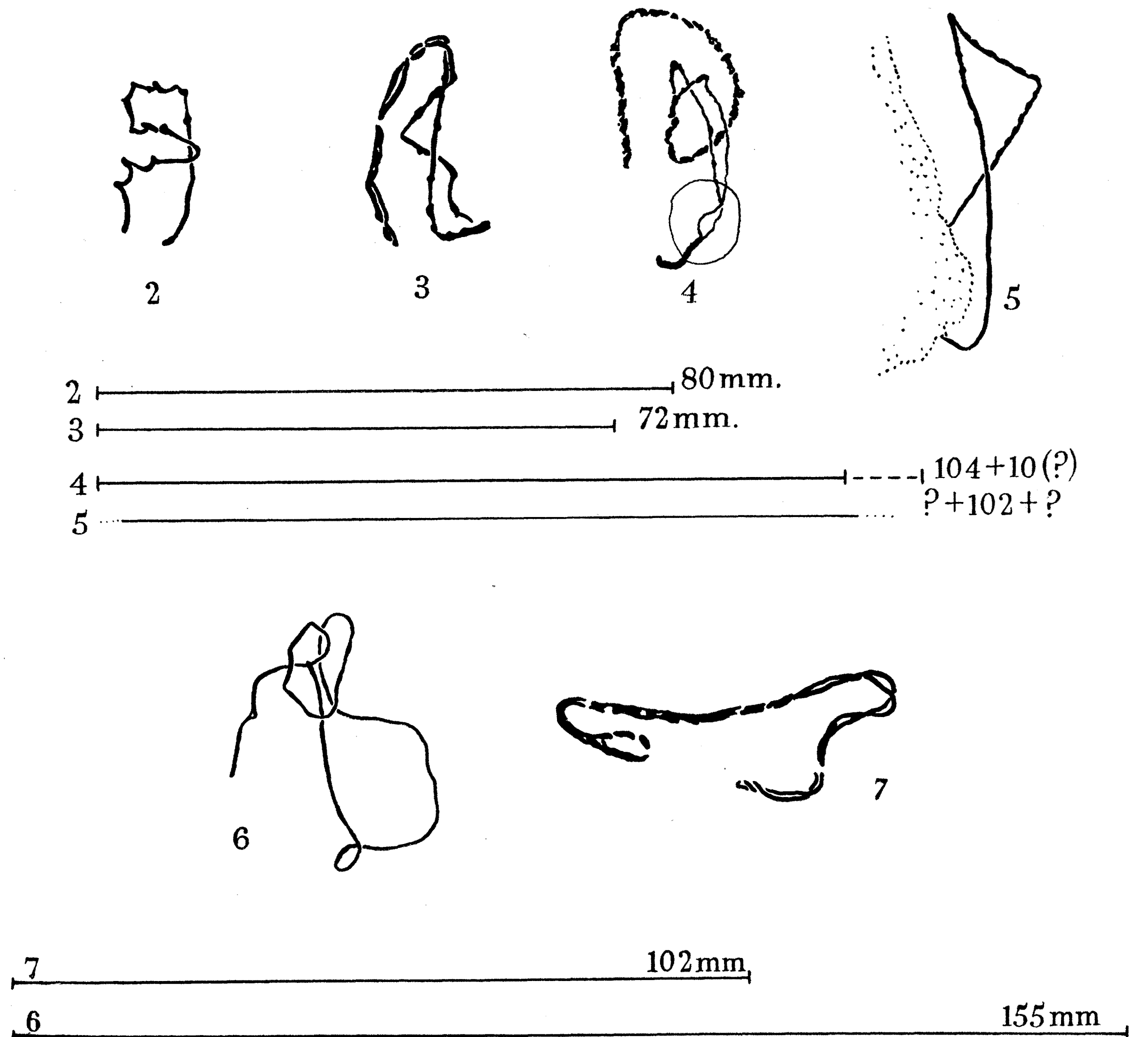
	Sections ×2000	Spirals ×3000	Sections in μ	Spirals in μ
Measured:				
Prophase	(fig. 24) 40 mm.	—	20	—
Pachytene (late)	(fig. 35) 36 mm.	—	18	—
Strepsitene	(fig. 37) 23 mm.	—	11·5	—
Calculated:				
Metaphase I	(fig. 41) 30·5 mm.	(fig. 64) 32·7 mm. (fig. 66) 31·0 mm. (fig. 68) 30·4 mm.	15	10·5
Anaphase I	(fig. 44) 21·8 mm.	(fig. 75) 24·6 mm. (fig. 73) 24·4 mm. (fig. 76) 24·6 mm.	11	8
Metaphase II	—	(fig. 69) 27·8 mm.	—	9
Anaphase II	(fig. 45) 30·7 mm.	(fig. 70) 26·7 mm.	15	—
Somatic metaphase	(fig. 46) 42·7 mm.	(fig. 71) 48·6 mm.	21	16
Somatic anaphase	(fig. 26) 41·1 mm. (fig. 47) 49·1 mm.	—	22·5	—

All these elements of uncertainty combine to prevent an *exact* determination of either the chromosome length itself or the extent of supercontraction. As shown in Appendix III, and as can be seen at a glance from Tables I and II, the estimates of supercontraction vary according to the technique and according to the stages used. Using the estimated somatic length in each case as a basis and comparing it with the length estimated for the first meiotic metaphase, the aceto-carmine series works out at 43 % contraction, the sectioned series as 33 % contraction, and the spiral structure series as 35 % contraction. If, however, the shortest length obtained, that of the first meiotic anaphase, is used, the estimate of contraction is higher. In the spiral structure series for which this stage has been most fully studied the estimate of contraction is then actually 50 %. The reasons for believing that this series is the most reliable for

* It must of course be remembered that the working of the ammonia treatment must certainly involve some changes in the dimensions of the chromosome thread. This must be supposed to shrink laterally, hence the visibility of the coils; what alteration, if any, occurs in its length is not clear. Greater reliability of the estimations is thus only a relative term applicable to relative lengths within the same technique. The actual sizes of the living chromosome are still uncertain.

relative sizes have already been given, and though the coincidence between this result and the known contractability of keratin and myosin must not be over-emphasized, the fact of chromosome shrinkage is abundantly clear.

With regard to the direct measurements, the general statement that chromosome length at an early somatic prophase is roughly equivalent to that at late pachytene



Text-figs. 2–7. Summary of the principal prophase measurements. Figs. 2–5 are camera lucida drawings × 4000.* Figs. 6 and 7 are merely diagrammatic representations of figs. 61 and 62, Plate 18. Fig. 2: early somatic prophase (cf. fig. 24, Plate 17, and fig. 80, Plate 19). Fig. 3: late pachytene (cf. fig. 35, Plate 17). Fig. 4: early pachytene (cf. fig. 34, Plate 17) with an uncertain allowance for foreshortening in the measurement. Fig. 5: the longest piece seen at zygotene, incomplete at both ends. Fig. 6: leptotene in aceto-carmine. Fig. 7: pachytene in aceto-carmine. *Note*: the actual sizes of the last two figures must not be directly compared with those preceding.

* A reduction of $\frac{1}{20}$ has been made in reproducing text-figs. 2–7. The measurements quoted refer to the unreduced drawings.

is not likely to be disputed, for it is based on measurements within the same technique. The leptotene elongation, on the other hand, is far more difficult to prove conclusively. The chief evidence for it has two serious disadvantages. The fact that only one chromosome has been measurable at leptotene has been felt so acutely that publication of this manuscript has been deferred for six months in the unfulfilled hope of a repetition. A further difficulty is in the technique used. Acetic acid has the peculiar property of practically doubling chromosome sizes at all stages. Is it not possible that the apparent extension at leptotene may really be an artefact? While this possibility cannot be denied, there is, nevertheless, no direct evidence to support it. In spite of the actual enlargement the general relations of the *relative* lengths at all other stages (see Table II) are still comparable with those obtained in the other techniques. It therefore seems unnecessary to assume that the effect will suddenly be different at leptotene.

TABLE II. SUMMARY OF NUMERICAL DATA FROM PLATE 18 (ACETO-CARMINE) EXPRESSED IN MILLIMETRES AT A MAGNIFICATION OF 1000. THESE ARE NUMERICALLY EQUIVALENT TO MICRONS IN THIS CASE, BUT MICRONS ARE NOT USED SINCE ONLY THE RELATIVE, NOT THE ACTUAL, SIZES ARE SIGNIFICANT

	mm.
Measured leptotene (fig. 48)	=73
Measured pachytene (fig. 49)	=48
Measured strepsitene (fig. 50*a*)	=21
Calculated metaphase I (fig. 53)	=25·6
Calculated anaphase I (fig. 54)	=18
Calculated anaphase I (fig. 79)	=18·4
Calculated anaphase II (fig. 58)	=25·5
Calculated somatic (fig. 63)	=45

The attempt to obtain confirmatory evidence without the use of acetic acid has not so far been fully successful owing to the impossibility with ordinary methods of observing whole chromosomes in this material at any stage earlier than early pachytene, and even here the effect of foreshortening is very difficult to allow for. The available evidence is, however, summarized in text-figs. 2–5, the measurements being indicated by ruled lines. With the smallest possible allowance for foreshortening (an allowance based on the diameter of the nucleolus) the length of the photographed specimen of early pachytene (fig. 34, Plate 17; text-fig. 4) is markedly greater than that of the measured early somatic prophase (text-fig. 2). And the same can be said of the longest piece of unpaired chromosome so far seen at zygotene (text-fig. 5), both ends of which are lost in the tangled mass. No room could be found in the plates for a photograph of this specimen, but this is no great loss since it is incomplete. The most that can be said about it is that it accords with the other evidence; it is not full confirmation.*

* The difference of 25% shown between text-figs. 5 and 2 (the measured somatic prophase) would of course be quite effective confirmation of the fact of elongation if it were clear that the measurements could be used at their face value. Caution in this is however necessary from the many sources of uncertainty regarding the exact somatic length.

While all emphasis is thus laid on the weakness of this part of the evidence it is nevertheless true that such facts as there are cannot easily be swept aside, for the single aceto-carmine specimen at leptotene (text-fig. 6) remains unrefuted. It is unfortunate that no comparable figures appear to exist for another plant. Leptotene measurements have, however, been made on one animal, *Dendrocoelum lacteum*, by Gelei (1922). With a particularly favourable object and by means of what appears to be most rare technical skill, Gelei succeeded in measuring every chromosome ($2n=14$) in two complete nuclei. Unfortunately, the comparable measurements for pachytene are not given and there is no knowledge of spiral structure in the premeiotic chromosomes. Even this work is therefore not readily usable for comparative purposes. If, however, it is assumed that "diplotän" is, as it appears to be, the equivalent of the plant pachytene, the general impression gained from Gelei's figures is not unlike that of *Osmunda*.

Even were the evidence from *Osmunda* above reproach it would, however, still be unsafe to attribute general significance to the conclusions as long as they refer to only one organism. For this reason it has seemed profitable to publish the facts as they stand in the hope that confirmation (or the reverse) may be obtainable by other workers on other material. Should this be forthcoming it will perhaps be possible to reply to the next question which has to be raised, namely, when exactly does the prophase elongation cease? Is it really completed before pairing begins or is the chromosome still lengthening during zygotene?

That such an apparently simple question should still be unanswered is symptomatic of our ignorance regarding these much studied processes. Far larger problems remain. Why, for instance, should a twisted shape be the unstressed state of the chromosome thread? Why does the equilibrium suddenly become altered during prophases so that untwisting occurs? What are the chemical influences necessary to produce the changes of length which seem to precede and to follow chromosome pairing? What is the physical basis of chromosome movement, which must certainly take place before even molecular forces of the type visualized could possibly affect behaviour? The only reply to all this is to confess how little we really know about the physics of the cell. It is therefore to the facts in this paper rather than to the conclusions that attention should be directed.

Acknowledgements

I am glad of this opportunity to express my appreciation of the immense debt which I, in common with every other worker in the Barker Cryptogamic Laboratory, owe to the personality of Professor Lang. On this occasion I am also indebted to Professor Hartree, F.R.S., and Mr Dearden, M.Sc., for advice and help with the calculations, to Dr Astbury and the Oxford Press for permission to reproduce text-fig. 1, and to Dr Astbury personally for information and advice.

Summary

I. From observations on the chromosomes of *Osmunda regalis* by three different technical methods (sections, aceto-carmine squashes and ammoniated smears), the following facts among others have been elucidated:

(1) The haploid complement is 22 chromosomes, closely similar in length and with terminal or subterminal (but not median) attachment constrictions.

