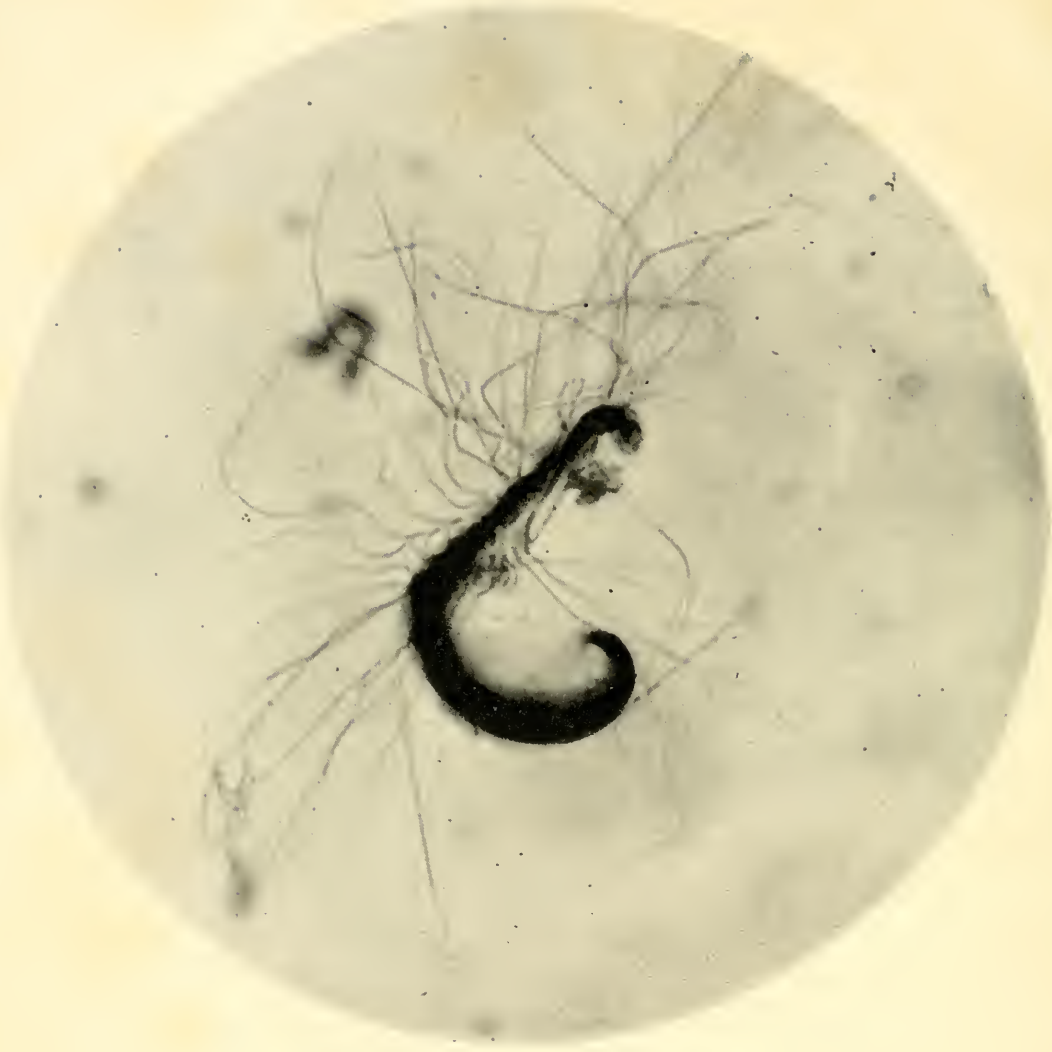
PROBLEMS OF CYTOLOGY AND EVOLUTION IN THE PTERIDOPHYTA



The motile spermatozoid of a fern (*Dryopteris Villarsii* from Cyprus) killed with osmic vapour and photographed with ultra-violet light at a magnification of three thousand diameters. The organs of locomotion are numerous cilia, each of which is a tuft of still finer threads. The body of the spermatozoid appears black because it is almost entirely composed of the nucleus which absorbs ultra-violet light strongly. In life the body is tightly coiled.

PROBLEMS OF CYTOLOGY AND EVOLUTION IN THE PTERIDOPHYTA

BY

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PREFACE

The publication of this book is one of the more harmless consequences of the Second World War. The scientific work which it contains was begun in 1932, at first with no clearly defined programme but rather as a hobby in relation to a field botanist's interest in some attractive British plants, although in part also as a salutary exercise and test of skill. For the Pteridophyta are difficult cytological material. They keep a cytologist on his mettle the whole time to an extent that the student of the more familiar Flowering Plants only rarely experiences, and only by patience, skill and the application of the most modern methods can success be achieved. It is, indeed, no accident that, in spite of three-quarters of a century of 'modern' cytological research by many workers to whom at first the Pteridophyta were familiar and much used material, there is at present practically no single species except *Osmunda regalis* for which the available published accounts can be accepted as accurate. This much will at once be clear to anyone who takes the trouble to compare the photographs reproduced in this book with the quotations from the literature in even a recent compilation such as that of Löve and Löve (1948). To remedy this state of affairs individually for each species to be handled has therefore been a problem in itself, and only after the inquiry had been in progress for some years did the several parts of it begin to take on the coherent form of a wider investigation on the lines indicated in the first two chapters. Some of the episodes, notably the study of autopolyploidy in *Osmunda* (Chapter 3), were at an advanced state of completion fairly early, since in this particular case the inquiry had been used as a preliminary study, on material of known origin and with fairly large chromosomes, to serve as a background to the elucidation of cognate problems in other groups. The published accounts which subsequently appeared, of the flowering plants *Biscutella* and *Nasturtium*, undoubtedly benefited greatly by this procedure, though the work on *Osmunda* itself remained unpublished. Other subjects, notably the taxonomic analysis of the Male Fern (Chapter 4), were undertaken as it were involuntarily, having arisen as an unexpected complication in an inquiry intended to elucidate the cytology of apogamy. This in turn led on to the wider consideration of the British species of *Dryopteris* (Chapter 5) and so on. Being incidental to other work, very little had been rounded off for publication before the war broke out and, indeed, the only significant item had been the appearance of a preliminary note on the Male Fern published in *Nature* in August 1939, in reply to a longer paper by Döpp on the same subject, which had been received in July of that year. Immediate publication of a fuller statement on the Male Fern work would have followed the appearance of the preliminary note, and this would have been succeeded by serial publication of the other subjects but for the outbreak of hostilities in September 1939. The gravity of the international situation then made it appear somewhat frivolous to continue such a programme at such a time, and as a matter of deliberate policy throughout the war years publication was only attempted for work of exceptional importance. As regards

the evolutionary studies in the Pteridophyta, this amounted only to a short 'Note on the Cytology of *Psilotum* with special reference to Vascular Prothalli from Rangitoto Island', which appeared in the *Annals of Botany* in 1942, since in that case the possible destruction by enemy action of the unique material supplied by the late Dr Holloway of New Zealand, or of the manuscript containing the cytological description of it, would have constituted a serious loss to science. As the war advanced, continuance of the observational work became more and more difficult, and a large programme of genetical inquiry which had been planned to amplify the results for the Male Fern and other species had largely to be abandoned. Many valuable plants were lost either in air raids or from neglect. Nevertheless, at the close of hostilities a sufficient thread of continuity had been maintained to produce the situation that whereas in 1939 some half-dozen draft manuscripts of projected papers had been held back for further work, in 1945 these and several other topics had been developed as far as could reasonably be expected with the material available in England. The congestion which would result from the simultaneous presentation of such a large number of papers to the depleted scientific journals of the immediately post-war years was, however, painfully apparent, and for this reason book form was first envisaged.

Anyone who has shouldered the task of reducing to manageable proportions the scientific notes of fifteen years' observation on more than this number of different topics will perhaps treat the present author with sympathetic forbearance. Without the constant help and encouragement of many friends and colleagues in the University of Manchester and more recently in Leeds, exhaustion might well have set in and the project have been abandoned. Not only has it been a matter of clinching the observations on literally hundreds of the troublesome details which remain uncertain long after the broad outlines of results have been safely established, but the mode of presentation is somewhat unfamiliar. The potential reader of a scientific paper can be judged with reasonable certainty and the style of writing chosen accordingly. The reader of a book is more unpredictable, and whatever modifications of style are adopted to adjust the subject-matter to book form it is almost inevitable that certain parts will be too simple for some readers and too difficult for others.

To what extent the author will have succeeded in the attempt to write simply is for others to judge, but in the hope of making the work understandable to as wide a circle of readers as possible, most of the essential concepts and such technical terms as are unavoidable are introduced by means of illustrative examples in the first three chapters, and the only mental equipment which is presupposed in the reader is an elementary acquaintance with simple Mendelism and some general awareness as to what is meant by a chromosome. To a botanist who has this equipment as a matter of course a glance at the illustrations will convey the factual content of Chapters 1–3, and the book may be said to begin in earnest at Chapter 4. A zoologist interested in general evolutionary studies, and similarly equipped but lacking an intimate acquaintance with plants, would do well to begin at Chapter 2. A field naturalist, however, whose primary interest is not the laboratory but the life of plants in the field, may need to read parts of the first three chapters twice over, after which he should have no more difficulty in understanding the rest of the book than in reading, for example,

Dr Turrill's volume on *British Plant Life* in the New Naturalist Series (No. 10, 1948), a book which may indeed be recommended as an excellent introduction to the present study.

With regard to the illustrations a word of explanation should perhaps be offered as to their purpose and use. In a group like the Pteridophyta, where the technical difficulties are so great that it has been my unfortunate lot to have to correct errors in the work of almost every previous investigator, the attainment of accuracy has been a primary task without which no valid general conclusions could have been drawn. For this reason the use of photography has assumed a special importance. The data themselves have in the first place been assembled as far as possible according to the principle that what cannot be photographed cannot be used as evidence. The photographic evidence so assembled has then been utilized to provide illustrations to the book on a scale which should enable the reader to repeat for himself upon the printed page enough of the observations quoted to judge of their validity. Only so can finality in the establishment of basic facts be hoped for, and that many errors have been removed or prevented by this procedure is certain. That some errors may still remain is, however, only too probable, although where uncertainty is known to exist it is unlikely to exceed the limits indicated in the text.

All details regarding technical methods are given in the Appendix under the two heads of cytology and photography. In cytology, more perhaps than in any other science, progress depends on manipulative skill and that type of low cunning which is needed to apply old methods to new uses. A considerable range of methods old and new will be found illustrated throughout the book, from which it will perhaps be clear that the shortcomings of previous workers, to which attention has so often to be directed, have been due in the main to the shortcomings of their tools. Standard techniques such as that of sections stained in haematoxylin or gentian violet are indispensable for certain purposes, and when used with precision in easier material can yield all the information required. This is not, however, the case in the Pteridophyta. Sections are indeed of great value as supplementary evidence and for comparative and morphological purposes, but except in very rare cases, the Osmundaceae and *Selaginella* being the only ones known to me, sections alone are inherently unsuitable for accurate cytological work. Only by the squash methods, either with Feulgen staining or with aceto-carmine or other reagents, can accuracy be reached. In some groups with large sporangia, such as the Osmundaceae, Eusporangiatae, Lycopods, Horsetails and so on, smear or squash methods as devised for flowering plant stamens can be used without modification. The Leptosporangiate Ferns, however, which form the subject-matter for more than half the book, have sporangia far too small for this. With only 8 or 16 mother cells in each it is impossible to handle a sporangium singly, and the fact that they grow in sori in which many developmental stages are always present together offers what at first sight seems like an insuperable obstacle to the use of the simpler smear methods. The success, such as it is, which is claimed in the present study is primarily due to the overcoming of these preliminary difficulties as a result of which the power of new techniques has become fully available, and it should be found that, undetected accidents apart, in the main where accuracy is claimed, even in species like *Cystopteris* with $n = 126$,

the facts are as fully authenticated and as firmly established as is the chromosome number of the Broad Bean.