(2) The number of coils made visible by the ammonia technique is 4 for the first meiotic division; 8, of smaller diameter, for the second meiotic division; and *ca.* 14 (i.e. 12–16) for a cell of the tapetum.

(3) The numbers of coils visible at early prophase of the archesporium and of the second meiotic division are in accord with the direct evidence from the ammonia method. In both divisions they indicate that uncoiling cannot have occurred at the previous telophase but is confined to prophase.

(4) Evidence is quoted to show that no torsional strain is associated with the spiral shape at any stage other than prophase. The spiral configuration is an equilibrium position of the chromosome thread to which it will spontaneously return if forcibly straightened. In some respects the simile of a spiral staircase is helpful, but certain geometrical differences between the spirals of mitotic and meiotic chromosomes are analysed.

(5) Observations on the stretching of a tapetal chromosome and on the stretching and recovery of the separating chromatids at the first meiotic division indicate that both chromosomes possess a "long-range" elasticity beyond what would be expected from their spiral configuration alone. This is thought to indicate the existence of a submicroscopic convolution in the chromosome thread. It is suggested as a pure hypothesis that this convolution may be of molecular dimensions.

(6) Direct measurement of chromosome length at an early somatic prophase agrees closely with the calculated length of the chromosomes at metaphase or anaphase, obtained from their apparent dimensions and known number of coils. The real length of the chromosome thread is therefore thought to be constant throughout the mitotic cycle.

(7) From direct measurement of one chromosome in the aceto-carmine series the chromosome length at leptotene is found to be of the order of 50% longer than that at any point in a normal cell division. This is regarded as a very important preliminary to chromosome pairing and as, possibly, the most fundamental difference between the prophases of mitosis and meiosis.

(8) At pachytene, when chromosome pairing has ceased, the normal somatic length is found to have been restored.

(9) Calculation of the true length of the final coiled chromosome at both meiotic divisions shows them to be in reality closely similar in spite of the difference in number of coils, but both are shorter than the somatic chromosomes by amounts which vary

from 33 to 50% according to the method of estimation. In all cases the calculated length is roughly equivalent to the visible length of the chromosome at strepsitene.

II. From these facts it is postulated that the molecular structure of the chromosome must in some way resemble that of other highly elastic fibres, notably the proteins keratin and myosin. The observed changes of length are commensurate with those produced in keratin and myosin by changes in molecular shape from the α to the β and subsequently to the supercontracted state. On this view the temporary straightening of a molecular convolution is postulated as a possible causal factor in chromosome pairing. Elastic recovery from this exceptional state is regarded as the cause of the cessation of pairing, and the subsequent shortening is interpreted as supercontraction in the same sense as in the fibrous proteins mentioned above.

APPENDIX I

Calculations of lengths of thread in spirally coiled chromosomes

An equation for obtaining the length of a spirally coiled object has been worked out for me by Mr Dearden, M.Sc., of the Physics Department of this University. The length required, L, is given by the expression

$$\frac{L}{l}=\left[1+\left(\frac{\pi n d}{l}\right)^2\right]^{\frac{1}{2}},$$

where l is the apparent length of the coil, d its diameter and n the number of turns. Mr Dearden has also evaluated the formula for the twenty specific cases enumerated below for which l, d and n were supplied by me. The measurements of l and d (which have been verified by several of my colleagues to whom I am grateful) were made on the negatives with the aid of a dissecting microscope and a finely engraved metal ruler graduated in half-millimetres. Where fractions other than half-millimetres are given the values are only approximate, but it does not seem possible to apply more accurate methods owing to the diffuseness of outline inseparable from work at these magnifications. In an attempt to minimize this "edge difficulty" some of the photographs were taken with the Zeiss apochromat. 1·4 instead of the Zeiss apochromat. 1·3. The better lens has a slightly higher resolving power and therefore produces a slightly sharper image. Unfortunately, it has such a short working distance that direct calibration of the magnification by the normal methods is not possible. The magnifying power is nominally the same in the two lenses, but the large photograph of the pachytene chromosome of fig. 62, Plate 18, which was the last to be taken, made it possible to detect a difference between them of the order of 6%. This difference is not appreciable on a casual inspection of a small object (compare fig. 24, Plate 17, and fig. 80*a*, Plate 19), it must, however, be allowed for in the calculations.

The dimensions and the results are expressed in the first instance in millimetres at the magnifications stated, but since millimetres at a magnification of 1000 are numerically equivalent to the true dimensions in microns the actual sizes for magnifications of 2000 and 3000 are readily obtained by dividing the results by 2 and by 3 respectively. All the estimates are summarized in the text in Tables I and II, pp. 203, 205.

Plate 17, *sections at a magnification of* × 2000

Metaphase I (4 *coils*).

(1) Fig. 41. $d = 2{\cdot}5$ mm. $l = 8{\cdot}5$ mm. Therefore $L = 32{\cdot}6$ mm. Applying lens correction, $L =$ **30·5** mm. $= 15\mu$.

Anaphase I (4 *coils*).

(2) Fig. 44. $d = 1{\cdot}8$ mm. $l = 5$ mm. Therefore $L = 23{\cdot}2$ mm. Applying lens correction, $L =$ **21·8** mm. $= 11\mu$.

Second meiotic division (8 *coils*).

(3) Fig. 45. $d = 1{\cdot}2$ mm. $l = 6$ mm. Therefore $L =$ **30·7** mm. $= 15\mu$.

Somatic divisions (14 *coils*).

(4) Fig. 46. $d = 1$ mm. (The diameter here is of a chromatid not the whole chromosome. This will be obvious on reference to the figure.) $l = 12$ mm. Therefore $L = 45{\cdot}5$ mm. Applying lens correction, $L =$ **42·7** mm. $= 21\mu$.

(5) Fig. 26. $d = 0{\cdot}9$ mm. $l = 11{\cdot}5$ mm. Therefore $L =$ **41·1** mm. $= 21\mu$.

(6) Fig. 47. $d = 1{\cdot}1$ mm. $l = 9$ mm. Therefore $L =$ **49·1** mm. = *ca.* 25μ. (This result is unexpectedly high and it is not impossible that the more superficial tissue of the tapetum may have reacted somewhat differently to the fixative than the more deeply seated archesporium of fig. 46. The result will, however, be retained if only to illustrate the sort of experimental difficulty which is encountered.)

Plate 18, *aceto-carmine at a magnification of* × 1000

Metaphase I (4 *coils*).

(7) Fig. 53. $d = 2$ mm. $l = 5$ mm. Therefore $L =$ **25·6** mm. $= 26\mu$.

Anaphase I (4 *coils*).

(8) Fig. 54. This is an exceedingly difficult measurement owing to the very irregular outlines. Approximate value for $d = 1{\cdot}4$ mm. (rather doubtful). Measurements of l in different chromosomes = 4, 4·5, 3·5, therefore average = 4 mm. Therefore L = approximately **18·0** mm. $= 18\mu$.

(9) Fig. 79 (Plate 19). $d = 1{\cdot}3$–$1{\cdot}5$ = mean value 1·4 mm. l = approximately 4·5 mm. Therefore $L =$ **18·4** mm. $= 18\mu$.

Second meiotic division (8 *coils*).

(10) Fig. 58. $d = 1$ mm. Several measurements of l in different chromosomes = 4·5, 4, 3·5, 4, 4·5 = average 4 mm. Therefore $L =$ **25·48** mm. $= 25\mu$.

Tapetum (14 *coils*).

(11) Fig. 63. $d = 1$ mm. $l = 8$ mm. Therefore $L =$ **44·6** mm. $= 45\mu$. (It is possible that this is too large owing to the fact that a metaphase not an anaphase was available.)

Plate 19, *spiral structure at a magnification of* × 3000

Metaphase I (4 *coils*).

(12) Fig. 64, but in this case the measurements are not taken from the figure itself but from another photograph at the correct magnification, not reproduced. $d = 2{\cdot}5$ mm. $l = 9$ mm. Therefore $L =$ **32·7** mm. $= 11\,\mu$.

(13) Fig. 66. $d = 2{\cdot}5$ mm. $l = 10$ mm. Therefore $L = 33{\cdot}0$ mm. Applying lens correction, $L =$ **31·0** mm. $= 10\,\mu$.

(14) Fig. 68. $d = 2{\cdot}5$ mm. $l = 8$ mm. Therefore $L = 32{\cdot}4$ mm. Applying lens correction, $L =$ **30·4** mm. $= 10\,\mu$.

Anaphase I (4 *coils*).

(15) Fig. 75. $d = 2$ mm. $l = 8$ mm. Therefore $L = 26{\cdot}4$ mm. Applying lens correction, $L =$ **24·6** mm. $= 8\,\mu$.

(16) Fig. 73. $d = 2$ mm. $l = 7$ mm. Therefore $L = 26{\cdot}1$ mm. Applying lens correction, $L =$ **24·4** mm. $= 8\,\mu$.

(17) Fig. 76. $d = 2$ mm. $l = 8$ mm. Therefore $L = 26{\cdot}4$ mm. Applying lens correction, $L =$ **24·6** mm. $= 8\,\mu$.

Metaphase II (8 *coils*).

(18) Fig. 69. $d = 1$ mm. (rather difficult). $l = 12$ mm. Therefore $L =$ **27·8** mm. $= 9\,\mu$.

(19) Fig. 70. $d = 1$ mm. $l = 13$ mm. Therefore $L = 28{\cdot}3$ mm. Applying lens correction, $L =$ **26·7** mm. $= 9\,\mu$.

Tapetum (14 *coils*).

(20) Fig. 71. $d = 1$ mm. $l = 27$ mm. Therefore $L = 51{\cdot}6$ mm. Applying lens correction, $L =$ **48·6** mm. $= 16\,\mu$.

APPENDIX II

Estimations of chromosome stretching

Plate 18, *fig.* 54 (*anaphase I in aceto-carmine*).

Chromosome length (Appendix I, 8) = *ca.* 18·0 mm.
Therefore length of one coil = 4·5 mm.
Measured length of stretched piece = 6 mm.
Therefore percentage stretching = **33** %.

Plate 19, *fig.* 72 (*early anaphase I, spiral structure*).

Measured length of one coil in left-hand V-shaped chromosome = *ca.* 7 mm.
Measured length of one coil in stretched chromosome marked with an arrow = *ca.* 12 mm.
Therefore percentage stretching = **71** %.
(*Note* that this is the most authentic estimate since the number of coils involved is actually visible.)

Plate 19, *fig.* 76 (*late anaphase, spiral structure*).

Length of chromosome (Appendix I, 17 without lens correction) = 26·4 mm.
Therefore length of one coil = 6·6 mm.
Measured length of stretched piece = *ca.* 7 mm.
Therefore this specimen demonstrates recovery from stretching.

Plate 19, *fig.* 79 (*tetraploid in aceto-carmine*).

Length of chromosome (Appendix I, 9) = 18·4 mm.

Therefore length of one coil = 4·6 mm.

Measured length of stretched portions = at least 14 mm. (This is a very conservative estimate but all the very thin places have been ignored as possible artefacts.)

Therefore percentage stretching = at least **200** %.

Plate 19, *fig.* 69 (*stretched second division chromosome*).

Length of chromosome (Appendix I, 18) = 27·8 mm.

Measured length of stretched chromosome = 43 mm.

Therefore percentage stretching = **56** %.

Plate 19, *fig.* 78 (*stretched somatic chromosome*).

Length of chromosome × 3000 (Appendix I, 20) = 48·6 mm.

Therefore length × 1500 = 24·3 mm.

Distance between ends of stretched specimen = 52 mm.

Therefore percentage stretching = **115** %.

(*Note* that this is a minimum estimate since the undulations in the stretched chromosome have been ignored as possible artefacts due to the action of the ammonia subsequent to the stretching. Some coils also probably remain unexpanded at the upper end of the specimen.)

APPENDIX III

Estimations of supercontraction

Plate 17 (*sections* × 2000).

Average somatic length (Appendix I, 4–6) = 45·8 mm. Metaphase I (Appendix I, 1) = 30·5 mm. Therefore contraction = 15·3 mm. Percentage contraction = **33** %.

Plate 18 (*aceto-carmine* × 1000).

Somatic length (Appendix I, 11) = 44·6 mm. Metaphase I (Appendix I, 7) = 25·6 mm. Therefore contraction = 19·0 mm. Percentage contraction = **43** %.

Plate 19 (*spiral structure* × 3000).

Somatic length (Appendix I, 20) = 48·6 mm. Metaphase I (Appendix I, 12–14) = 31·4 mm. Therefore contraction = 17·2 mm. Percentage contraction = **35** %.

Plate 19.

Somatic length (Appendix I, 20) = 48·6 mm. Anaphase I (Appendix I, 15–17) = 24·5 mm. Therefore contraction = 24·1 mm. Percentage contraction = **50** %.

APPENDIX IV

Note on methods of measuring prophase chromosomes

A number of different ways of determining the length of the very tortuous lines representing prophase chromosomes were tried. They include: (1) Direct measurement of the prints with a ruler. (2) Measurement with a ruler of the actual specimens projected on to the ground glass and traced with a pencil on tracing paper (text-figs. 2–5). In this method the photographic apparatus

is treated like a camera lucida. (3) Construction of a model in flexible copper foil placed on edge on the back of the negative and bent up to superpose on the image; the model when complete, straightened out and measured. (4) The image carefully cut out of a spare print or prints and the cut edge followed round with fine string or thick cotton which was then measured. (5) Projection of the photographs on to a lantern screen, the shadow traced with a pencil on paper pinned to the screen and the length then determined with a map measurer. The additional magnification introduced by the lantern has to be determined concurrently by projecting a photograph of a graduated millimetre scale; for the lantern used here the total magnification came to 30,000.

In most cases the result quoted is an average of several estimations by several of these methods though preference was naturally given to the easiest method for each particular specimen. Thus for the leptotene measurement the easiest method is undoubtedly the last (the lantern). For the aceto-carmine pachytene the most accurate method was undoubtedly the copper model. For the others direct measurement seemed satisfactory though the actual specimen was always collated with the result from the photographs. For all measurements based on photographs with the 1·4 lens a correction of 6% has to be applied as previously explained (Appendix I). As in Appendix I the results are given in millimetres at the magnifications stated and in microns. The most important are summarised in text-figs. 2–7 and collated with the results of Appendix I in Tables I and II, pp. 203, 205.

Summary of measurements

Pachytene in aceto-carmine:

Measurement of fig. 49 (× 1000) = 48 mm.

Measurement of fig. 62 (× *ca.* 2000) = 102 mm. = 96 mm. with lens correction.

Therefore length of aceto-carmine pachytene at 1000 = **48** mm. = 48 μ.

Leptotene in aceto-carmine:

Measurement of fig. 48 (× 1000) = 73 mm.

Measurement of fig. 61*a* (× *ca.* 2000) = 156–158 mm. = 148 mm. with lens correction.

Therefore length of aceto-carmine leptotene × 1000 = **73** mm. = 73 μ.

Strepsitene in aceto-carmine, fig. 50*a*, × 1000 = **21** mm. = 21 μ.

Somatic prophase from sections:

Measurement of composite drawing from figs. 80*a–c* (× *ca.* 2000) = 43 mm., = 40 mm. with lens correction.

Measurement based on fig. 24 (× 2000) = 39–41 mm.

Measurement of camera lucida drawing of text-fig. 2 (× 4000) = 80 mm.

Therefore length of somatic prophase chromosome × 2000 = **40** mm. = 20 μ.

Late pachytene from sections:

Measurement of fig. 35 (× *ca.* 2000) = 37–39 mm., = approximately 36 mm. with lens correction.

Measurement of camera lucida drawing of text-fig. 3 (× 4000) = 72 mm.

Therefore length of late pachytene chromosome × 2000 = **36** mm. = 18 μ.

Early pachytene from sections:

Measurement of camera lucida drawing of text-fig. 4 ($\times 4000$) $= 104$ mm.

The allowance for foreshortening can be roughly estimated as equivalent to the diameter of the nucleolus which disappears entirely during focussing = *ca.* 10 mm.

Therefore approximate length of early pachytene chromosome $\times 2000$ = **57** mm. = 28μ.

Zygotene from sections:

Measurement of camera lucida drawing of text-fig. 5 ($\times 4000$) $= 102$ mm.

This is incomplete at both ends, therefore length of zygotene chromosome $\times 2000$ = more than **51** mm. = more than 25μ.

Strepsitene in sections:

Measurement of fig. 37 ($\times$ *ca.* 2000) = *ca.* 24 mm., = *ca.* **23** mm. with lens correction = *ca.* 11μ.

References

Astbury, W. T. 1933 *Fundamentals of fibre structure.* London.

Baranetzky, J. 1880 Die Kerntheilung in den Pollenmutterzellen einiger Tradescantien. *Bot. Ztg*, **38**, 283.

Bělař, K. 1928 *Die cytologische Grundlage der Vererbung*, p. 171. Berlin.

Belling, J. 1928 Contraction of chromosomes during maturation divisions in *Lilium* and other plants. *Univ. Calif. Publ. Bot.* **14**, 335.

Darlington, C. D. 1935 Internal mechanics of the chromosomes. *Proc. Roy Soc.* B, **87**, 33–96.

— 1937 *Recent advances in cytology*, 2nd ed. London.

Digby, L. 1919 On the archesporial and meiotic mitosis of *Osmunda. Ann. Bot., Lond.*, **33**, 135.

Dixon, H. 1900 The first mitosis of spore mother cells of *Lilium. Proc. R. Irish Acad.* **6**, 1–12.

Farmer, J. B. and Moore, J. E. S. 1904 On the meiotic phase (reduction divisions) in animals and plants. *Quart. J. Micr. Sci.* N.S. **48**, 489.

Fujii, K. 1926 *Jap. Assoc. Adv. Sci.* (in Japanese), quoted by Kuwada (1927).

Geitler, L. 1938 *Chromosomenbau.* Berlin.

Gelei, J. 1922 Weitere Studien über die Oogenese des *Dendrocoelum lacteum.* II. Die Langskonjugation der Chromosomen. *Arch. Zellforsch.* **16**, 88.

Grégoire, V. 1907 La formation des gemini hétérotypiques dans les végétaux. *Cellule,* **24**, 369–420.

— 1910 Les cinèses de maturation dans les deux règnes. *Cellule*, **26**, 223–422.

Guignard, L. 1898 Le Développement du Pollen et la Réduction chromatique dans le *Naias major. Arch. Anat. micr.* **2**, 477.

Huskins, C. L. and Smith, S. G. 1935 Meiotic chromosome structure in *Trillium erectum. Ann. Bot., Lond.*, **49**, 120–50.

Iwata, J. 1935 Chromosome structure in *Lilium. Mem. Coll. Sci. Kyoto*, B, **10**, 275.

Janssens, F. A. 1924 La Chiasmatypie dans les insectes. *Cellule*, **34**, 135–359.

Koshy, T. K. 1933 Chromosome Studies in *Allium. J. R. Micr. Soc.* **53**, 299.

— 1937 Number and behaviour of chromosomes in *Aloe litoralis. Ann. Bot., Lond.*, N.S. **1**, 43–58.

Kuwada, Y. 1921 On the so-called longitudinal split of chromosomes at telophase. *Bot. Mag., Tokyo*, **35**, 99.

Kuwada, Y. 1926 On the structure of the anaphase chromosomes in the somatic mitosis in *Vicia faba*, with special reference to the so-called longitudinal split of chromosomes in the telophase. *Mem. Coll. Sci. Kyoto*, B, **11**, no. 1.
— 1927 On the spiral structure of chromosomes. *Bot. Mag., Tokyo*, **41**, 100.
— 1932 The double-coiled spiral structure of chromosomes. *Bot. Mag., Tokyo*, **46**, 257–8.
Kuwada, Y. and Nakamura, T. 1933 Behaviour of chromonemata in mitosis. I. Observations of pollen mother cells in *Tradescantia reflexa*. *Mem. Coll. Sci. Kyoto*, B, **9**, 129.
— — 1935 Behaviour of chromonemata in mitosis. IV. Double refraction of chromosomes. *Cytologia, Tokyo*, **6**, 314.
— — 1938 Behaviour of chromonemata in mitosis. VII. A chromosome study by the artificial uncoiling method of the chromonema spiral. *Cytologia, Tokyo*, **9**, 21.
Kuwada, Y. and Sugimoto, T. 1926 On the structure of the chromosomes of *Tradescantia virginica* (preliminary note). *Bot. Mag., Tokyo*, **40**, 19.
La Cour, L. 1931 Improvements in everyday technique in plant cytology. *J. R. Micr. Soc.* **51**, 119.
— 1935 Technic for studying chromosome structure. *Stain Tech.* **10**, 57.
Lang, W. H. 1924 On some deviations from the normal morphology of the shoot in *Osmunda regalis*. *Mem. Manchr Lit. Phil. Soc.* **68**, 53.
Lawson, A. A. 1911 The phase of the nucleus known as synapsis. *Trans. Roy. Soc. Edinb.* **47**, 591.
Maeda, T. 1928 The spiral structure of chromosomes in the sweet-pea, *Lathyrus odoratus*. *Bot. Mag., Tokyo*, **42**, 191–5.
Manton, I. 1932 A case of induced apospory in *Osmunda regalis* L. *J. Genet.* **25**, 423–30.
— 1935 Some new evidence on the physical nature of plant nuclei from intraspecific polyploids. *Proc. Roy. Soc.* B, **118**, 522–47.
— 1936 Spiral structure of chromosomes in *Osmunda*. *Nature, Lond.*, **138**, 1058.
— 1937 The problem of *Biscutella laevigata*. II. *Ann. Bot., Lond.*, N.S. **1**, 439.
McClintock, B. 1929 A method of making aceto-carmine smears permanent. *Stain Tech.* **4**, 53.
— 1930 A cytological demonstration of an interchange between non-homologous chromosomes of *Zea mays*. *Proc. Nat. Acad. Sci., Wash.*, **16**, 791.
— 1931 Cytological observations of deficiencies, etc., in *Zea mays*. *Bull. Univ. Mo. Coll. Agric. Res.* no. 163.
Naithani, S. P. 1937 Chromosome studies in *Hyacinthus orientalis*. II. *Ann. Bot., Lond.*, N.S. **1**, 257.
Nebel, B. R. 1932 Chromosome structure in *Tradescantia*. *Z. Zellforsch.* **16**, 251.
Newton, W. C. F. 1925 Chromosome studies in *Tulipa* and some related genera. *J. Linn. Soc.* (*Bot.*), **47**, 339–54.
Sakamura, T. 1927 Fixierung von Chromosomen mit siedendem Wasser. *Bot. Mag., Tokyo*, **41**, 58.
Sargant, E. 1897 The formation of sexual nuclei in *Lilium Martagon*. *Ann. Bot., Lond.*, **11**, 187.
Sax, K. 1930 Chromosome structure and the mechanism of crossing over. *J. Arnold Arbor.* **11**, 193–220.
— 1935 Chromosome structure in the meiotic chromosomes of *Rhoeo discolor* Hance. *J. Arnold Arbor.* **16**, 216.
Sax, K. and Humphrey, L. M. 1930 Structure of meiotic chromosomes in microsporogenesis of *Trandescantia*. *Bot. Gaz.* **96**, 354.
Sax, H. J. and Sax, K. 1935 Chromosome structure and behaviour in mitosis and meiosis. *J. Arnold Arbor.* **16**, 423–39.

Sharp, L. W. 1934 *Introduction to cytology*, 3rd ed. New York.
Shinke, N. 1930 On the spiral structure of chromosomes of some higher plants. *Mem. Coll. Sci. Kyoto*, B, **5**, 239.
Strasburger, E. 1894 The periodic reduction in the number of the chromosomes in the life history of living organisms. *Ann. Bot., Lond.*, **8**, 281.
— 1900 Über Reduktionstheilung, Spindelbildung, etc. *Histol. Beitr.* **6**, 77.
Szakien, B. 1927 La formation des chromosomes hétérotypiques dans l'*Osmunda regalis*. *Cellule*, **37**, 369.
Upcott, M. 1935 Chromosomes of the tulip in mitosis. *Nature, Lond.*, **135**, 957.
— 1937 Relic and relational coiling in pollen grains. *Cytologia, Tokyo*, **8**, 398.
White, M. J. D. 1937 *The chromosomes*. London.

Description of Plates

Plate 16

General views of the last three sporangial divisions in *Osmunda regalis*. Magnification × 1000. The fixative for all except fig. 21 is La Cour's 2 BD (1931) after very brief dipping in alcohol; fig. 21 is from a sporangium fixed in chrom-acetic-formalin. Except for fig. 21 only three pinnae are involved; the first contains all stages of the premeiotic division, figs. 1–10; the second was removed from the same frond two days later and shows early meiotic prophases up to zygotene, figs. 11–14; the third pinna contains all stages from pachytene to the tetrad, figs. 15–22. Sections were cut at 10 or 12μ and both gentian violet and Heidenhain's haematoxylin were used as stains.