In an extended inquiry all aids to accuracy which can relieve the observer of personal fatigue are of great importance. In this connexion the photographic methods (Appendix 2) used for the making of black and white diagrams are perhaps of some interest. Practically none of the numerous black and white illustrations of fern morphology and of chromosomes has been based on a drawing of the usual type, and in dispensing entirely with the old-fashioned camera lucida both speed and accuracy have been greatly improved.

With regard to the identification of material, this has not often been in any doubt, although as a precaution close contact has been maintained with the herbaria at Kew and the British Museum throughout the work. Since, however, it may perhaps be of importance to later investigators to know more about the actual specimens used, a herbarium specimen has been retained for almost every species, access to which may be had on request either direct to the author or to the authorities at Kew.

With regard to nomenclature, a perennial difficulty, this has become more acute during the preparation of the manuscript owing to the numerous changes in taxonomic usage regarding familiar species which have occurred since the work began. The *Check List of British Vascular Plants*, published under the auspices of the British Ecological Society (Clapham, 1946) in preparation for the compilation of new British Floras, has emended terminology partly in accordance with preliminary communication of the cytological results (as in the Male Fern complex, Manton, 1939) but in part independently of this. More extended works, such as the *Genera Filicum* of Copeland (1947) or the *Revised Classification of the Leptosporangiate Ferns* by Holttum (1947), have similarly affected the views previously current on questions of phylogeny. Since one of the aims of the cytological study is to provide independent evidence on taxonomic matters it has been felt that to revise the work constantly to incorporate these various developments as they appeared was neither necessary nor desirable. As a basis of discussion any system of classification and naming will suffice provided only that it is familiar and unambiguous. Both these conditions are fulfilled by the very familiar phyletic views of F. O. Bower and by the system of nomenclature of a standard Flora such as Babington's *Manual of British Botany* (11th edition, 1922). At the time the work began these were all that were available, and they still seem suitable as a framework on which the cytological facts can be disposed. Emendations which seem necessary in the light of the cytology itself can then be clearly distinguished and their agreement or disagreement with newer views based on other evidence assessed. For ease of reference, however, the more important cases in which the integrity of species has been disturbed by the cytological facts are listed in Appendix 3, and a complete list of new chromosome numbers is added in Appendix 4.

That the work is incomplete is self-evident and a defect for which apology cannot be made. The stopping place in an extended inquiry is in a sense arbitrary and could be indefinitely postponed if it were made to wait upon completion of every item raised. One can only repeat with Goethe that 'Die Kunst ist lang; und kurz ist unser Leben', and since the ground which can be covered by the labours of one worker is

narrowly circumscribed of necessity, one should perhaps be thankful that in the midst of the difficulties of this atomic age any progress has been made at all.

It would be invidious, in a work which owes such a heavy debt to others, to select many names for special mention. An exception must, however, be made for Professor W. H. Lang, without whom the work would not have been done at all. And of the many contributors who are not my colleagues, I must record a special debt to Dr R. L. Praeger of Dublin, Mr F. Ballard of Kew, Mr A. H. G. Alston of the British Museum, and Dr B. T. Cromwell of Hull. For personal assistance I am specially indebted to Professor Lang's former research assistant, Mr Ashby, for help over the many years I spent in Manchester, and, more recently, to Mr B. Clarke for similar assistance in Leeds. Finally, I am indebted to the Oxford University Press, for permission to reproduce various figures from my own papers in the *Annals of Botany* and the *Journal of Experimental Botany*, to the Royal Society for similar permission regarding papers in their *Proceedings* and *Transactions*, to Professor R. Nordhagen of Oslo and Professor P. Martens of Louvain for permission to use Figs. 49 and 142 respectively, and to various friends and colleagues for the originals of photographs which have been reproduced as figures, notably to Professor Lang for Figs. 8*a*, 12 and 143, to Dr H. F. Dovaston for Fig. 98 and to Mr Ashby for many of the natural-sized and low-power photographs throughout the book.

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CHAPTER 1

INTRODUCTION TO THE METHOD

A scientific historian writing at some future time about the growth of biology in the nineteenth, twentieth and perhaps twenty-first centuries might be expected to begin his account somewhat as follows:

'The nineteenth century was a period of great intellectual activity in biology, carried on it is true by a limited number of people but containing among them some of the most powerful minds of the age. The perfecting of the compound microscope was the most significant technical development, by means of which, for the first time, animal histology and plant anatomy, together with the descriptive facts of life history of the principal organisms of both kingdoms, could be adequately explored. On the theoretical level the theory of evolution, coming as it did concurrently with this, and based as it was in the hands of Darwin on the accumulated content of several centuries of morphological observation of living and extinct plants and animals, wound up as it were the purely descriptive phase of these sciences and paved the way for the experimental period which was to follow.

'The effect of the theory of evolution on human thought as a whole was revolutionary and in many ways catastrophic, but this aspect of the history of ideas need perhaps not be discussed by the merely scientific historian. Within biology itself the effect, though actually profound, was superficially less marked. The great achievements of the pre-Darwinian period, namely, the establishment of workable principles of nomenclature, description and classification, together with the recognition of the nature of fossils and of their use for the compilation of the geological time scale, remained almost unaffected in their factual content although profoundly influenced by the new conception of their purpose. The quest for phylogenetic significance became a conscious aim to be pursued almost to the exclusion of all else towards the close of the century, and under it the descriptive aspects of morphological biology expanded rapidly with the renewed vigour and interest which the change of aim awakened. The methods by which this aim were pursued remained, however, substantially the same as those available to Darwin himself until, with the twentieth century in sight, the change from observation to experiment set in.

'This change was closely associated with the gradual recognition, not by isolated individual workers but by the scientific world as a whole, of the need for an objective study of the facts of variation as the next step required by the Darwinian theory of evolution. This need was first effectively voiced by Bateson in the celebrated introduction to the *Materials for the Study of Variation* of 1894, and it led directly to the rediscovery in 1900 of the work of Gregor Mendel.

'The effect of Mendel's papers at this their second appearance was at first to obscure the connexion between evolution and the study of variation owing to the necessary preoccupation of geneticists for the next few decades with establishing the rules of

their craft by an extension of the Mendelian experimental methods. The evolutionary context, however, came back into the picture after the unification of genetics with the sister science of cytology, which occurred in the period between the first two world wars. This was made possible in the first instance by the introduction of team work into genetics, notably by the American school of workers on *Drosophila* founded by Morgan in the 1910's, but the final proof that the chromosomes are the seat of Mendelian inheritance was first conclusively given in 1931 simultaneously for an animal, *Drosophila* (Stern, 1931), and a plant, *Zea mays* (Creighton and McClintock, 1931).

'The resulting establishment of cytogenetics as an exact science must be recognized as one of the biggest intellectual achievements of the first half of the twentieth century. The roots of cytology, as of genetics, can be discerned in the nineteenth century, some well-known landmarks being 1875, the date of the publication of *Zellbildung und Zelltheilung* by the botanist Strasburger with its factual description of mitosis,* and 1894, the numerical demonstration of chromosome reduction by the same author. Nevertheless, we know that cytology no less than genetics was a twentieth-century science from the fact that almost everything in Strasburger's 1894 paper was erroneous except the basic numerical conclusion. It required the pioneer work of Grégoire in the years preceding 1910, Janssens, Belling and others in the 1920's, and many more workers, both earlier and later, to stabilize technique and to elucidate the fundamental descriptive facts of meiosis * without which cytogenetics could not have been established.

'By this means as the century advanced, a new tool of great and unexpected power was made available for students of evolution. It became possible to investigate the nature of species, or at least of some species, experimentally, to diagnose their mode of origin and trace with precision some significant parts of their genealogy. The preoccupation of biologists with tracing phylogeny on the grand scale gave place to the attempt to analyse some actual evolutionary mechanisms by experimental means. The success which attended these efforts was, in the first place, of importance as a direct proof, if proof were needed, that the idea of evolution represented not a theory but a fact, and in the second place to shatter the Darwinian conception as to ways and means.

'The destructive effects of the new knowledge on the compelling simplicity of "Darwinism" came from the recognition that its apparent simplicity was oversimplification, and that what was next required was not one generalization to account

* An elementary acquaintance on the part of the reader with the basic facts of mitosis and meiosis is here presupposed, although it is sufficient at this stage to know merely that mitosis is another name for nuclear division of the ordinary kind, while meiosis is a peculiar form of nuclear division, sometimes referred to as reduction division, which occurs at one point only in the life cycle of every species which reproduces by sexual means. In mitosis the chromosomes split longitudinally and their number remains constant throughout the process. In meiosis there are always two nuclear divisions in rapid succession, in the first of which the chromosomes pair and then separate to opposite poles so that their number is halved in each of the resulting nuclei. A detailed knowledge of the mechanism of meiosis is not required for the purpose of this book, but such parts of it as are essential, notably some details of chromosome pairing, will be described in relation to illustrative examples in Chapters 1 and 2 and especially Chapter 3.

for the origin of species in the singular, but a painstaking analysis of numerous special cases of the origins of species in the plural, carried out objectively and without undue deductive reasoning until sufficient wealth of well-authenticated individual cases should have been assembled to make a fresh generalization possible. This programme of work took the rest of the twentieth century to carry out and was still occupying the attention of many minds in the middle of the twenty-first.

'By this time', our historian might continue, 'two further events had occurred. The effect of the visual light microscope on the scope of work of the nineteenth century was repeated at a different level in the twentieth by the perfecting of the electron microscope. This brought the field of molecular structure under direct observation, and made possible for the first time a full description of organic materials. Simultaneously, a synthesis was effected between cytogenetics and the previously independent science of enzymology, so that the dynamics of living matter could at last be explored. The consequences of these developments were naturally not fully appreciated until the twenty-first century was well under way, although the shadow of big things to come was clearly discernible by the middle of the twentieth.'

Taking leave of our historian while yet there is time and before he intoxicates us with a preview of distant scenes, we may ask in sober earnest what has in fact happened to the theoretical background of knowledge in almost a century which has elapsed between publication of *The Origin of Species* in 1859 and the present day? As I see it, the change in our attitude to theories of evolution rests principally in a new precision which can now be attached to the words 'variation' and 'variability'. In Darwin's day these concepts were so ill-defined that plausible assumptions, uncontrolled by any reference to experiment, could at any time be made without serious challenge except on *a priori* grounds. At present we possess enough exact knowledge about both terms to be able to discuss the basic concepts of this or that general theory of evolution, not on logical grounds alone, but to some extent on a basis of fact. The underlying assumptions required by simple Lamarckism (evolution by use and disuse), or simple Darwinism (adaptive evolution by gradual accumulation of minute heritable differences), or the mutation theory first voiced by Darwin's earliest opponent, Richard Owen, and later much elaborated in the hands of de Vries and others (evolution by sudden leaps), can *all* now be seen in their original form to be over-simplifications. They may contain a greater or less germ of truth, but they cannot be the whole truth, for we now know enough to be certain that variation is not one process but many; different types of variation have widely different causes and consequences, and all follow their own laws of behaviour which must first be elucidated before they can safely be built into any theoretical scheme. The task before us is therefore seen to be something quite different from that with which the theorists of the last century were concerned. We have a new field of knowledge to explore, and the exploration is only just beginning. Exactly where it will end cannot at this date be wholly foreseen, and it may therefore be well at the outset to disinterest ourselves from general theories in order to concentrate the better on a limited number of rather fundamental questions. How many types of evolutionary activity can we actually detect? How do these differ and what are their characteristics? What proportion do the analysable cases bear to the unanalysable?

And what, lastly, are the accumulated effects of each type of evolutionary change likely to be if carried on and repeated over a span of millions of years? Only when some reply has been obtained to this last question shall we be in a position to assess the real power of our present tools and to judge whether or not a generalized evolutionary theory can in fact be constructed.