Fig. 1. Telophase preceding premeiotic division showing polarized arrangement of chromosomes in the upper nucleus. Detail of chromosomes in fig. 27, Plate 17. Gentian violet.

Fig. 2. Very early prophase of the premeiotic division. Nuclear shape oval with several nucleoli. Chromosomes diffuse and not traceable in detail (see fig. 23, Plate 17). From the same slide as the preceding. Gentian violet.

Fig. 3. Middle prophase of the premeiotic division. Nuclear shape as before but chromosomes quite distinct and much convoluted. For details see fig. 24, Plate 17, and fig. 80, Plate 19. From the same slide as the preceding. Gentian violet.

Fig. 4. Late prophase of the premeiotic division. Nuclei quite spherical and dominated by nuclear sap in which chromosomes float. Polarized arrangement still visible in upper nucleus (cf. fig. 1). From the same slide as the preceding. For details see fig. 25, Plate 17.

Fig. 5. Prometaphase of the premeiotic division. Nuclear sap has disappeared and chromosomes in process of orientation on the spindle. From the same slide as the preceding. Gentian violet.

Fig. 6. Early metaphase of the premeiotic division, stained in haematoxylin.

Fig. 7. Anaphase. The somatic chromosome number ($2n = 44$) can be roughly counted in the top left-hand nucleus. Detail of chromosomes in fig. 26, Plate 17. Gentian violet.

Fig. 8. Early telophase before formation of wall, from the same slide as the preceding. Gentian violet.

Fig. 9. Late telophase of the premeiotic division after formation of the wall, from the same slide as the preceding. Gentian violet. The nuclear outline has ceased to be angular but it is not yet full size. The polarized arrangement of the chromosomes is visible in the left-hand nucleus. Detail of chromosomes is given in fig. 28, Plate 17.

Fig. 10. Resting nucleus before onset of meiosis, showing diffuse chromosomes and very slight staining capacity. This can be judged by comparing the archesporial nuclei with the tapetal nucleus in the bottom corner. From the same slide as the preceding. Gentian violet. For details of the chromosomes see fig. 29, Plate 17.

Fig. 11. Fairly early preleptotene. Nuclear substance a mass of fine closely coiled threads (cf. fig. 30, Plate 17). Stain haematoxylin.

Fig. 12. Late preleptotene. Nucleus larger and almost spherical owing to increase of nuclear sap. Chromosomes still coiled but looser (cf. fig. 31, Plate 17). Gentian violet.

[P. T. O.

Fig. 13. Leptotene. The nucleus is a dense tangle of very fine threads with nuclear sap outside the mass (for comments on the "synaptic knot" condition see p. 191). Chromosome movement has begun, as shown by displacement and coalescence of nucleoli (see text, p. 192). Details of the chromosomes can only be made out on the larger scale of fig. 32, Plate 17. From the same slide as fig. 11. Haematoxylin.

Fig. 14. Zygotene. Nuclear sap more abundant. Chromosomes more spread with paired and unpaired segments easily detectable but only at the higher magnification of fig. 33, Plate 17. From the same slide as the preceding. Haematoxylin.

Fig. 15. Early pachytene. Chromosomes thicker and almost filling nucleus, the nuclear size being temporarily diminished. Pairing probably ended though incomplete (see fig. 34, Plate 17). Whole chromosomes can be made out for the first time since the end of the preceding division, but only with difficulty. Haematoxylin.

Fig. 16. Late pachytene or early strepsitene. The nucleus has enlarged and the chromosomes have shortened and spread more evenly through the nucleus. Whole chromosomes are more easily observed (fig. 35, Plate 17). From the same slide as the preceding. Haematoxylin.

Fig. 17. Diplotene. Chiasmata visible (cf. figs. 38, 38*a*, Plate 17, and fig. 51, Plate 18). From the same slide as the preceding. Haematoxylin.

Fig. 18. Diakinesis. For details see fig. 39, Plate 17, and compare with fig. 52, Plate 18. From the same slide as the preceding.

Fig. 19. First meiotic metaphase with polar and side views visible, from the same slide as the preceding. For details see figs. 40, 41, Plate 17.

Fig. 20. Very late anaphase and interkinesis. Compare with figs. 54–56, Plate 18. From the same slide as the preceding.

Fig. 21. The second meiotic metaphase showing the reduced number of chromosomes (22). Fixed in chromacetic formalin, stained in gentian violet.

Fig. 22. Anaphase of the second meiotic division and early tetrad. For detail of chromosomes see fig. 45, Plate 17. From the same slide as figs. 15–20. Haematoxylin.

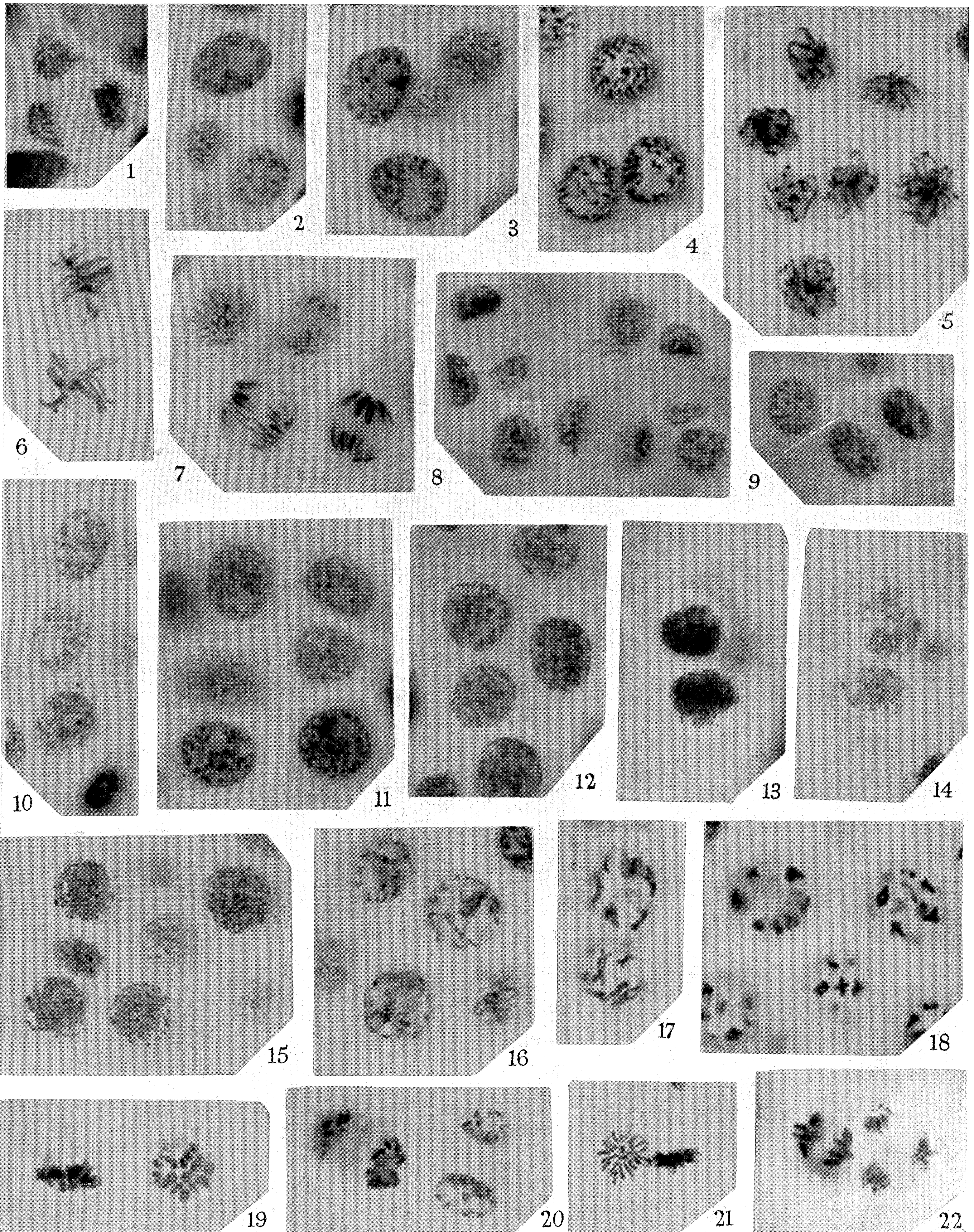

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22

Plate 17

Detail of chromosome appearances at principal stages of mitosis and meiosis, from the sections used for Plate 16. Magnification throughout the plate × 2000.

Fig. 23. Early premeiotic prophase, chromosomes still diffuse. From the sporangium of fig. 2, Plate 16. Gentian violet.

Fig. 24. Middle premeiotic prophase. Chromosomes distinct and still spirally coiled. Other focal levels of the measured chromosome from the upper nucleus are given in figs. 80*a*–*c*, Plate 19, and a general view of the tissue in fig. 3, Plate 16. Gentian violet.

Fig. 25. Late premeiotic prophase from a nucleus of fig. 4, Plate 16. The chromosomes apparently shorter and thicker and visibly double. The original spiral uncoiling as new spiral forms in each chromatid. Gentian violet. Zeiss 1·4*.

Fig. 26. Detail of anaphase chromosomes for size and demonstration of subterminal attachments, from the sporangium of fig. 7, Plate 16. Gentian violet.

Fig. 27. Detail of early telophase showing appearance of spirals in the chromosomes, from the sporangium of fig. 1, Plate 16. Gentian violet. Zeiss 1·4*.

Fig. 28. Late premeiotic telophase from the sporangium of fig. 9, Plate 16, showing the spirals. Gentian violet. Zeiss 1·4*.

Fig. 29. Rest before meiosis. Chromosomes diffuse and staining very faintly, from the sporangium of fig. 10, Plate 16. Gentian violet.

Fig. 30. Early preleptotene showing closely convoluted threads. Gentian violet. Zeiss 1·4*.

Fig. 31. Late preleptotene from the sporangium of fig. 12, Plate 16. The convolutions straightening. Gentian violet. Zeiss 1·4*.

Fig. 32. Leptotene. Convolutions disappeared and chromosome movement begun. Compare this with fig. 13, Plate 16, and fig. 48, Plate 18. Gentian violet. Zeiss 1·4*.

Fig. 33. Zygotene from the sporangium of fig. 14, Plate 16, showing parts of paired and unpaired chromosomes side by side. Stain haematoxylin. Zeiss 1·4*.

Fig. 34. Early pachytene from the sporangium of fig. 15, Plate 16, showing change of texture. Haematoxylin. Zeiss 1·4*.

Fig. 35. Late pachytene from the sporangium of fig. 16, Plate 16, showing a whole chromosome incompletely paired. The ends are slightly out of focus. A drawing is given in text-fig. 3, p. 204. Haematoxylin. Zeiss 1·4*.

Fig. 36. Another piece of chromosome from the same sporangium as the preceding, showing the mutual twist of the paired chromosomes more clearly. Zeiss 1·4*.

Fig. 37. A single chromosome at strepsitene showing the remains of the pachytene coiling and considerable shortening. Gentian violet. Zeiss 1·4*.

Figs. 38 and 38*a*. Diplotene. Two paired chromosomes cut out of one photograph. Fig. 38 is a single-chiasma configuration with a cut piece of another chromosome superimposed; fig. 38*a* is a double chiasma figure. Gentian violet. Zeiss 1·4*.

Fig. 39. Diakinesis with single and double chiasma figures. Haematoxylin. For fuller information see fig. 52, Plate 18.

* The asterisk means that owing to the substitution of the better lens (Zeiss apochromat. 1·4 instead of Zeiss apochromat. 1·3) the magnification is 6 % greater than stated. This difference is not appreciable on a casual inspection but has to be allowed for in measurements (see p. 208).

[P. T. O.

FIG. 40. The first meiotic metaphase in side view showing some chromosomes on the spindle. Haematoxylin. Zeiss 1·4*.

FIG. 41. Late metaphase of the first meiotic division, the spirals just detectable. From the same sporangium as the preceding. Haematoxylin. Zeiss 1·4*.

FIG. 42. Early anaphase of the first meiotic division, the chromatids beginning to separate laterally (both ends of the left-hand chromosome are out of focus, hence apparent shortness). From the same sporangium as the preceding. Zeiss 1·4*.

FIG. 43. Anaphase of the first meiotic division. Lateral separation of chromatids precedes unloosening of chiasmata. (The upper part of the middle chromosome is out of focus.) From the same sporangium as the preceding. Zeiss 1·4*.

FIG. 44. Late anaphase of the first meiotic division showing the effect of an interstitial chiasma which has delayed and distorted one side of a pair of chromosomes. For fuller details compare this with fig. 54, Plate 18, and figs. 72, 76 and 79, Plate 19. The specimen is fixed in 2BD and stained in brazilin. Zeiss 1·4*.

FIG. 45. Anaphase of the second meiotic division for comparison of chromosome size and shape with the preceding. From the sporangium of fig. 22, Plate 16.

FIG. 46. Late metaphase in a tapetal nucleus, the remains of mutual coiling of the chromatids visible in the bottom chromosome. From a sporangium with its archesporium in preleptotene. Zeiss 1·4*.

FIG. 47. Anaphase in a tapetal cell. Details of spiral structure in a somatic chromosome have only been obtained in the tapetum (fig. 71, Plate 19) but the chromosome size is strictly comparable with that in the archesporium (fig. 26).

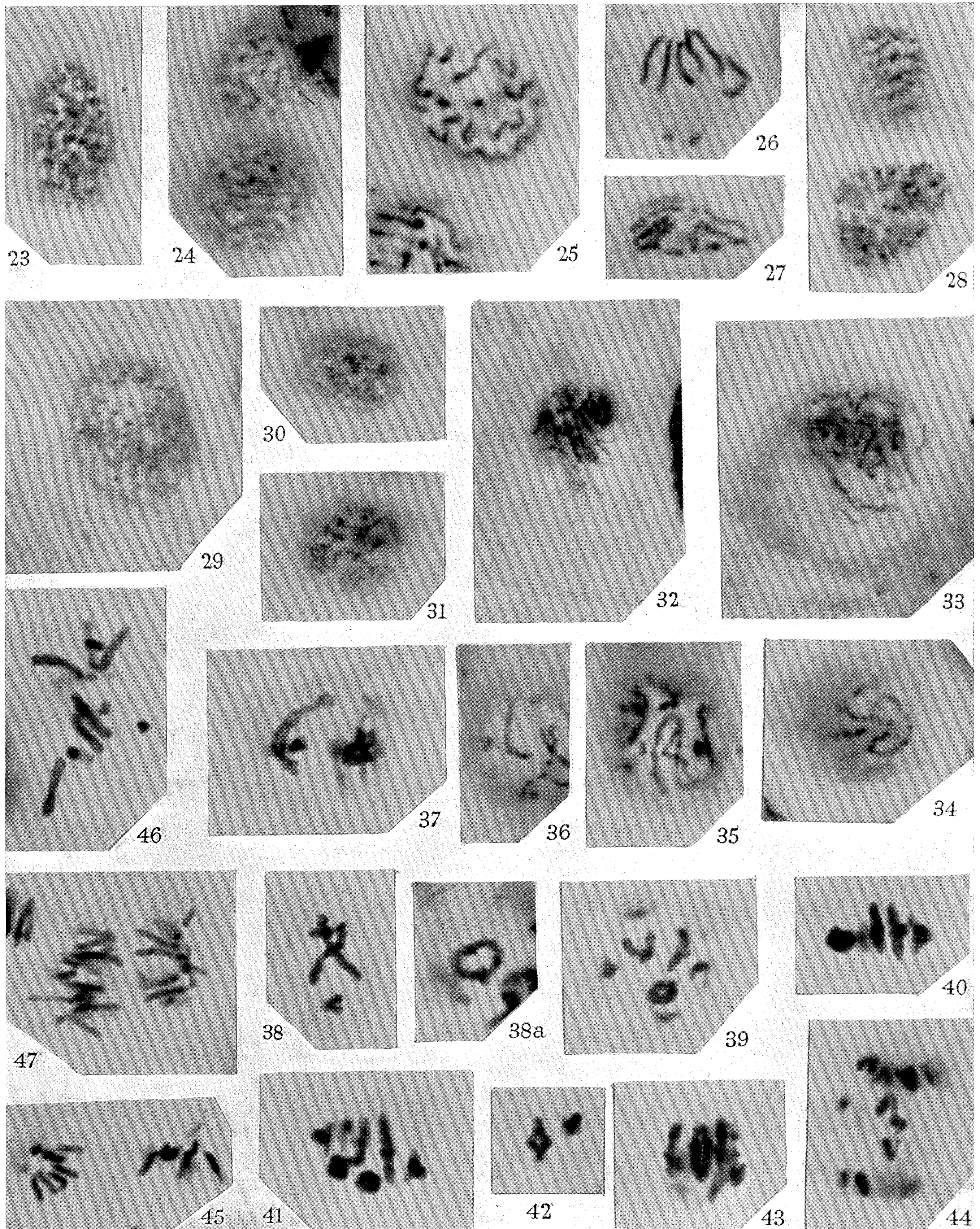

23
24
25
26
27
28
29
30
31
32
33
46
37
36
35
34
47
38
38a
39
40
45
41
42
43
44

Plate 18

Magnification × 1000 for all except figs. 61 and 62 which are × 2000*. All are permanent aceto-carmine preparations by a modification of McClintock's method (cf. Manton 1937). Figs. 48, 60 and 61 are from triploid *O. regalis*, figs. 55 and 59 from *O. gracilis*. All other figures are of diploid *O. regalis*, as in the previous plates.

Fig. 48. Leptotene in a triploid showing a whole chromosome, of which a drawing is given in text-fig. 6, p. 204. This chromosome is enlarged more highly in figs. 61 *a*, *b*.

Fig. 49. Pachytene showing one pair of chromosomes completely. This chromosome is given at a higher magnification in fig. 62.

Fig. 50 *a*, *b*. Two focal levels of an unsquashed nucleus at strepsitene. This is the earliest stage at which whole nuclei can be completely analysed. Its characteristics are shortening of the chromosomes and untwisting of the pachytene coiling.

Fig. 51. Diplotene in unsquashed nuclei.

Fig. 52. Diakinesis in a squashed nucleus showing number and position of chiasmata in all the 22 pairs of chromosomes.

Fig. 53. The first meiotic metaphase. The marked chromosome is the basis of measurements, since it is comparable in shape to that of fig. 65 on the spiral structure plate (Plate 19).

Fig. 54. Anaphase of the first meiotic division, showing V-shaped chromosomes before and after separation. For details of separation compare figs. 41–44, Plate 17, and for explanation of "necks" shown by the left-hand chromosome see figs. 72 and 76, Plate 19.

Fig. 55. Telophase after first meiotic division in *O. gracilis*. V-shaped chromosomes becoming diffuse.

Fig. 56. Prophase of second meiotic division, showing remains of spirals and V shape. For earlier prophase stage see fig. 77, Plate 19.

Fig. 57. Early metaphase of second meiotic division, in side view.

Fig. 58. Anaphase of second meiotic division showing 22 chromosomes (the haploid complement) at each pole.

Fig. 59. Telophase from a tetrad of *O. gracilis* showing 22 rod-shaped chromosomes becoming diffuse.

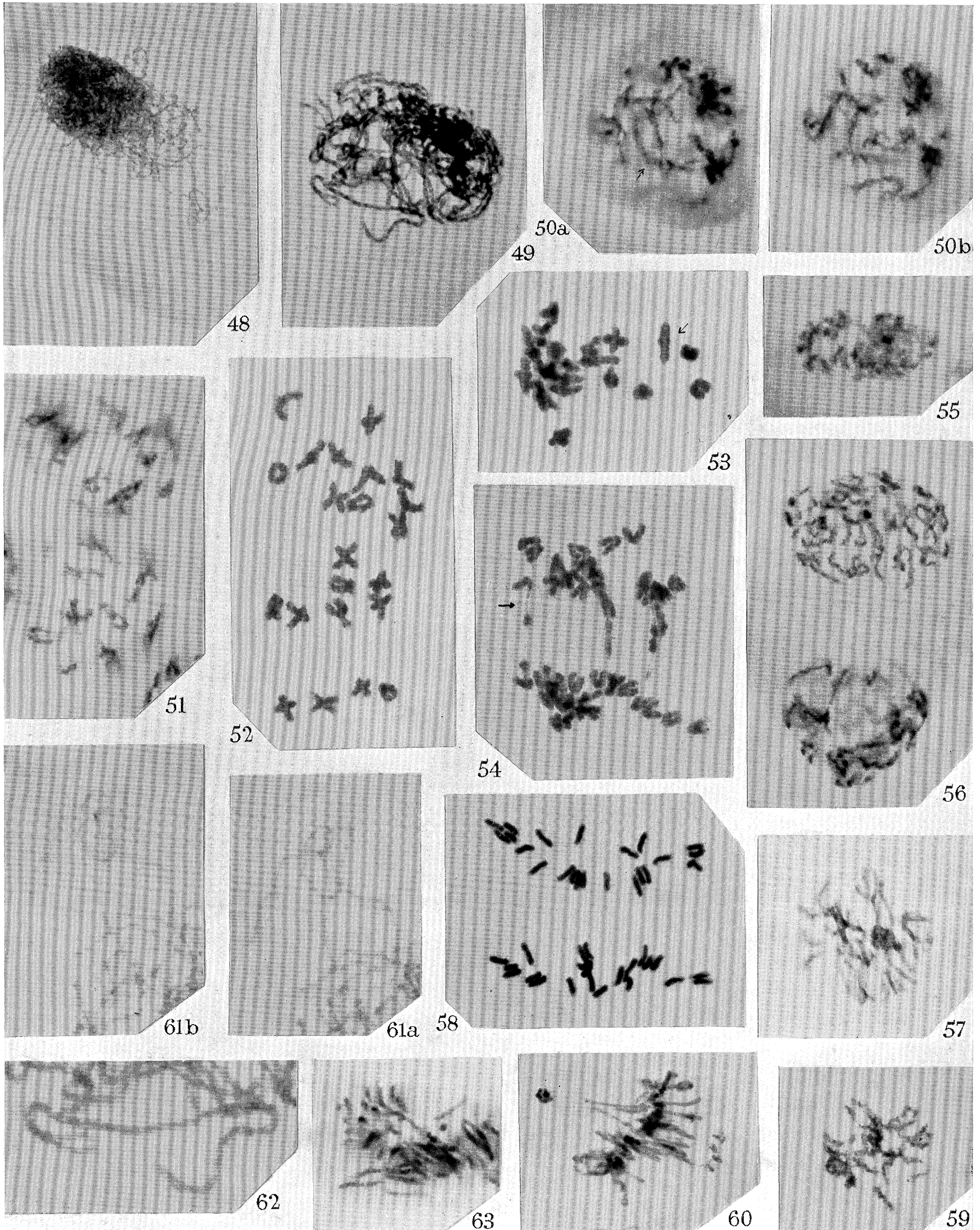
Fig. 60. Early metaphase of second meiotic division in triploid *O. regalis* showing the retention of coils by chromosomes lost at the previous division. These are lying in the cytoplasm near the two sides of the picture.

Figs. 61 *a*, *b*. Two different focal levels of the leptotene chromosome of fig. 48 at a magnification of × 2000*.

Fig. 62. The pachytene chromosome of fig. 49 at a magnification of × 2000* showing coiling and incomplete pairing.

Fig. 63. Portion of a tapetal division, to show the size of somatic chromosomes in this technique, the chromosome used is the isolated one at the top. × 1000.

* The asterisk means that owing to the substitution of the better lens (Zeiss apochromat. 1·4 instead of Zeiss apochromat. 1·3) the magnification is 6 % greater than stated. This difference is not appreciable on a casual inspection but has to be allowed for in measurements (see p. 208).



48
49
50a
50b
53
55
51
52
54
56
61b
61a
58
57
62
63
60
59

PLATE 19

Spiral structure in *Osmunda regalis*. Most of the figures are prepared by Sax and Humphrey's method and photographed at a magnification of × 3000.

FIG. 64. The first meiotic metaphase, showing spiral structure of chromosomes in position on the spindle. Magnification slightly less than × 3000 but identical with that of fig. 72.

FIG. 65. An isolated pair of chromosomes from the first meiotic division, with one sub-terminal chiasma at the end away from the spindle attachment. × 3000*.

FIG. 66. Similar to the last but with a chiasma at the same end as the spindle attachment. Magnification × 3000*.

FIG. 67. Similar to the last but with a chiasma at each end. × 3000*.

FIG. 68. An unpaired chromosome at the first meiotic division in a triploid. × 3000*.

FIG. 69. General view of the second meiotic division in a triploid treated for spiral structure. A damaged chromosome is visible on the left but details of the others are not easy to make out (compare therefore fig. 70).

FIG. 70. Clear details of a single chromosome at the second meiotic division in a triploid. The specimen is a lagging chromosome at late anaphase and shows 8 small coils. × 3000*.