It may be said at once that this stage will not be reached in the course of this book, nor, in the opinion of the writer, is it to be looked for for many years to come. In the meanwhile we may cultivate our garden, but before doing so it may perhaps be of help to the uninformed reader, if such there be, to explain a little more precisely what it is that cytogenetics at its present stage of development can do.

Genetics alone can contribute much to an understanding of the differences which separate natural units of less than specific rank. It is true that inquiry is much restricted by the difficulty in most cases of getting behind the necessarily vague concept 'genic mutation'. Sometimes we can determine the place on a chromosome where a 'mutation' has occurred. In other cases we can measure some statistical facts about its frequency of recurrence and can sometimes alter this frequency by deliberate interference (induced mutations). As a rule we do not know at all *what* has occurred unless a piece of chromosome large enough to be seen has become lost or misplaced. We are likewise generally ignorant of how the mutation acts to produce its visible effect. The effects can, however, be studied, their distribution in the progeny of crosses analysed and predicted, both under controlled conditions and to some extent in natural populations, and the accumulated knowledge so gained can in favourable cases give us the basis of a numerical idea of the relative complexity of genetical differences which separate one natural form from another. These natural forms are, however, very rarely, if ever, species, and as a general rule, genetics, unaided by cytology, is unable to extend its analysis beyond the level which Goldschmidt has fittingly labelled 'Microevolution' to contrast it with 'Macroevolution', on which alone the attention of the older evolutionists was bent.

Cytology is, however, in somewhat better case. With the knowledge that the chromosomes are the seat of genetically active materials and that changes in these are the physical basis of evolution, the comparative study of chromosomes, if informative at all, can be used to give evolutionary information of a type which no other morphological detail can supply.

The comparison of chromosome *numbers* between related forms can in favourable instances be a conclusive guide to phylogeny. The classic case of *Spartina Townsendii* is a well-known example. This putative hybrid detected in Southampton Water in 1870,* and variously listed by Wallace, Hooker and others as an endemic variety or species, or as an interspecific hybrid of spontaneous local origin, was conclusively proved to be the cross between our native *S. stricta* and a locally introduced alien from

* A good general account of the history of the discovery and spread of *S. Townsendii* on the British and French coasts will be found in Stapf (1927), from which it appears that the first person to propose a hybrid origin for the species was a French botanist, Foucaud, in 1894. Other early references to the plant are Wallace's *Island Life*, 2nd ed. (1892), and Hooker's *Student's Flora*. Huskins (1931) contains the cytological facts and final diagnosis.

North America, *S. alterniflora*, as soon as an accurate chromosome count could be made (cf. Huskins, 1931) of the three forms in question. The endemic species *S. Townsendii* could then be seen to contain twice the sum of the gametic chromosome numbers of the two putative parent species and was therefore clearly the hybrid between those species, which had doubled its chromosomes and become fertile and true breeding in consequence.

Comparative *morphology* of chromosomes may also give highly significant information, some very striking examples being the work of Babcock (1947) and his colleagues and collaborators on *Crepis* in plants and the numerous studies on various genera of flies, notably *Drosophila* and *Sciara*, some of which have been summarized recently by Dobzhansky (1937) and by White (1945). From these it is clear how important a part gross structural changes have played in the evolution of species in these groups.

A still more powerful tool lies in the accurate analysis of chromosome *pairing*, either at meiosis or, in the special case of the Diptera, in the salivary glands. The detection of failure of pairing or irregular pairing in a wild plant or animal will often reveal a hybrid with certainty where other evidence is inconclusive or misleading. The classic cases of *Drosera obovata* (Rosenberg, 1909), *Rosa* (Blackburn and Harrison, 1921), *Hieracium* (Rosenberg, 1917), etc., are examples which could be indefinitely multiplied. This type of inquiry has been of enormous benefit to pure taxonomy wherever natural barriers between species have been obscured by the existence of unrecognized hybrid forms, some of which, as in the case of *Rosa*, may have been perpetuated from remote ages by the adoption of a non-sexual mode of reproduction. The presence of extra chromosomes is revealed by the formation of multivalent groups. These may occur singly as in the sex chromosomes of *Rumex acetosa*, in which the male plant has a trivalent group in place of the bivalent pair of the female, in this case betokening perhaps no more than the fragmentation of one sex chromosome. The presence of multivalent groups on a considerable scale more commonly denotes the presence of duplicated sets of chromosomes which constitutes the phenomenon of polyploidy.* This may be autopolyploid, *i.e.* due to exact duplication of identical gametic sets with at most only a minor amount of mutational difference of 'genic' origin to distinguish them. Tetraploid *Biscutella laevigata* (see below) in the Swiss and Austrian Alps is an example of this

* Polyploidy (Winkler, 1916) is the name given to the state of a cell, organism, or tissue, in which the number of chromosomes contained in the nucleus is a simple multiple of the chromosome number of some other, related, cell, organism or tissue. When the nuclear cycle of the higher plants was first worked out at the beginning of the century the words haploid and diploid were introduced to designate the reduced and unreduced nuclear states of the same organism. When it was later realized that other multiples of the basic gametic number could exist, the term haploid has in certain contexts given place to monoploid, and additional terms such as triploid, tetraploid, pentaploid, hexaploid and so on have had to be introduced to describe the various members of a series, which collectively represents the state of polyploidy. The word heteroploid is sometimes used as a synonym for polyploid (*e.g.* by Sharp, 1934), as also is euploid (Täckholm, 1922). Special types of polyploidy, namely, autopolyploidy and allopolyploidy (Kihara and Ono, 1926), are explained in the text on pp. 8–9; these have recently been abbreviated to autoploidy and alloploidy (Clausen, Keck and Hiesey, 1945).

The opposite condition in which the nuclei of related cells differ by some quantity which is not that of a simple multiple of a basic or monoploid set of chromosomes is designated aneuploidy (Täckholm, 1922) or dysploidy (see Clausen, Keck and Hiesey, 1945).

kind. If, on the other hand, the sets are so different from each other that their chromosomes cannot pair together, multivalents are not formed and we have allopolyploidy as in *Spartina Townsendii*, or to quote a more recently analysed case, *Nasturtium uniseriatum* (cf. p. 10). In both types of polyploidy precise phylogenetic evidence can be obtained if the polyploid forms are crossed with others of lower or higher valency and

Fig. 1. Diploid form of *Biscutella* from central France (*B. arvernensis* Jord.) showing young fruits, from a herbarium specimen collected in July, near Clermont Ferrand in the Auvergne. Natural size.

the chromosome pairing in such crosses analysed. This method can indeed be applied to all species or strains closely enough related to be crossed at all, and detailed information can then be compiled about the relative homologies of their respective chromosomes which may give a surprising insight into past history. Occasionally some similar evidence can be obtained more directly by inducing apogamy or parthenogenesis in a normally sexual organism and observing the presence or absence of chromosome pairing in the supposedly haploid set.

All these methods and others will meet us in the chapters which follow, but in order to give the reader some preliminary insight into the type of observations actually involved, it may be helpful to reproduce some of the photographs used in the analyses of *Biscutella* and *Nasturtium* listed above.

Biscutella laevigata L. is a fragrant little yellow-flowered member of the cabbage family, well known to tourists in Switzerland, France, Germany and Spain on account of its curiously shaped fruits (Fig. 1), which the Latin name of *Biscutella* compares to two shields, although at least one popular name ('Brillenschoten' in German) makes the comparison rather with a pair of spectacles. The species *B. laevigata* does not occur in Britain, but a number of different strains of it are met with as limestone rock plants in the lowlands or in subalpine meadows in the mountains of central and southern Europe. It is, however, by no means uniform throughout its range. The German and French lowland types have 18 chromosomes except in their pollen grains and embryo sacs, where the reduced number of 9 is found. In Switzerland and Austria, on the other hand, the plants all have twice as many chromosomes, 36 being found in their roots and 18 in most of their reproductive cells. Both types are, however, still interfertile, and hybrids between them, possessing the intermediate chromosome number of 27, are spontaneously formed when suitable plants are grown together in a garden. Fig. 2*a–c* shows the somatic chromosome numbers of these three types of plants, which may be taken as the first illustrative example of a polyploid series. The number 9 which they all share in varying degrees is the gametic number of the lowest member. This gametic number, which is fundamental to the whole series, is conveniently designated the monoploid number, in respect of which the plant with 18 chromosomes (Fig. 2*a*) is diploid, the one with 27 (Fig. 2*b*) triploid and that with 36 (Fig. 2*c*) tetraploid.

Chromosome pairing at meiosis in diploid, triploid and tetraploid *B. laevigata* is shown in Fig. 2*d–f*. In the diploid (Fig. 2*d*), pairing occurs in the simplest manner possible and nine pairs can easily be seen in the photograph. In the tetraploid of Fig. 2*f*, however, pairing is more complex, for the presence of four instead of two monoploid sets of chromosomes has led to the formation of numerous quadrivalent groups easily recognizable as such, where the four component chromosomes of a quadrivalent are joined in a ring, as may be seen in many places to the right of the figure. In the triploid (Fig. 2*e*), where there are three duplicate sets of chromosomes, trivalents and not quadrivalents are formed. These are also easily recognizable by their shapes, and in the cell figured there are five trivalents, four pairs and four univalents, the pairs and univalents representing potential trivalents which have fallen apart at an earlier stage into 2 + 1. Falling apart into lower valency components is liable always to affect a certain proportion of potential multivalent groups, since the successful cohesion among a group depends not only on homology (that is ability to pair) but also on the number and relative positions of the chiasmata which form after pairing has taken place and by means of which cohesion up till metaphase is made possible. Since the position of chiasmata is, to some extent, determined at random, the precise numbers of effective multivalents which appear at metaphase will vary somewhat from cell to cell. The numbers of multivalent groups visible in Fig. 2*e* and *f* are,

however, sufficiently high to be indicative of *auto*polyploidy in the cytological sense, by which is meant the duplication of identical, i.e. 'homologous', sets of chromosomes throughout the series, although the existence of slight genetical differences between the sets is not thereby excluded.

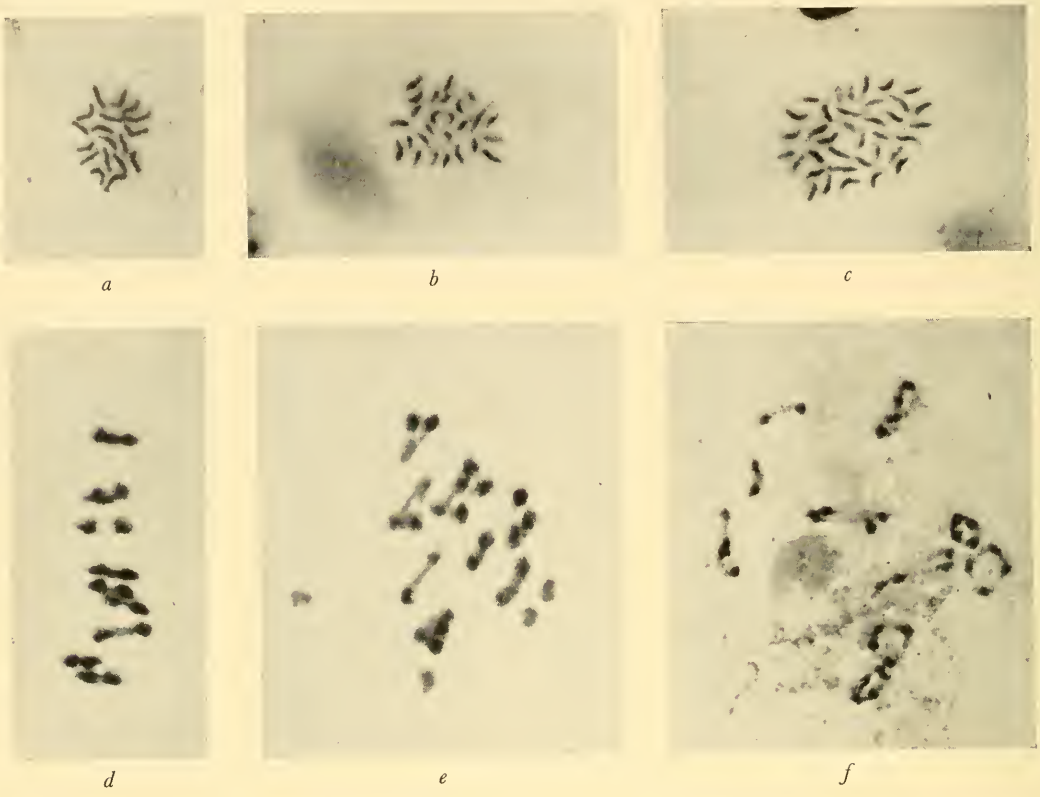


Fig. 2. Polyploidy in *Biscutella laevigata* L. *a–c* from Manton (1935*a*); *d–f* from Manton (1937). *a*. A diploid root of subsp. *alsatica* showing 18 chromosomes, from a section stained in gentian violet. × 2000. *b*. A triploid root of a hybrid between subsp. *alsatica* and subsp. *longifolia* showing 27 chromosomes, from a section stained in haematoxylin. × 2000. *c*. A tetraploid root of subsp. *longifolia* showing 36 chromosomes, from a section stained in gentian violet. × 2000. *d*. Chromosome pairing in the diploid showing 9 pairs, from a permanent acetocarmine preparation. × 1500. *e*. Chromosome pairing in the triploid showing trivalents, pairs and univalents, permanent acetocarmine. × 1500. *f*. Chromosome pairing in the tetraploid showing quadrivalents and pairs, permanent acetocarmine. × 1000.

From the evolutionary point of view these facts show that the natural populations of diploid and tetraploid *B. laevigata* in different parts of Europe are closely related phyletically and that the diploids are the older type. The first of these conclusions could have been deduced by the normal procedure of comparative morphology but the second could not, and in this particular case the association of newer and older forms with glaciated and unglaciated areas of central Europe respectively made it possible to suggest some rough approximations regarding their relative ages and the paths of

migration into their present habitats in terms of the Quaternary Ice Age (Manton, 1934, 1937).

The case of watercress (*Nasturtium*) is slightly more intricate because the chromosomes are more numerous and smaller, and there are also a greater number of types of plant to consider. A selection only of the relevant photographs is given in Fig. 3*a–f*, the magnification being the same as for *Biscutella*, but, to facilitate the interpretation in view of their smaller size, some explanatory diagrams are added (Fig. 4*a–d*). The monoploid chromosome number (which is also the 'haploid' or gametic number of the lowest form) is here 16, and the polyploid series again consists of diploids, triploids and tetraploids which, in this case, possess chromosome numbers of 32, 48 and 64 respectively. Sample views of the somatic chromosomes showing the diploid and tetraploid chromosome numbers in the unpaired state are contained in Fig. 3*a* and *b*, and the only further point of importance to add about the origin of the series is that in this case all three members of it are wild plants widespread in Europe, although the triploid, as in *Biscutella*, is the hybrid between diploid and tetraploid which, in the watercress, has occurred spontaneously.

Chromosome pairing at meiosis in wild plants of diploid, triploid and tetraploid watercress is shown in Fig. 3*c–e*, with an artificially produced autotetraploid (Fig. 3*f*), obtained from the diploid by treatment with colchicine, added for comparison. In the *auto*tetraploid (Fig. 3*f*), chromosome pairing closely resembles that in tetraploid *Biscutella*, allowance being made for the smaller size and greater total number of the chromosomes; numerous quadrivalents are formed. In the *wild* watercress polyploids, on the other hand, multivalent groups are completely absent. The tetraploid (Figs. 3*e*, 4*c*) forms 32 pairs and the triploid (Figs. 3*d*, 4*b*) invariably develops 16 pairs and 16 univalents, whether the plant studied be the wild triploid or an artificially synthesized hybrid between the wild tetraploid and the diploid. This means that polyploidy in the wild watercress cannot be simply due to the multiplication of identical sets of chromosomes. There must be two different sorts of monoploid sets contained in the polyploids, one of which is identical with that of the low-numbered *Nasturtium officinale* R.Br., and which can pair readily with the chromosomes of that species when hybrids are formed (as in the triploid) but the other of which is not homologous and which completely fails to pair in the triploid and does not form quadrivalents in the wild tetraploid. The origin of this second set of 16 chromosomes is still unknown, though, from the morphology of the fruits in the wild tetraploid, it is suspected to be a species of *Cardamine*.

The watercress series in the wild state is, therefore, not an autopolyploid series as in *Biscutella* but an *allo*polyploid one, and the wild tetraploid must be recognized as in origin an interspecific, or in this case probably an intergeneric, hybrid since the genus *Nasturtium* contains no other known species. This hybrid, at some former period, doubled its chromosomes and became fertile and stable, as in the case of *Spartina Townsendii*. It is therefore desirable in the case of watercress to separate the wild tetraploid taxonomically from the diploid and to relegate it to a separate species to which the name *Nasturtium uniseriatum* has been given as a descriptive title to record the most distinctive morphological difference by which the tetraploid can be recognized in the field without a chromosome count, namely, the arrangement of seeds in the

fruit. The genus *Nasturtium* thus now contains not one single species as had previously been thought but two separate species, one of which is older than the other, and partly, though not wholly, parental to it. The two species from the taxonomist's point of view

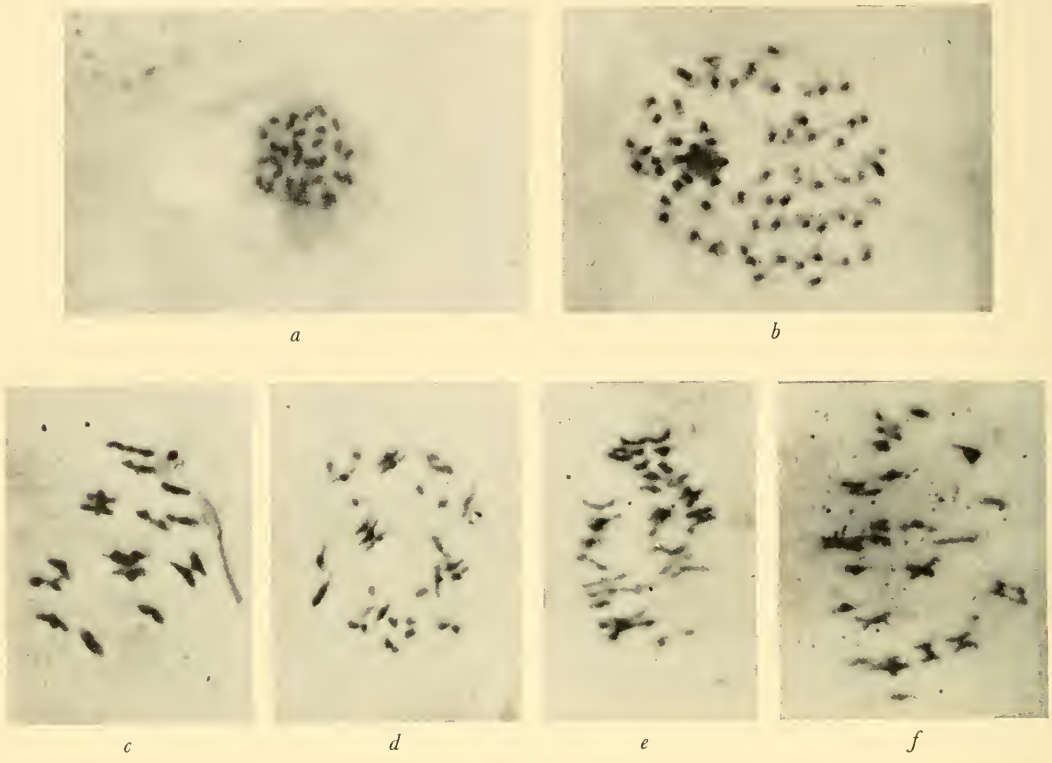


Fig. 3. Polyploidy in watercress (*Nasturtium*). *a* after Manton (1935), the rest after Howard and Manton (1946). *a*. A diploid root in the true watercress (*N. officinale* R.Br.) showing 32 chromosomes, from a section stained in gentian violet. ×2000. *b*. Tetraploid cell in prophase from the tapetum of a plant derived from the diploid by colchicine treatment showing 64 chromosomes, permanent acetocarmine. ×1500. *c*. Chromosome pairing in the diploid showing 16 pairs, permanent acetocarmine. ×1500. *d*. Chromosome pairing in the triploid hybrid between the diploid and the wild tetraploid showing 16 pairs and 16 univalents, permanent acetocarmine. ×1500. For explanatory diagram see Fig. 4*b*. *e*. Chromosome pairing in the wild tetraploid (*N. uniseriatum* How. & Mant.) showing 32 pairs, permanent acetocarmine. ×1500. For explanatory diagram see Fig. 4*c*. *f*. Chromosome pairing in the autotetraploid of *b* showing quadrivalents and pairs. For explanatory diagram see Fig. 4*d*.

are conveniently designated as *N. officinale* R.Br. and *N. uniseriatum* Howard & Manton, respectively.

Since *N. uniseriatum** (unlike *Spartina Townsendii*) has existed for long enough to

* Some important new facts have recently been added to this subject by H. K. A. Shaw (1947) from a study of the literature and herbarium material which appears to show that the tetraploid species is probably more widespread than had previously been thought since specimens attributed to the new species have recently been reported from as far afield as America, Africa and Afghanistan. The name

extend its geographical range throughout Great Britain and over a part at least of the continent of Europe (and perhaps farther afield) it is possible that it may be on the way towards the production of yet other new forms; for the triploid hybrid between *Nasturtium uniseriatum* and *N. officinale*, which is also widespread in Europe, is quite capable of giving rise to a variety of new types by strictly cytological means. In any case the triploid itself must be recognized as a type of watercress which is necessarily still younger than its parent species and which is at present spreading vegetatively.

Fig. 4. Explanatory diagrams to Fig. 3. ×2000. After Howard and Manton (1946). *a*. Diploid watercress (Fig. 3*c*). *b*. Triploid hybrid (Fig. 3*d*). *c*. Wild tetraploid (*Nasturtium uniseriatum* How. & Mant.) (Fig. 3*e*). *d*. Autotetraploid watercress (Fig. 3*f*).

Looking again to the past, however, it may be suggested that the low-numbered *N. officinale* itself may not be quite as simple as it seems, for a 'haploid' as high as $n = 16$ is unusual among its nearer relatives, the majority of which (in the genus *Cardamine* and others) possess $n = 8$. It may therefore be suspected that *Nasturtium officinale* may in turn have originated at some still earlier period and at a lower cytological level by a repetition of the processes which have since produced *N. uniseriatum*. The last is specula-

N. uniseriatum may therefore have to be superseded on grounds of priority by one of two other possible synonyms. For the sake of simplicity these emendations have for the moment been disregarded, since they are at present not based on the same type of cytological evidence as that quoted in the text, which, for convenience, follows Howard and Manton (1946), though without prejudice to the ultimate adoption of Shaw's proposals should they be further authenticated.

tion, but it is sufficient to indicate how a factual record of evolution within a small group could be pieced together and to see how the periodic repetition of a few simple processes such as hybridization and chromosome doubling can give rise to a cluster of species of different ages which, it may be said in passing, have no doubt been subjected at every stage to natural selection but have not been caused by that process.

We could multiply examples, choosing other and better instances, but this is perhaps enough to introduce the subject to an impatient reader who, at this point, may legitimately be expected to ask 'What, after all, does this amount to? You have shown, it is true, some facts about the mode of origin of a few rather trivial species and have traced some events in their past history, extending some paltry thousands of years beyond our little human lifetime, but is this Macroevolution?'