FIG. 71. Tapetal chromosomes, *ca.* 14 coils visible in the marked one. × 3000*.

FIG. 72. Anaphase of the first meiotic division at the same magnification as fig. 64 (i.e. × slightly less than 3000).

FIG. 72*a*. Another focal level of the preceding at two-thirds the magnification, i.e. × *ca.* 2000.

FIG. 73. Anaphase in an unpaired chromosome in a triploid (cf. fig. 68 for the metaphase appearance) which has divided prematurely at the first division. The polar group to which the half-chromosome is going is visible to the right of the figure. The other half is in a comparable position at the other end of the cell but there is no room to include it in the plate. The long axis of the spindle is placed vertically. × 3000*.

FIG. 74. Late anaphase at the first meiotic division in a normal plant, the details of a V-shaped chromosome very clearly shown × 3000.

FIG. 75. Similar to the last but with spirals more distinct. Recovery from temporary deformation, of the type shown in the V-shaped chromosome on the left of fig. 72, complete. × 3000*.

FIG. 76. A chromosome at late anaphase in a triploid which has not only straightened but stretched in coming loose from its fellow. Recovery from stretching is much slower than recovery from straightening. For other examples of this see centre of fig. 72, fig. 79; fig. 54, Plate 18, and fig. 44, Plate 17. × 3000*.

FIG. 77. Very early prophase of the second meiotic division showing the spirals. These are very easily observed at this stage and the specimen has not been specially treated for spiral structure. It is from the ordinary sectioned series of Plates 16 and 17. The stage depicted comes in between those of figs. 55 and 56, Plate 18. × 2000*.

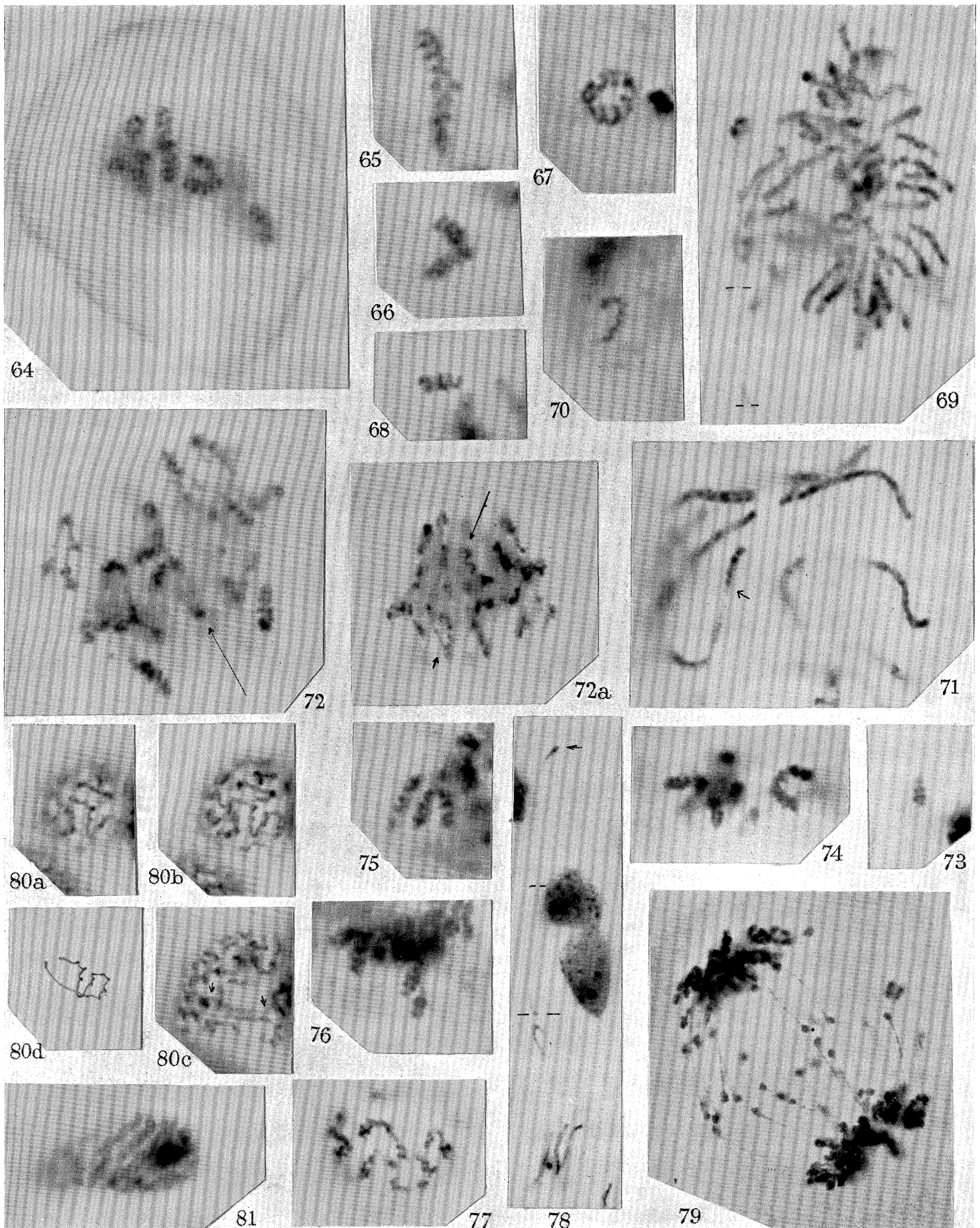
FIG. 78. Part of the same tapetal cell as fig. 71, at a lower magnification, showing undamaged chromosomes at the bottom and mechanically stretched chromosomes at the top. × 1500.

FIG. 79. Anaphase of the first meiotic division in a tetraploid cell showing exaggerated development of "necks" due to enhanced difficulty of disjunction in multivalent groups (cf. fig. 76 and fig. 54, Plate 18). × 1000.

FIG. 80*a–c*. Three focal levels of a somatic chromosome at middle prophase from the nucleus of fig. 24, Plate 17. Fig. 80*d* is a drawing of the whole chromosome. × 2000*.

FIG. 81. Fairly early prophase in a root of *Allium ursinum* to illustrate the mutual coiling of split halves of a chromosome at this stage. × 2000*.

* The asterisk means that owing to the substitution of the better lens (Zeiss apochromat. 1·4 instead of Zeiss apochromat. 1·3) the magnification is 6 % greater than stated. This difference is not appreciable on a casual inspection but has to be allowed for in measurements (see p. 208).


64
65
66
67
68
69
70
71
72
72a
73
74
75
76
77
78
79
80a
80b
80c
80d
81

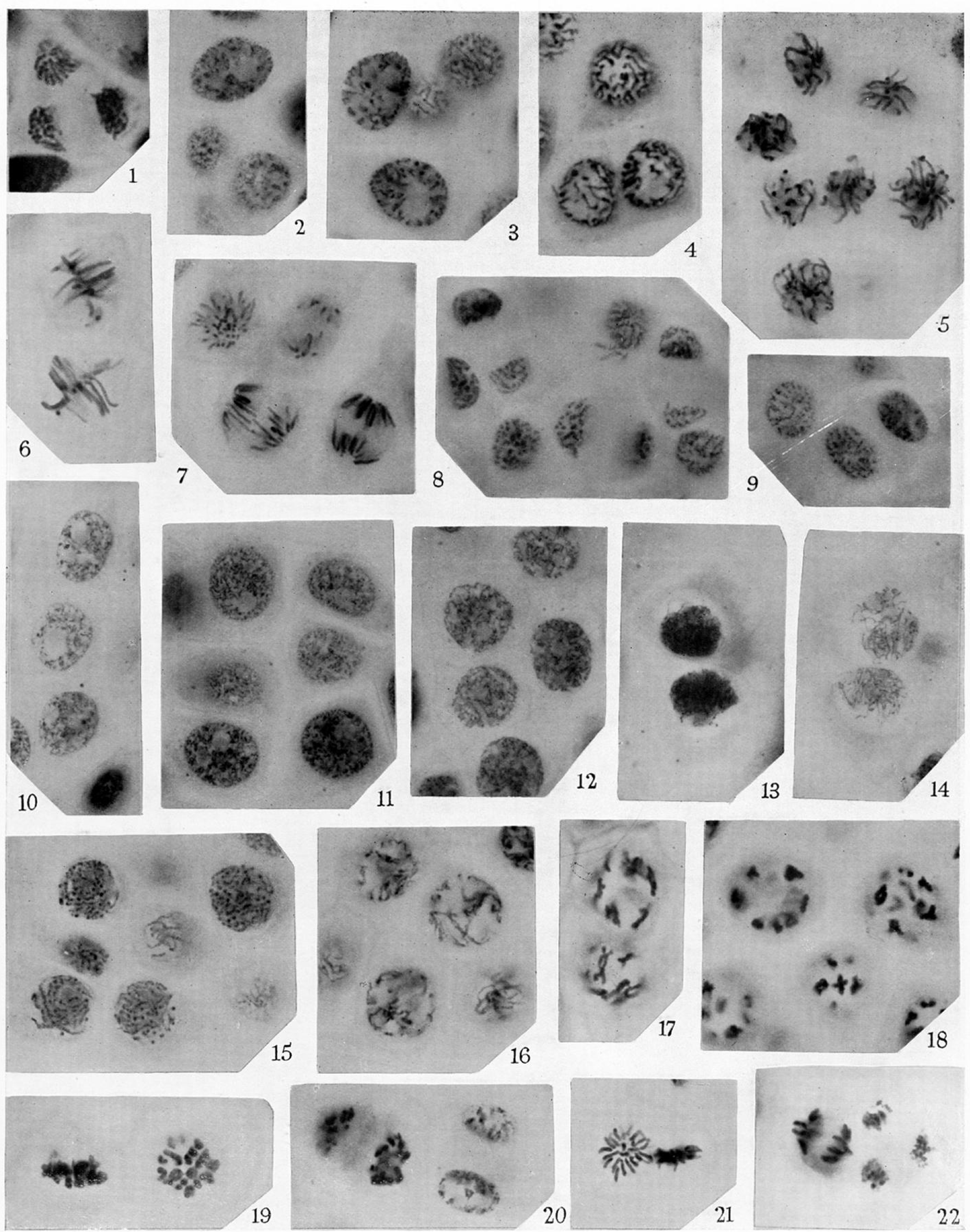


Plate 16

General views of the last three sporangial divisions in *Osmunda regalis*. Magnification × 1000. The fixative for all except fig. 21 is La Cour's 2 BD (1931) after very brief dipping in alcohol; fig. 21 is from a sporangium fixed in chrom-acetic-formalin. Except for fig. 21 only three pinnae are involved; the first contains all stages of the premeiotic division, figs. 1–10; the second was removed from the same frond two days later and shows early meiotic prophases up to zygotene, figs. 11–14; the third pinna contains all stages from pachytene to the tetrad, figs. 15–22. Sections were cut at 10 or 12 μ and both gentian violet and Heidenhain's haematoxylin were used as stains.

Fig. 1. Telophase preceding premeiotic division showing polarized arrangement of chromosomes in the upper nucleus. Detail of chromosomes in fig. 27, Plate 17. Gentian violet.

Fig. 2. Very early prophase of the premeiotic division. Nuclear shape oval with several nucleoli. Chromosomes diffuse and not traceable in detail (see fig. 23, Plate 17). From the same slide as the preceding. Gentian violet.

Fig. 3. Middle prophase of the premeiotic division. Nuclear shape as before but chromosomes quite distinct and much convoluted. For details see fig. 24, Plate 17, and fig. 80, Plate 19. From the same slide as the preceding. Gentian violet.

Fig. 4. Late prophase of the premeiotic division. Nuclei quite spherical and dominated by nuclear sap in which chromosomes float. Polarized arrangement still visible in upper nucleus (cf. fig. 1). From the same slide as the preceding. For details see fig. 25, Plate 17.

Fig. 5. Prometaphase of the premeiotic division. Nuclear sap has disappeared and chromosomes in process of orientation on the spindle. From the same slide as the preceding. Gentian violet.

Fig. 6. Early metaphase of the premeiotic division, stained in haematoxylin.

Fig. 7. Anaphase. The somatic chromosome number ($2n = 44$) can be roughly counted in the top left-hand nucleus. Detail of chromosomes in fig. 26, Plate 17. Gentian violet.

Fig. 8. Early telophase before formation of wall, from the same slide as the preceding. Gentian violet.

Fig. 9. Late telophase of the premeiotic division after formation of the wall, from the same slide as the preceding. Gentian violet. The nuclear outline has ceased to be angular but it is not yet full size. The polarized arrangement of the chromosomes is visible in the left-hand nucleus. Detail of chromosomes is given in fig. 28, Plate 17.

Fig. 10. Resting nucleus before onset of meiosis, showing diffuse chromosomes and very slight staining capacity. This can be judged by comparing the archesporial nuclei with the tapetal nucleus in the bottom corner. From the same slide as the preceding. Gentian violet. For details of the chromosomes see fig. 29, Plate 17.

Fig. 11. Fairly early preleptotene. Nuclear substance a mass of fine closely coiled threads (cf. fig. 30, Plate 17). Stain haematoxylin.

Fig. 12. Late preleptotene. Nucleus larger and almost spherical owing to increase of nuclear sap. Chromosomes still coiled but looser (cf. fig. 31, Plate 17). Gentian violet.

Fig. 13. Leptotene. The nucleus is a dense tangle of very fine threads with nuclear sap outside the mass (for comments on the "synaptic knot" condition see p. 191). Chromosome movement has begun, as shown by displacement and coalescence of nucleoli (see text, p. 192). Details of the chromosomes can only be made out on the larger scale of fig. 32, Plate 17. From the same slide as fig. 11. Haematoxylin.

Fig. 14. Zygotene. Nuclear sap more abundant. Chromosomes more spread with paired and unpaired segments easily detectable but only at the higher magnification of fig. 33, Plate 17. From the same slide as the preceding. Haematoxylin.

Fig. 15. Early pachytene. Chromosomes thicker and almost filling nucleus, the nuclear size being temporarily diminished. Pairing probably ended though incomplete (see fig. 34, Plate 17). Whole chromosomes can be made out for the first time since the end of the preceding division, but only with difficulty. Haematoxylin.

Fig. 16. Late pachytene or early strepsitene. The nucleus has enlarged and the chromosomes have shortened and spread more evenly through the nucleus. Whole chromosomes are more easily observed (fig. 35, Plate 17). From the same slide as the preceding. Haematoxylin.

Fig. 17. Diplotene. Chiasmata visible (cf. figs. 38, 38*a*, Plate 17, and fig. 51, Plate 18). From the same slide as the preceding. Haematoxylin.

Fig. 18. Diakinesis. For details see fig. 39, Plate 17, and compare with fig. 52, Plate 18. From the same slide as the preceding.

Fig. 19. First meiotic metaphase with polar and side views visible, from the same slide as the preceding. For details see figs. 40, 41, Plate 17.

Fig. 20. Very late anaphase and interkinesis. Compare with figs. 54–56, Plate 18. From the same slide as the preceding.

Fig. 21. The second meiotic metaphase showing the reduced number of chromosomes (22). Fixed in chromacetic formalin, stained in gentian violet.

Fig. 22. Anaphase of the second meiotic division and early tetrad. For detail of chromosomes see fig. 45, Plate 17. From the same slide as figs. 15–20. Haematoxylin.

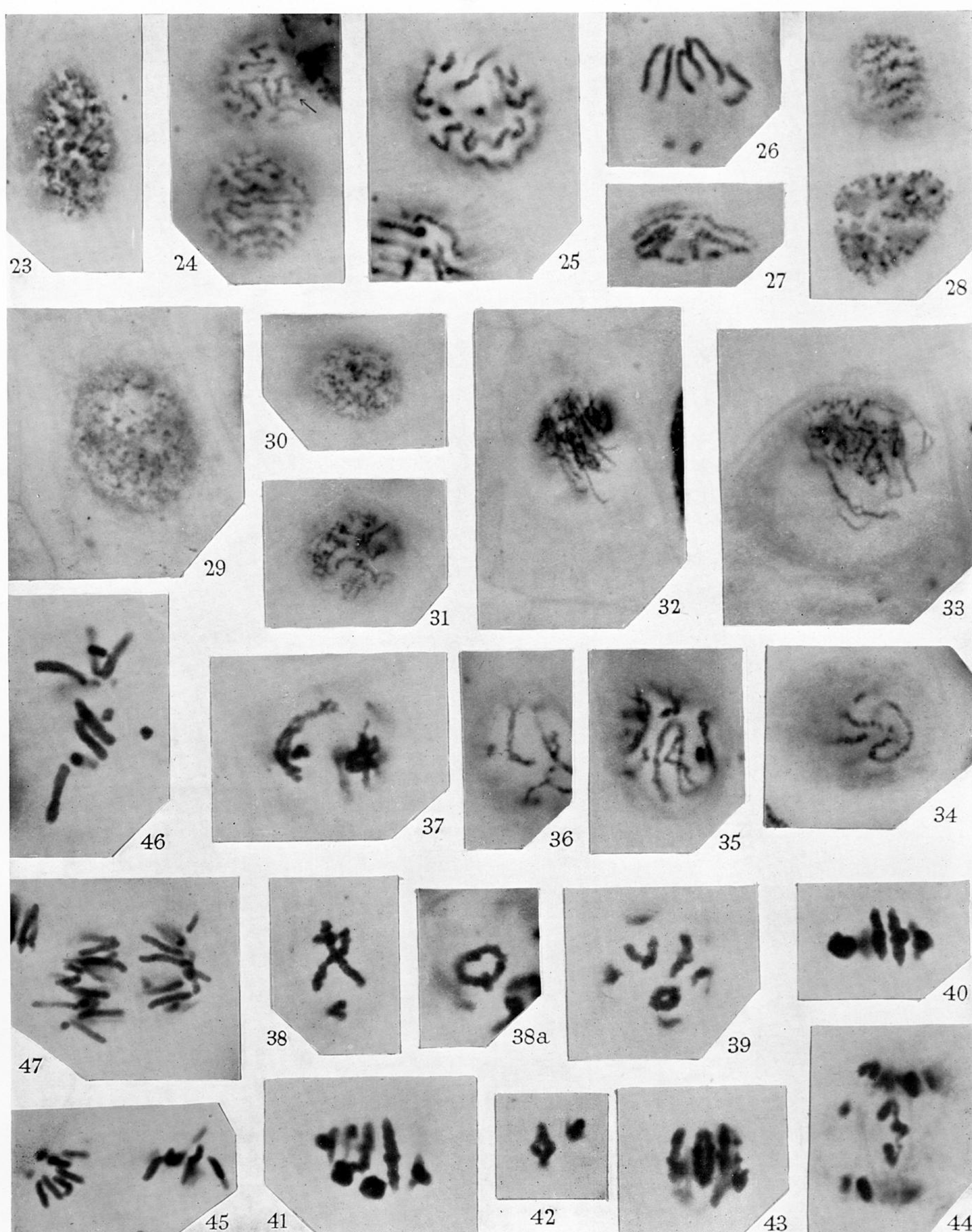


PLATE 17

Detail of chromosome appearances at principal stages of mitosis and meiosis, from the sections used for Plate 16. Magnification throughout the plate × 2000.

FIG. 23. Early premeiotic prophase, chromosomes still diffuse. From the sporangium of fig. 2, Plate 16. Gentian violet.

FIG. 24. Middle premeiotic prophase. Chromosomes distinct and still spirally coiled. Other focal levels of the measured chromosome from the upper nucleus are given in figs. 80*a–c*, Plate 19, and a general view of the tissue in fig. 3, Plate 16. Gentian violet.

FIG. 25. Late premeiotic prophase from a nucleus of fig. 4, Plate 16. The chromosomes apparently shorter and thicker and visibly double. The original spiral uncoiling as new spiral forms in each chromatid. Gentian violet. Zeiss 1·4*.

FIG. 26. Detail of anaphase chromosomes for size and demonstration of subterminal attachments, from the sporangium of fig. 7, Plate 16. Gentian violet.

FIG. 27. Detail of early telophase showing appearance of spirals in the chromosomes, from the sporangium of fig. 1, Plate 16. Gentian violet. Zeiss 1·4*.

FIG. 28. Late premeiotic telophase from the sporangium of fig. 9, Plate 16, showing the spirals. Gentian violet. Zeiss 1·4*.

FIG. 29. Rest before meiosis. Chromosomes diffuse and staining very faintly, from the sporangium of fig. 10, Plate 16. Gentian violet.

FIG. 30. Early preleptotene showing closely convoluted threads. Gentian violet. Zeiss 1·4*.

FIG. 31. Late preleptotene from the sporangium of fig. 12, Plate 16. The convolutions straightening. Gentian violet. Zeiss 1·4*.

FIG. 32. Leptotene. Convolutions disappeared and chromosome movement begun. Compare this with fig. 13, Plate 16, and fig. 48, Plate 18. Gentian violet. Zeiss 1·4*.

FIG. 33. Zygotene from the sporangium of fig. 14, Plate 16, showing parts of paired and unpaired chromosomes side by side. Stain haematoxylin. Zeiss 1·4*.

FIG. 34. Early pachytene from the sporangium of fig. 15, Plate 16, showing change of texture. Haematoxylin. Zeiss 1·4*.

FIG. 35. Late pachytene from the sporangium of fig. 16, Plate 16, showing a whole chromosome incompletely paired. The ends are slightly out of focus. A drawing is given in text-fig. 3, p. 204. Haematoxylin. Zeiss 1·4*.

FIG. 36. Another piece of chromosome from the same sporangium as the preceding, showing the mutual twist of the paired chromosomes more clearly. Zeiss 1·4*.

FIG. 37. A single chromosome at strepsitene showing the remains of the pachytene coiling and considerable shortening. Gentian violet. Zeiss 1·4*.

FIGS. 38 and 38*a*. Diplotene. Two paired chromosomes cut out of one photograph. Fig. 38 is a single-chiasma configuration with a cut piece of another chromosome superimposed; fig. 38*a* is a double chiasma figure. Gentian violet. Zeiss 1·4*.

FIG. 39. Diakinesis with single and double chiasma figures. Haematoxylin. For fuller information see fig. 52, Plate 18.

FIG. 40. The first meiotic metaphase in side view showing some chromosomes on the spindle. Haematoxylin. Zeiss 1·4*.

FIG. 41. Late metaphase of the first meiotic division, the spirals just detectable. From the same sporangium as the preceding. Haematoxylin. Zeiss 1·4*.

FIG. 42. Early anaphase of the first meiotic division, the chromatids beginning to separate laterally (both ends of the left-hand chromosome are out of focus, hence apparent shortness). From the same sporangium as the preceding. Zeiss 1·4*.

FIG. 43. Anaphase of the first meiotic division. Lateral separation of chromatids precedes unloosening of chiasmata. (The upper part of the middle chromosome is out of focus.) From the same sporangium as the preceding. Zeiss 1·4*.

FIG. 44. Late anaphase of the first meiotic division showing the effect of an interstitial chiasma which has delayed and distorted one side of a pair of chromosomes. For fuller details compare this with fig. 54, Plate 18, and figs. 72, 76 and 79, Plate 19. The specimen is fixed in 2BD and stained in brazilin. Zeiss 1·4*.

FIG. 45. Anaphase of the second meiotic division for comparison of chromosome size and shape with the preceding. From the sporangium of fig. 22, Plate 16.

FIG. 46. Late metaphase in a tapetal nucleus, the remains of mutual coiling of the chromatids visible in the bottom chromosome. From a sporangium with its archesporium in preleptotene. Zeiss 1·4*.

FIG. 47. Anaphase in a tapetal cell. Details of spiral structure in a somatic chromosome have only been obtained in the tapetum (fig. 71, Plate 19) but the chromosome size is strictly comparable with that in the archesporium (fig. 26).