And this, of course, is the unsolved question. The interest of cytogenetic analysis lies in the fact that it does indeed permit of *some* extension backwards in time beyond our own very limited experience, and that it provides *some* phyletic information regarding more important natural units than the artificially produced varieties of domesticated animals and cultivated plants. But the gulf between this and macroevolution in the literal sense is enormous. The tremendous changes recorded in the rocks and in the taxonomic systems of the plant and animal kingdoms are so greatly different in degree from anything which our existing analysis has so far touched that we cannot with certainty know whether they may not also be different in kind. In that case we should have in the end to admit that all our present tools can only touch the fringe of the subject, and that such knowledge as we can acquire of the origin of species does not necessarily provide the clue that we are seeking.

A decision as to this, however, may perhaps be left in the hands of our imaginary historian who, from his vantage point in the twenty-first century or later, ought to be able to see things in better perspective than is attainable by us. For ourselves we may be satisfied with the knowledge that with some new tools in our hands we have a large new field to explore, and if our exploration does not resolve the major problems of the organic world, we may at least look forward to some enjoyable experiences which may enhance our interest in some familiar and common plants.

CHAPTER 2

INTRODUCTION TO THE PROBLEM

The object of this book is nothing more ambitious than to assemble for the first time some material for a preliminary comparison of evolutionary processes, as revealed by cytology, in an ancient and a modern group of plants. The modern group which will be kept particularly in mind, on grounds both of suitability and the accident of close personal acquaintance, is the family of Flowering Plants, the Cruciferae. To this group both the examples discussed in detail in the last chapter belong and a good deal of other cytogenetic information is already available in published form. The ancient group, as will already be obvious, is the assemblage of ferns and fern-like plants known as the Pteridophyta. For these, so little of the new knowledge has previously been acquired, partly owing to technical difficulty, that the first purpose of the chapters which follow will be to elicit the fundamental data.

The Crucifers are a family of about 220* genera and 1900* species of Dicotyledons, neither outstandingly primitive nor specially advanced. They are common in Europe but are spread to some extent the world over. Of fossil record they have little or none, but they may reasonably be assumed to have arisen, or at least to have become established, during the Tertiary period. That they are still actively evolving is suggested first by the prolific development of new forms of domesticated species such as *Brassica oleracea*, the Cabbage, and secondly by the relatively numerous cases of wild taxonomic species of undoubtedly recent origin which cytogenetic analysis has already detected. Many of the classic 'Jordanian' or 'microspecies' of *Erophila verna* first studied in the 1850's by Alexis Jordan, and in modern times by Winge (1940), are certainly of this nature, and other examples have already been mentioned in the preceding chapter.

That hybridization and polyploidy have been potent sources of species formation in the family is known both from observations of the type already quoted for *Nasturtium* and *Biscutella* and also from the beautiful experimental work of investigators such as Karpechenko (1928 and earlier), who, in the famous case of *Raphanobrassica*, induced the formation of closely comparable new forms artificially. That polyploidy itself has been initiated repeatedly throughout the family is known from the simple evidence of comparative chromosome numbers among related species in many of the genera (cf. Jaretzky, 1932; Manton, 1932*a*).† Such simple comparison cannot diagnose the type of polyploidy involved (whether auto- or allo-), nor determine whether the numerical change preceded, followed, or caused the emergence of the species affected by it. It does, however, show beyond dispute that polyploidy in some form or other has entered into the evolutionary history of at least thirty out of the eighty-odd genera examined. This is certainly an underestimate of its frequency, for in several genera

* Willis's *Dictionary*, 1925.

† See also general lists of chromosome numbers by Tischler, Gaiser, Darlington and Ammal, and Maude.

it must have occurred more than once, since different species are polyploid in different degree, while in others it may have so far escaped detection owing to an insufficient number of species available for study. That a proportion, possibly a high proportion, of these cases are allopolyploids of the type of *Nasturtium uniseriatum* seems highly probable both from experience within the family (e.g. *Erophila*, *Raphanobrassica*, etc.) and from the sum of evidence from other flowering plants.

There are, however, undoubtedly other methods of species formation in operation. Thus, polyploidy apart, the diploid species of *Biscutella* of the *Laevigatae* section appear at present to be evolving by purely genetical means. Many of them have a distinctive morphology, which is retained in cultivation, and a characteristic ecological habitat: the high alpine *B. mollis*, endemic to Austria, and the French *B. Lamottii* confined to extinct volcanic ash heaps of Tertiary age in the Auvergne, being good examples. Some of the morphological and probably also physiological characteristics of these species (or ecotypes as they may perhaps more properly be called) are undoubtedly adaptive and enable their possessors to colonize successfully types of habitat which are completely closed to the parental forms which, in both cases, are almost certainly still present in the same geographical area though far removed from the actual sites. This at once confronts us with something closely resembling simple Darwinism, but the species in question are all still sufficiently akin to be interfertile if brought artificially into contact and have completely regular chromosome pairing in their hybrids.

Experience has, however, shown that speciation by genetical means uncomplicated by other cytological changes is either relatively unusual or only a passing phase. Sooner or later in most species, and probably also at some future time in *Biscutella*, internal changes in the chromosomes set in so that homology is lost. Chromosome pairing in hybrids then ceases to occur, and we have the type of sterility barrier which is the taxonomist's greatest ally in determining natural specific boundaries. We know very little, unfortunately, about the mechanisms by which sterility barriers due to a loss of chromosome homology are brought about.* The pioneer in drawing attention to the need for such knowledge was again Bateson in 1913. A beginning has, however, been made by cytogenetic work carried out largely in America, Russia, and to a lesser extent in Great Britain, on a wide range of experimental plants and animals in which the effects on chromosome pairing of visible lesions such as fragmentation, translocation, segmental interchange, etc., have been studied. The exact role of these processes in species formation is less easily demonstrated than are the other methods previously discussed, though the work on the species of *Drosophila* and *Crepis* mentioned on p. 5 is sufficient indication that in certain cases their importance is considerable. In the Cruciferae there is practically no work directly devoted to such studies, but the mere observation that species hybrids here, as elsewhere, are more commonly sterile than fertile is sufficient ground for believing that here also an important effect of intrachromosomal structural changes must be envisaged.

A different type of nuclear upheaval to which the intrachromosomal changes just mentioned may perhaps provide a clue, is that of aneuploidy (sometimes termed

* An outstanding exception is to be found in the work of Tobgy and Gerassimowa on *Crepis*, summarized by Babcock (1947*a*).

dysploidy)* by which is meant a numerical change of some type other than that of a mere multiplication of whole gametic sets. The genus *Biscutella*, from which so much information has already been obtained, may again be quoted in illustration. In the *Laevigatae* section one species of very limited geographical range in southern Spain has a haploid chromosome number not of 9 but of 6. At first sight it might be suggested that the numerical relation between 9 and 6 is such that both could be parts of a polyploid series on 3. In this case, however, there is no direct evidence to support such a contention and much which directly contradicts it. A gametic number of 3 is not known in the Cruciferae at all, and the species in question has all the appearance of being recent. Moreover, all other species of *Biscutella* in sections other than the *Laevigatae*, together with species of a number of neighbouring genera, have a basic haploid chromosome number not of 3, 6 or 9 but of 8. It therefore seems almost certain that the *Laevigatae* section itself arose at some remote period, probably in the Tertiary, by an aneuploid nuclear change which produced a numerical difference of one chromosome in the basic haploid count. This change presumably at first was associated with the production of one new species. This species must, however, have been somewhat more different from its fellows than usual,† for when sufficient time had elapsed for further new forms to develop from it in ways that we have recently touched upon, the taxonomists studying the group with only morphology as a guide were constrained to segregate them together into a separate section. In other cases, no doubt, the size of the unit might have been not a section or subgenus but a genus or larger group. Here, therefore, we are confronted suddenly with something very like the old mutation theory in one of its simplest forms. And the least that we may conclude is that new species are not all equally potent as evolving units, but that the precise means by which they have come about may have a decisive influence on their subsequent fate.

The difference between the relative importance of aneuploidy and polyploidy was probably the most significant general conclusion which the work on the Cruciferae brought out (cf. Manton, 1932*a*), and the evidence can be repeated again and again in other families. Polyploidy in the flowering plants abounds as a means of formation of the type of species which do not fundamentally break new ground and the chromosome numbers reached may be high; in the Cruciferae some of the highest known are $9n=81$ in nonaploid *Biscutella* and $8n=120$ in some varieties of *Crambe*, in which the basic haploid number appears to be $n=15$. The aneuploid changes, on the other hand, are, with very few exceptions, characteristically associated not with species but with groups of species, i.e. subgenera, genera, or larger units. They are far less frequent

* See footnote on p. 5 for further definition of terms.

† Very important recent evidence from *Crepis* summarized by Babcock (1947) indicates that sometimes the initiation of sterility barriers and morphological changes in the form of an organism may occur in the reverse order to that postulated above, the sterility barrier associated with aneuploidy being produced at first without external morphological changes which only ensue subsequently as a result of the isolation imposed. If this can be shown to be the usual order, it will represent a considerable advance in our knowledge of evolutionary mechanisms, though the essential point which is being made above, namely, that the morphological changes ultimately associated with aneuploidy are of a more far-reaching kind than those accompanying polyploidy, would remain unaffected.

than the polyploid changes, and whilst clearly being more important, they have another remarkable characteristic, namely, that the changes involved are always of a low numerical order. The fundamental haploid numbers of genera known in the family are limited to 5, 6, 7, 8, 9, 11 and 15, and though one might have expected that the polyploid species could in turn have experienced comparable changes to give rise to new genera with, say, basic haploid numbers of the order of 82 or 121, they do not, in actual fact, appear to have done so. The two types of change would thus appear to be not merely different in kind and in importance but in some sense, and for reasons which are not at once self-evident, to be mutually incompatible.

Since macroevolution would seem at the very least to require the successive origin of genera rather than the blind multiplication of species, this antagonism between two different types of evolutionary processes was felt to be possibly a matter of considerable importance. It was also a matter on which further light might be expected to be thrown if some comparative data relating to a longer period of evolutionary activity could be obtained. This was the reason why attention was first directed to the Pteridophyta.

In contrast to the Cruciferae, which may be regarded as a representative sample of the dominant vegetation of to-day, all relatively new in a geological sense and still in active development, the Pteridophyta are a mixture of newish forms such as some of the leptosporangiate ferns, and forms of extreme antiquity, the last survivors of the dominant vegetation of periods before the flowering plants existed. It is only necessary to recall the obvious structural affinity between *Equisetum* and the Carboniferous *Calamites*, or between the existing Lycopods and the fossil Lepidodendroids, or the still more distant Devonian *Drepanophycus* and Silurian *Baragwanathia*; or the very probable affinity between the living *Psilotales* and the Devonian *Psilophytales*; or between all the ferns and the Carboniferous *Coenopterideae*, to realize that in this group we have a record which cannot be equalled by that of any other types of living plants, and that they have existed in some form since the earliest times from which the vegetation of the land has been preserved.

Here, therefore, if anywhere, should the cumulative effects of different sorts of evolutionary mechanisms, working over long periods of time, be discernible.

To the non-botanical reader, if such there be, to whom the above enumeration provides merely a list of names, it may be helpful to mention briefly some of the principal characteristics which unite an otherwise very varied collection of plants into one great group. The members of the Pteridophyta are all vascular plants, possessing a water-conducting system composed of characteristic lignified cells known as tracheids. This lignified tissue differentiates them at once from the lower groups of land plants such as the mosses or fungi and accounts for their relative ease of fossilization; the tracheidal structure is, however, a relatively primitive type of tissue, and is certainly more ancient than the system of continuous tubes, the wood vessels, to be found in the Flowering Plants.

Seeds also are absent from the Pteridophyta, thereby distinguishing them from both the higher groups of land plants which reproduce by seed, namely, the Gymnosperms and Flowering Plants. Reproduction in the Pteridophyta is by spores which are liberated from sporangia borne on a variety of organs. They may be on the backs or

edges of the leaves in ferns, on peculiar lateral appendages in the Psilotales, on the upper surfaces of leaves in the Lycopods or attached to special members known as 'sporangiophores' of possibly axial nature and aggregated into cones in the Equisetales; lastly, in the ancient and undoubtedly primitive fossil Psilophytales the sporangia terminate the ordinary, dichotomously branched, vegetative axes.

'Flowers' are absent, the relative inconspicuousness of the spore-bearing members being associated with a complete absence of anything comparable to insect pollination. Instead, the male sexual cells are themselves endowed with active powers of self-motility, and these swimming spermatozoids (see Frontispiece) are the immediate means by which fertilization is brought about. The dependence on free water which this mechanism entails is thought to be a primitive feature, possibly inherited from an algal ancestor and certainly shared by other lowly land plants such as the mosses and liverworts; in the higher plants it has been lost.

The two sex organs which respectively either liberate the spermatozoids or retain the egg during and after fertilization, are not borne on the same plant as the sporangia referred to above. The sporangia liberate unicellular spores which germinate without the intervention of any sexual process into little plants known as prothalli which are always both smaller in size and surprisingly different in structure from the individual from which the spores were derived. Unlike the parent plant a prothallus is never differentiated into stem, leaves and roots and is devoid of vascular tissue (except in a few abnormal cases). It is generally attached to the soil by delicate unicellular hairs, the rhizoids, and it may or may not be green. Where the green pigment is present the prothallus supports itself by the same physiological mechanism, involving photo-synthesis, as the parent plant; this is the case in *Equisetum*, most ferns and some Lycopods. Where chlorophyll is absent the prothallus may either live in symbiotic relation with a fungus, as a saprophyte, a mode of life adopted by the prothalli of the Psilotales, many Lycopods and the Ophioglossaceae among the ferns; or the prothallus may be so short lived that it is able to complete its entire development on the food reserves laid up for it in the original spore, this is the case in all the 'heterosporous' members of the Pteridophyta, namely, *Isoetes*, *Selaginella* and the Hydropterideae among the ferns.

Sooner or later, a prothallus of any of these varied types becomes sexually mature. Antheridia are then produced, either superficially or sunk in the tissue, which will liberate many swimming male gametes if water is provided at the right time. The female gametes or eggs are large, non-motile cells, borne singly in characteristic female sex organs, the archegonia. The basal part of an archegonium, containing the egg, is sunk in the tissue of the prothallus which thereby adds to its protection, the rest of the organ being in the form of a projecting multicellular neck which is capable of opening when wetted, to give free access from the outside world to the naked surface of the egg cell. When the union of the gametes has been achieved, it is immediately followed by germination of the fertilized egg *in situ*. At first, it remains enclosed and nourished by the prothallial tissue, but as soon as the multicellular embryo has become large enough for the first organ (stem, leaf or root as the case may be) to assume its proper function, the prothallial tissue is broken through and an independent organism with the morphology of the spore-bearing plant is re-established.

This type of life history in which sexual and asexual modes of reproduction are separated on different individuals which succeed each other in regular order is known as alternation of generations. In the animal kingdom such a life history is rare and altogether absent from the higher forms, but in plants alternation of generations is so deeply established in all the principal land plants except the fungi, that its effects are still easily to be recognized even in the highest seed plants. In these, however, the sexual, haploid generation or gametophyte is so much reduced that for many purposes its existence may be ignored, and it commonly is so by geneticists. The flowering plants

Fig. 5. The sporophyte generation of a horsetail (*Equisetum*). *a*. Sterile and fertile shoots of *E. limosum* Willd. in the month of May. Natural size. *b*. Ripe spores of *E. palustre* L. × 100. *c*. Section of a young sporangium of *E. robustum* A.Br. with meiosis in progress. × 100.

can then be treated exactly like the higher animals as diploid organisms in which the nuclear reduction (meiosis) takes place as part of the maturation process of the gametes. In the Pteridophyta the complete independence of the two generations for most of their lives makes it necessary to retain the technically correct terminology appropriate to botany or misunderstandings will follow. The conspicuous generation here (as technically also in the flowering plants) is a diploid organism reproducing by asexual means and known in consequence as the sporophyte. The haploid, sexual plant (in the case of the Pteridophyta the inconspicuous but free-living prothallus) is the gametophyte. Sporophytes and gametophytes occur in all the higher groups of plants, though

the special type of gametophyte to which the name prothallus is given is confined to the Pteridophyta. In none of these cases is meiosis a maturation for the gametes, but it

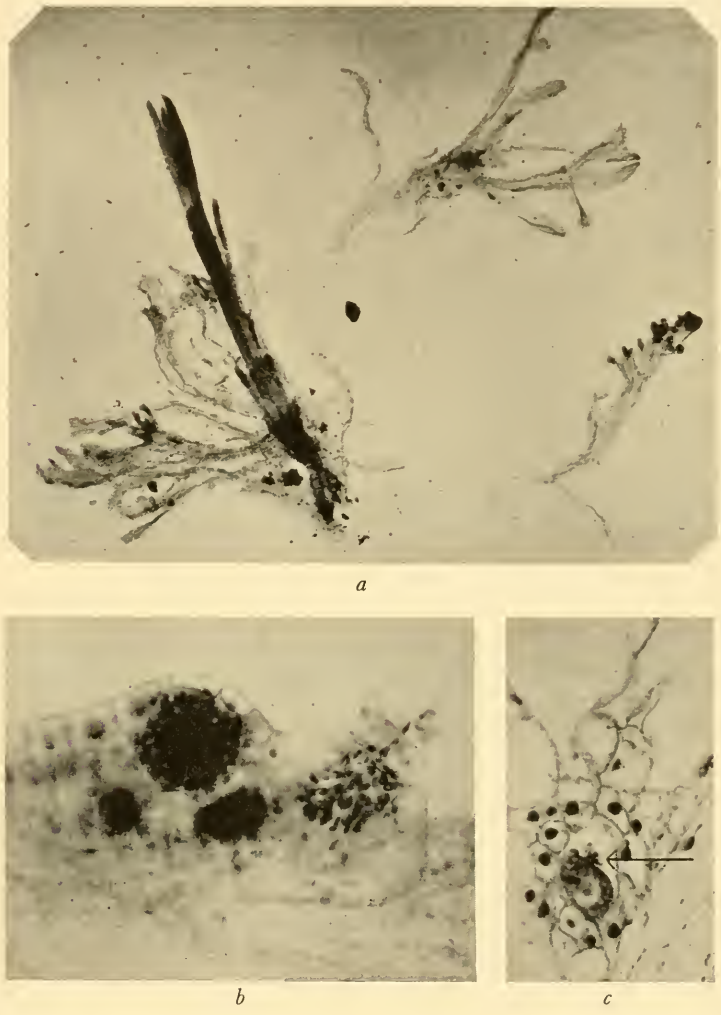


Fig. 6. The gametophyte generation of a horsetail (*Equisetum*). *a*. Three prothalli of *E. sylvaticum* L. of various ages mounted in glycerine jelly. × 10. The largest specimen bears a young sporophyte, the uppermost specimen is a young female with a recently fertilized archegonium containing an embryo, and the right-hand specimen is a male with numerous antheridia which appear dark owing to density of contents. *b*. Antheridia of various ages from a whole mount stained in braziline. × 200. Spermatozoids are being extruded from the right-hand antheridium. *c*. A recently fertilized archegonium with several spermatozoids in contact with the egg cell, the archegonial neck appearing closed and partly shrivelled, perhaps owing to the treatment, from a section stained in haematoxylin, fixed within an hour of insemination. × 200.

takes an essential part in the formation of the asexual spores and in so doing forms the point of demarcation between sporophyte and gametophyte, the other point of demarcation (between gametophyte and sporophyte) being the syngamous nuclear fusion. Some

of these facts may perhaps be helpfully summed up by a glance at Figs. 5 and 6, which show characteristic views of both generations, together with their respective reproductive organs for one particular member of the Pteridophyta, the Horsetail (*Equisetum*).

All this will be so familiar as to be second nature to a botanical reader; nevertheless, even for such, it may perhaps be convenient and save risk of confusion if two other

Fig. 7. Some examples of apogamy in ferns. *a*. Obligate (or 'direct') apogamy in *Dryopteris Borreri* Newm. 'var. *polydactyla* Wills' (for further details see Chapters 5 and 11) showing a young apogamous outgrowth from the region of the prothallus where the central cushion bearing archegonia should be. × 10. *b*. Induced apogamy in an abnormal prothallus of *Osmunda regalis* L. derived from a spore produced by the triploid (see Chapter 3) and too unbalanced genetically to be able to reproduce normally; after several years' sterility a leaf is being produced from the apex of the prothalloid tissue, but so far the specimen has no roots. × 2. *c*. The same as *b* in other examples. Natural size.

terms, without exact zoological equivalents, be referred to at this point. 'Apogamy' and 'aposporγ' are two aberrations of life history by which the regular sequence of sexual and asexual reproduction just explained is modified. 'Apogamy' is the production of a sporophyte from a gametophyte without the intervention of a sexual process. A special case of apogamy is 'parthenogenesis', a name used by both botanists and zoologists to denote the development of an egg without the act of fertilization. This is unknown in the Pteridophyta, although it undoubtedly occurs under exceptional

circumstances in the Flowering Plants and is not uncommon in the Thallophyta. In other groups, if fertilization is prevented, the egg itself invariably dies, but other parts of the gametophyte may, under suitable conditions, proliferate directly into sporophytic tissue. Sometimes, through a genetical aberration, such proliferation may become the rule, and one or both of the normal sex organs may become eliminated from the life history in consequence; several examples of this in ferns will be examined cytologically below. In other cases a genetically normal prothallus can, by prolonged prevention of fertilization through the withholding of free water, be induced to develop apogamously as an exceptional circumstance; some examples of this will also be examined. In both

a *b*

Fig. 8. Some examples of apospory in ferns. *a*. Apospory induced by malnutrition in an otherwise normal young plant of *Osmunda regalis* L. var. *cristata* Moore; the petiole of a juvenile leaf, shown at the base of the specimen, after forking twice has grown out into a forked prothallus covered with rhizoids. From a photograph supplied by Prof. W. H. Lang. ×8 approximately. *b*. Apospory in *Athyrium Filix-femina* (L.) Roth var. *clarissima* Jones. Portions of an adult frond laid on soil and kept moist for several weeks have proliferated into prothalli, some of which are already bearing apogamous young plants. Natural size.

cases the result is the same, a sporophyte with the gametophytic chromosome number is produced.

'Apospory' is the aberration on the part of a sporophyte by which proliferation of gametophytic tissue takes place from it without the intervention of normally constructed spores. As in the case of apogamy, it is sometimes possible to induce apospory in genetically normal plants by appropriate experimental treatment. Cutting off the leaves of juvenile plants and laying them on soil is a well-known method which, if successful, will result in the proliferation of prothalli from the tips of the leaf or from its cut surface. In other cases, owing to genetical peculiarity, the same process can be carried out even with the large leaves of fully mature plants; examples of this are certain well-known horticultural 'varieties' such as '*Lastrea pseudo-mas*' var. *cristata-*

apospora Cropper or '*Asplenium Filix-foemina* var. *clarissima* Jones'. In all these cases, whether genetically normal or not, the result of the aposporous development is a prothallus with the same chromosome number as the parent sporophyte. Such prothalli, in the case of the horticultural varieties, are generally apogamous, and nuclear change in them is eliminated from the life cycle. Where apospory has been induced in genetically normal plants, the sexual function of the induced prothalli may be unimpaired; the consequence of this is a polyploid series.

A special case of abnormal life history to which the name apospory cannot strictly be applied is sometimes found in association with permanent apogamy in ferns. *Pteris cretica* and *Cyrtomium falcatum* are the classic examples, and in these the morphology of the spores is retained, but the meiotic process is rendered ineffectual by a sequence of unusual and highly interesting cytological happenings in the sporangium. Spores with the unreduced chromosome number are formed, and these give rise to apogamously reproducing prothalli. Again the nuclear basis for alternation of generations has been eliminated.