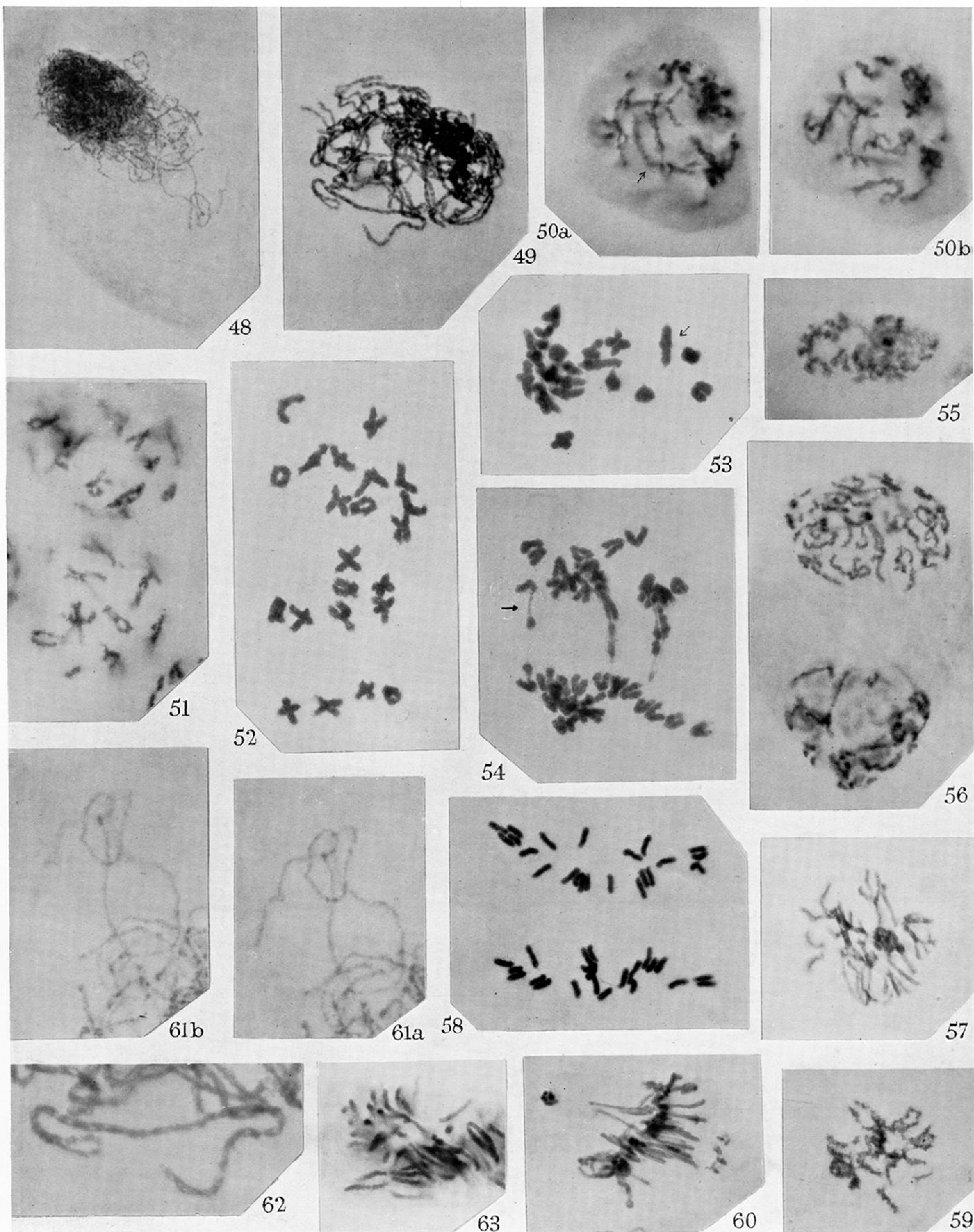


PLATE 18

Magnification ×1000 for all except figs. 61 and 62 which are ×2000*. All are permanent aceto-carmine preparations by a modification of McClintock's method (cf. Manton 1937). Figs. 48, 60 and 61 are from triploid *O. regalis*, figs. 55 and 59 from *O. gracilis*. All other figures are of diploid *O. regalis*, as in the previous plates.

FIG. 48. Leptotene in a triploid showing a whole chromosome, of which a drawing is given in text-fig. 6, p. 204. This chromosome is enlarged more highly in figs. 61 *a*, *b*.

FIG. 49. Pachytene showing one pair of chromosomes completely. This chromosome is given at a higher magnification in fig. 62.

FIG. 50 *a*, *b*. Two focal levels of an unsquashed nucleus at strepsitene. This is the earliest stage at which whole nuclei can be completely analysed. Its characteristics are shortening of the chromosomes and untwisting of the pachytene coiling.

FIG. 51. Diplotene in unsquashed nuclei.

FIG. 52. Diakinesis in a squashed nucleus showing number and position of chiasmata in all the 22 pairs of chromosomes.

FIG. 53. The first meiotic metaphase. The marked chromosome is the basis of measurements, since it is comparable in shape to that of fig. 65 on the spiral structure plate (Plate 19).

FIG. 54. Anaphase of the first meiotic division, showing V-shaped chromosomes before and after separation. For details of separation compare figs. 41–44, Plate 17, and for explanation of "necks" shown by the left-hand chromosome see figs. 72 and 76, Plate 19.

FIG. 55. Telophase after first meiotic division in *O. gracilis*. V-shaped chromosomes becoming diffuse.

FIG. 56. Prophase of second meiotic division, showing remains of spirals and V shape. For earlier prophase stage see fig. 77, Plate 19.

FIG. 57. Early metaphase of second meiotic division, in side view.

FIG. 58. Anaphase of second meiotic division showing 22 chromosomes (the haploid complement) at each pole.

FIG. 59. Telophase from a tetrad of *O. gracilis* showing 22 rod-shaped chromosomes becoming diffuse.

FIG. 60. Early metaphase of second meiotic division in triploid *O. regalis* showing the retention of coils by chromosomes lost at the previous division. These are lying in the cytoplasm near the two sides of the picture.

FIGS. 61 *a*, *b*. Two different focal levels of the leptotene chromosome of fig. 48 at a magnification of ×2000*.

FIG. 62. The pachytene chromosome of fig. 49 at a magnification of ×2000* showing coiling and incomplete pairing.

FIG. 63. Portion of a tapetal division, to show the size of somatic chromosomes in this technique, the chromosome used is the isolated one at the top. ×1000.

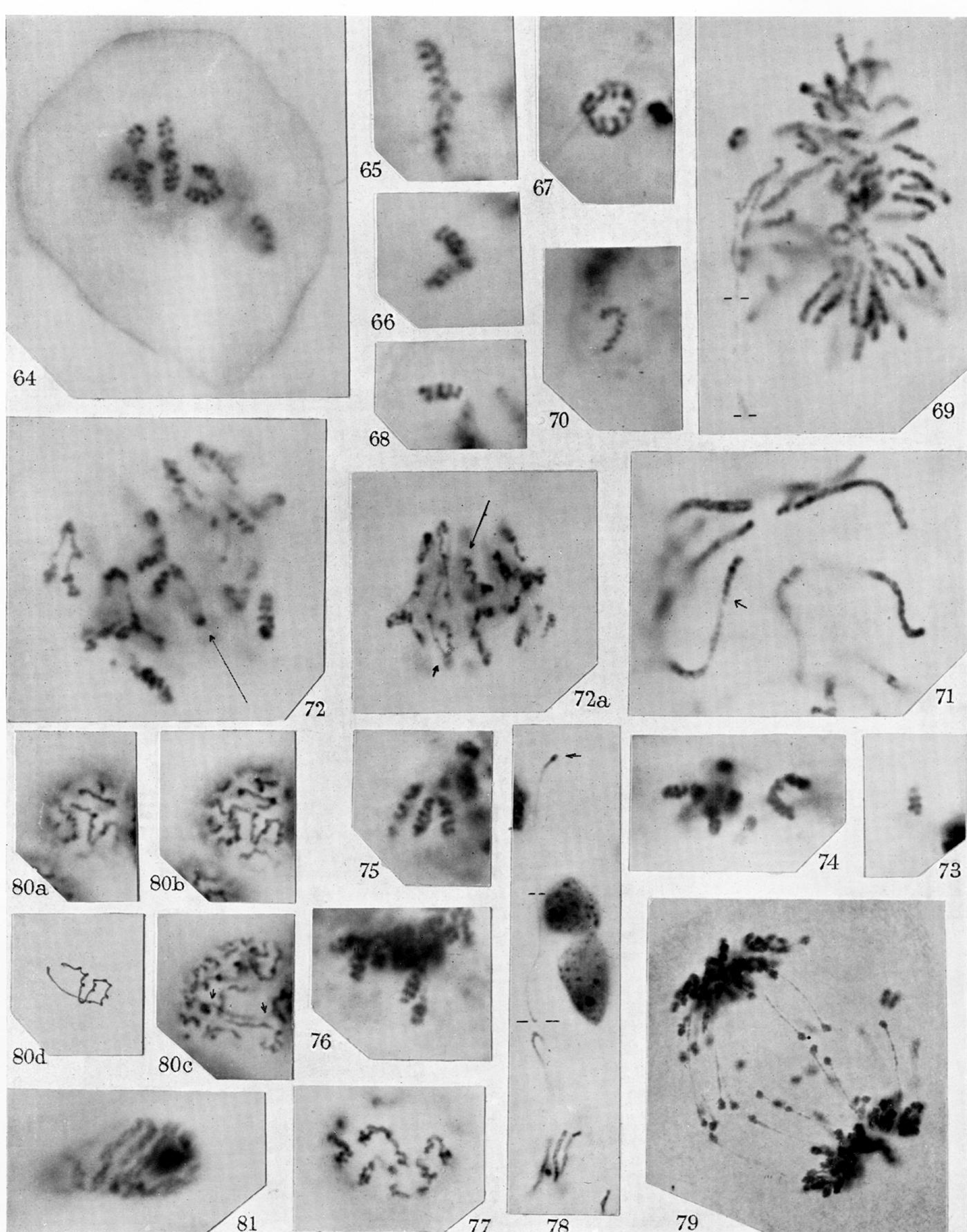


PLATE 19

Spiral structure in *Osmunda regalis*. Most of the figures are prepared by Sax and Humphrey's method and photographed at a magnification of × 3000.

FIG. 64. The first meiotic metaphase, showing spiral structure of chromosomes in position on the spindle. Magnification slightly less than × 3000 but identical with that of fig. 72.

FIG. 65. An isolated pair of chromosomes from the first meiotic division, with one sub-terminal chiasma at the end away from the spindle attachment. × 3000*.

FIG. 66. Similar to the last but with a chiasma at the same end as the spindle attachment. Magnification × 3000*.

FIG. 67. Similar to the last but with a chiasma at each end. × 3000*.

FIG. 68. An unpaired chromosome at the first meiotic division in a triploid. × 3000*.

FIG. 69. General view of the second meiotic division in a triploid treated for spiral structure. A damaged chromosome is visible on the left but details of the others are not easy to make out (compare therefore fig. 70).

FIG. 70. Clear details of a single chromosome at the second meiotic division in a triploid. The specimen is a lagging chromosome at late anaphase and shows 8 small coils. × 3000*.

FIG. 71. Tapetal chromosomes, *ca.* 14 coils visible in the marked one. × 3000*.

FIG. 72. Anaphase of the first meiotic division at the same magnification as fig. 64 (i.e. × slightly less than 3000).

FIG. 72*a*. Another focal level of the preceding at two-thirds the magnification, i.e. × *ca.* 2000.

FIG. 73. Anaphase in an unpaired chromosome in a triploid (cf. fig. 68 for the metaphase appearance) which has divided prematurely at the first division. The polar group to which the half-chromosome is going is visible to the right of the figure. The other half is in a comparable position at the other end of the cell but there is no room to include it in the plate. The long axis of the spindle is placed vertically. × 3000*.

FIG. 74. Late anaphase at the first meiotic division in a normal plant, the details of a V-shaped chromosome very clearly shown × 3000.

FIG. 75. Similar to the last but with spirals more distinct. Recovery from temporary deformation, of the type shown in the V-shaped chromosome on the left of fig. 72, complete. × 3000*.

FIG. 76. A chromosome at late anaphase in a triploid which has not only straightened but stretched in coming loose from its fellow. Recovery from stretching is much slower than recovery from straightening. For other examples of this see centre of fig. 72, fig. 79; fig. 54, Plate 18, and fig. 44, Plate 17. × 3000*.

FIG. 77. Very early prophase of the second meiotic division showing the spirals. These are very easily observed at this stage and the specimen has not been specially treated for spiral structure. It is from the ordinary sectioned series of Plates 16 and 17. The stage depicted comes in between those of figs. 55 and 56, Plate 18. × 2000*.

FIG. 78. Part of the same tapetal cell as fig. 71, at a lower magnification, showing undamaged chromosomes at the bottom and mechanically stretched chromosomes at the top. × 1500.

FIG. 79. Anaphase of the first meiotic division in a tetraploid cell showing exaggerated development of "necks" due to enhanced difficulty of disjunction in multivalent groups (cf. fig. 76 and fig. 54, Plate 18). × 1000.

FIG. 80*a–c*. Three focal levels of a somatic chromosome at middle prophase from the nucleus of fig. 24, Plate 17. Fig. 80*d* is a drawing of the whole chromosome. × 2000*.

FIG. 81. Fairly early prophase in a root of *Allium ursinum* to illustrate the mutual coiling of split halves of a chromosome at this stage. × 2000*.