Some photographs in illustration of the phenomena of apogamy and apospory are appended in Figs. 7 and 8, and examples of all these types of abnormal life history will be examined below. Some of them are very instructive from an evolutionary point of view.

With this amount of introduction we may turn to the plants themselves. Fig. 9 is a reproduction, translated into English where necessary, of a fairly recent summary of the probable relationships of the main living and fossil types. It is not necessary to analyse it in detail, for certain obvious general conclusions can be obtained at a glance. The preponderance of extinct compared with living forms is striking and would doubtless be still more marked if account could be taken of extinct forms which have perished without trace. It is also obvious that, in the living forms, we are dealing with the end-products of phyletic lines which have long been separated. Of their somewhat impoverished modern representatives only the ferns can compare at all favourably with the wealth of the known or presumed ancestral types, and the ferns ('*Pteropsida*') enormously outnumber all the other living representatives added together.

The living members of the Pteridophyta may be listed in the somewhat more familiar terminology adopted by Bower as follows:

Psilotales: two living genera, *Psilotum* and *Tmesipteris*, both mainly tropical or subtropical, each with one, or at most two, species.

Lycopodiales (Clubmosses): four living genera. *Lycopodium*, world-wide distribution, about 185* species. *Phylloglossum* confined to Australia and New Zealand, one species. *Selaginella*, world-wide distribution, over 700 species. *Isoetes*, world-wide distribution, 50 species.

Equisetales (Horsetails): one living genus, *Equisetum*, world-wide distribution, except Australasia, about 25 species.

* These numbers and the following are quoted from Willis's *Dictionary* (1925).

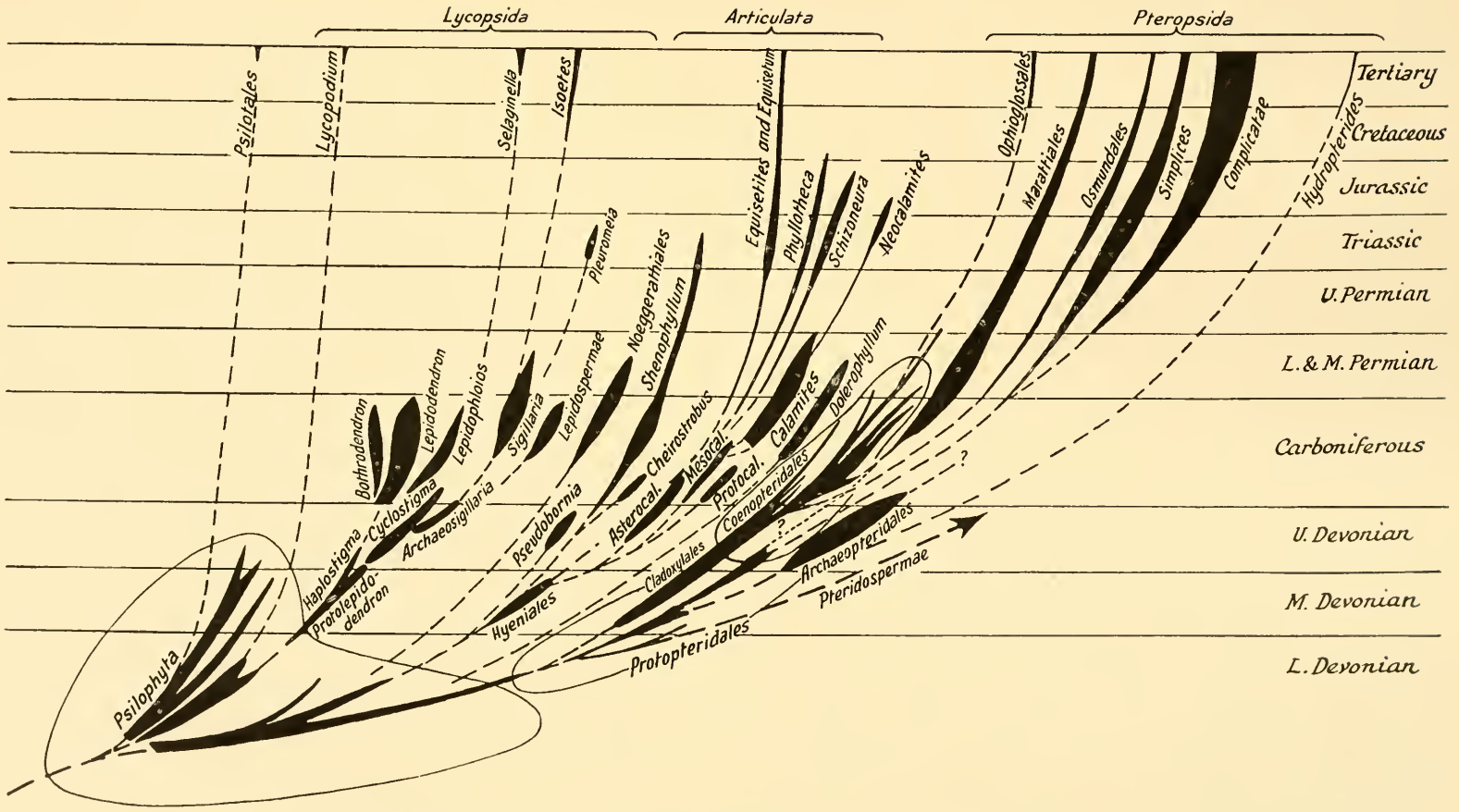


Fig. 9. Phylogeny of the Pteridophyta, redrawn after Zimmermann (1938).

Filicales (Ferns): about 150 genera and 6000* species grouped into orders and families, of which the Ophioglossaceae, Hymenophyllaceae, Osmundaceae, Polypodiaceae and Marsiliaceae occur in Britain.

Some idea of the taxonomic and phyletic complexity of the ferns may be obtained by a glance at Fig. 10 reproduced from Bower (1935). It is obvious that the ferns alone could easily provide material for cytological study for more than a lifetime, and one of the consequences of this book may perhaps be to suggest that such a study would be

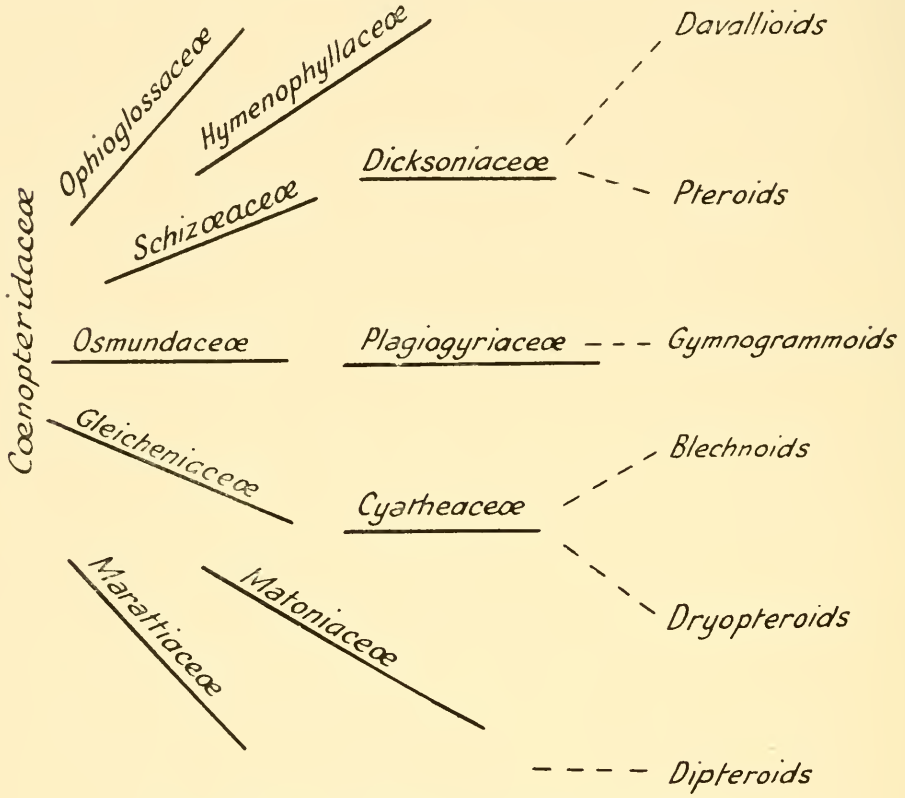


Fig. 10. Phylogeny of the ferns, redrawn after Bower (1935).

worth pursuing. The little that is presented here in the first few chapters may, however, also supply a wholesome warning. The ground must be conquered step by step with the utmost labour, and a rapid attack on all the intricacies of phylogeny cannot be hoped for. It will therefore not be attempted at this stage except incidentally and in small details, and attention will primarily be directed to those larger general questions for which the smaller but perhaps equally ancient or older living groups are as important as the ferns.

* These numbers are taken from Verdoorn's *Manual of Pteridology*, in which Christensen (1906) is quoted. Since then Christensen (Supplement 1934) emended these figures to 213 genera and 9387 species. Other estimates will be found in Copeland (1947).

These general questions are, first, to determine whether any of the evolutionary mechanisms shown to be operating in the Cruciferae can also be detected in the Pteridophyta. If this is answered in the affirmative the second question is to inquire whether any further light can be thrown upon such mechanisms by observing the accumulation of their effects in a longer period of geological time than the Flowering Plants have yet had at their disposal. In precise terms this amounts to asking, in the first place, whether the processes of genic mutation, aneuploidy, polyploidy, hybridization and the rest, have or have not played a recognizable part in species formation in the Pteridophyta; and secondly, to see if any of the predictions aroused by the data already obtained from the Cruciferae can be confirmed or extended by comparison with the older group. Consideration of this second question will be left to the end of the book. The earlier chapters will primarily be devoted to ascertaining the facts relevant to the first, and since the particular evolutionary processes of polyploidy and hybridization are the easiest to demonstrate, these will be uppermost in mind in the early stages of the inquiry.

CHAPTER 3

THE POLYPLOID SERIES IN *OSMUNDA*

Osmunda regalis, the Royal Fern,* is such a delightful cytological object that it may be well to prepare the way for the evolutionary analysis which follows by devoting a preliminary chapter to it. This will enable us to amplify the brief introduction to polyploidy given in Chapter 1 and thereby to set up a standard of reference from within the group itself, against which the facts for other plants of unknown origin can be assessed.

The reasons for the selection of *Osmunda* are well known. It is easily grown, indeed it is remarkably hard to kill, even in the centre of an industrial city. Both generations can be treated as perennials. The spores are produced in abundance and will germinate at once if scattered on to soil or tap water. The chromosomes are large and few in number ($n = 22$, one of the lowest known numbers in the ferns) and they are readily accessible, for both roots and sporangia are freely exposed in quantity with a minimum of protective covering. Fixation, moreover, with modern fixatives presents no difficulty, and all the more important special methods of treatment, including that for spiral structure (Manton, 1939), can be applied with success. Finally, in its morphology, the sporophyte at least has shown itself to be remarkably plastic under experimental treatment.

The origin of the autopolyploid series was apospory induced experimentally in a normal strain of the species. The basic facts of its production were described in some detail by Lang in 1924, and the cytology recorded in a preliminary form by Manton in 1932. Briefly, the process consisted in the persistent depauperation of young plants by mutilation and malnutrition, either deliberate or accidental, enhanced by prolonged neglect during the war of 1914–18. As a result, the normal adult type of foliage failed to develop, and a variety of abnormalities affected the younger leaves which had otherwise reverted to the juvenile type. Examples of some of these unusual growths are shown in Fig. 11 *a–d*, which are reproduced from Lang's original drawings, and in Fig. 12, which is one of Lang's original photographs. They represent various types of cylindrical processes, vegetative buds and prothalli which had developed from, or replaced, the leaf lamina or petiole. Prothalli were somewhat infrequent but were detected more than once (see also Fig. 8*a*, Chapter 2). Such prothalli could produce rhizoids and some sex organs whilst still attached to the parent plant or, if removed and laid on soil, they grew on as normal-looking gametophytes and gave rise to numerous young plants. Owing to their perennial habit the prothallial cultures could be kept growing indefinitely and they are now over 20 years old and still continue to produce young plants. The prothalli are, however, diploid and the young plants tetraploid.

* Anyone unfamiliar with the general appearance of this large and characteristic fern will find some leaves of a smaller species illustrated in Chapter 16, Fig. 276*a*, *b*, p. 275.

Fig. 11. Leaf abnormalities in starved and mutilated young plants of *Osmunda regalis* L., after Lang (1924). *a*. Part of an uninjured but starved specimen showing a bud (*b*) on one of the petioles. × 6. *b*. The bud of *a* more highly magnified. × 20. *c*. One leaf from a similar plant showing more extreme modification; there is no lamina, but instead the petiole forks and one fork bears a prothallus (*p*) at its tip and a bud laterally. × 7. *d*. Whole plant found detached and without roots in a crowded pan. × 6. Of the two expanded leaves one is normal and the other bears five prothalli at the tips of the veins. This leaf, after being detached and laid on soil, was the start of the polyploid series in *Osmunda*. For other examples of apospory of this type see the photographs in Figs. 8*a* and 12.

Since 1932 apospory of similar type has occurred spontaneously on several occasions in young tetraploid sporophytes. The resulting prothalli are also tetraploid, but, unlike the diploids, they appear to be sterile, for they are now over 10 years old and are still barren in spite of liberal watering.

The triploid sporophytes and gametophytes, which complete the series, have required somewhat more careful preparation. The first triploid sporophytes were already fully grown and fertile when their chromosomes were first examined in 1932. They occurred intermixed with the tetraploids which had been grown on from the first experiments, and two were found among seven plants. Their origin was at that time

Fig. 12.

Fig. 13.

Fig. 12. The origin of the polyploid prothalli. Two 'leaves' from a young plant of *Osmunda regalis* L., the one on the left showing the normal juvenile lamina, but the one on the right abnormal in shape and ending in a heart-shaped prothallus. From a photograph kindly supplied by Prof. W. H. Lang, after Lang (1924). Twice natural size.

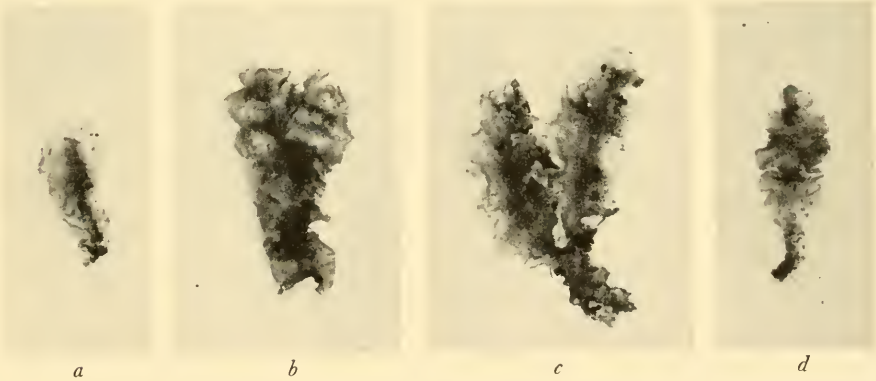
Fig. 13. The origin of the polyploid sporophytes. Haploid and diploid prothalli fertilized on the same day and grown on together. The normal (diploid) young plants on the right have grown much faster than the tetraploid young plants on the left which therefore appear much smaller. Several unfertilized prothalli can be seen creeping over the soil in the left half of the pot. Half natural size.

uncertain but has since been traced to contamination of the original diploid prothallial cultures with some normal prothalli from self-sown spores. Triploids have been synthesized on several occasions since by inseminating diploid archegonia with haploid spermatozoids, and they are not produced if the diploid prothallial cultures are kept pure. Though more troublesome to produce in large numbers than are the tetraploids, such young triploid plants can be made to become aposporous in the same way as the others. The method adopted by Mr Ashby, who has been entirely responsible for this part of the work, was to mutilate a young triploid plant by repeatedly removing its roots until the depauperate condition associated with leaf abnormalities was achieved. One aposporous prothallus was developed which has since been grown

on and subdivided. The triploid prothallial culture so obtained is now about 6 years old. It has not yet produced a young plant, but it is perhaps still possible that in time it may do so. The parent plant has been allowed to return to normal.

a *b* *c*

Fig. 14. Comparative leaves from the polyploid series of *Osmunda*; terminal leaflets from sterile fronds of comparable stature. *a*, diploid; *b*, triploid; *c*, tetraploid. The triploid shows gigantism, but the tetraploid is depauperate and also abnormal in shape. Half natural size.



a *b* *c* *d*

Fig. 15. The polyploid prothalli of *Osmunda*. Natural size, photographed at the end of the growing season, in autumn. *a*, *b* and *d* photographed at the same time under strictly comparable conditions; *c* from a different year. *a*, haploid; *b*, diploid; *c*, triploid; *d*, tetraploid. The diploid and triploid both seem to show gigantism, but the tetraploid is small and abnormally indented at the edge. Triploid and tetraploid are unable to reproduce, but haploid and diploid are very fertile.

While there is still some chance that the series may be extended in the future should either of the high-numbered prothalli become fertile, until they do so further progress is blocked. We must therefore be content with diploid, triploid and tetraploid sporophytes and haploid, diploid, triploid and tetraploid gametophytes.

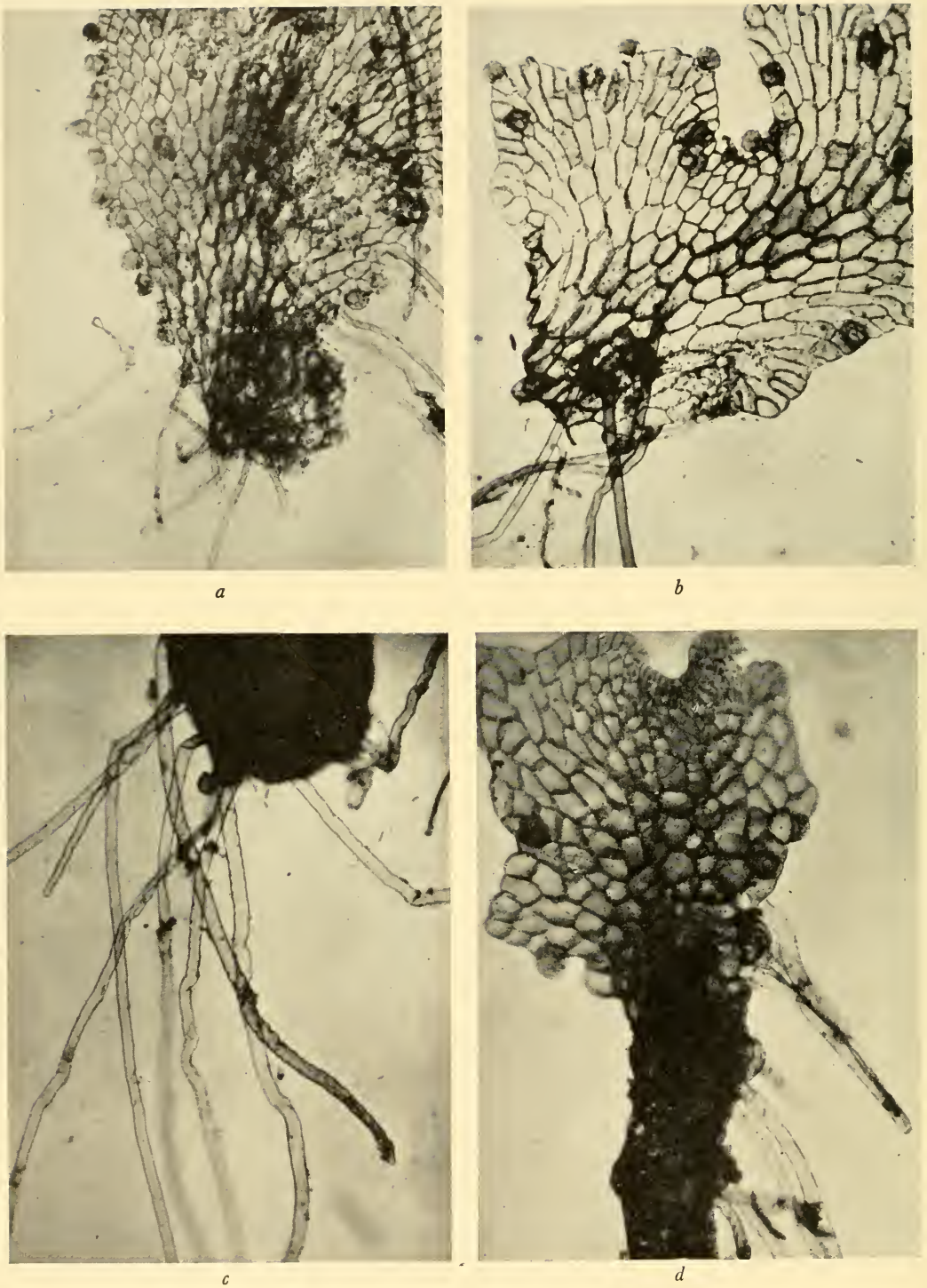
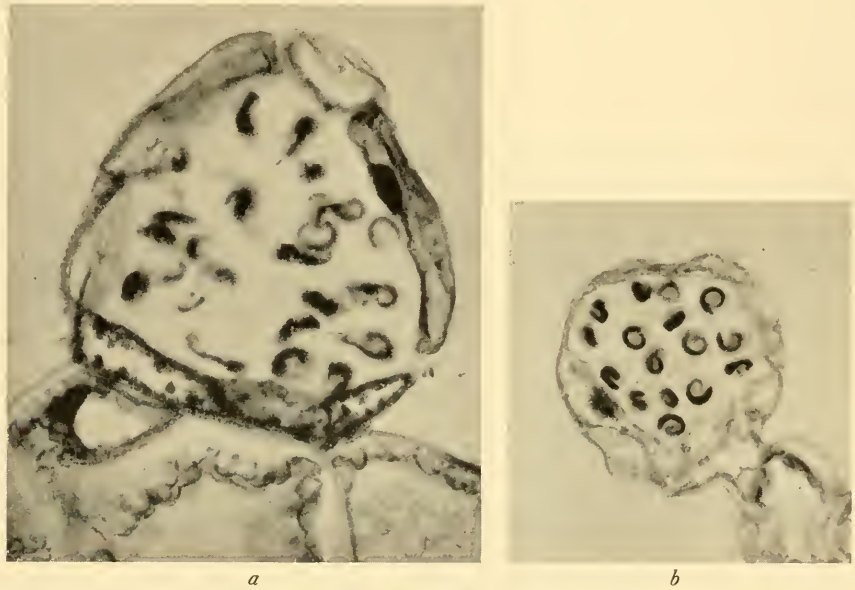


Fig. 16. Comparative series of cell sizes, especially rhizoid diameters in the polyploid prothalli of *Osmunda*. ×50. *a*, haploid; *b*, diploid; *c*, triploid; *d*, tetraploid.

Some idea of the gross morphology of the various members of both generations may be obtained from Figs. 14 and 15. Each member of the series, even a sterile prothallus, is a complete individual provided with all the appropriate vegetative and reproductive organs, though perhaps the female sex organs may not always be structurally perfect. Differences in size, shape, growth rate and no doubt other physiological processes nevertheless accompany polyploidy.

The first increase in chromosome number produces gigantism; this is conspicuously the case for the triploid sporophytes and the diploid gametophytes, both of which are larger than the corresponding normal individuals. With further increase of chromosome number size is again reduced. The triploid prothalli are almost as large as



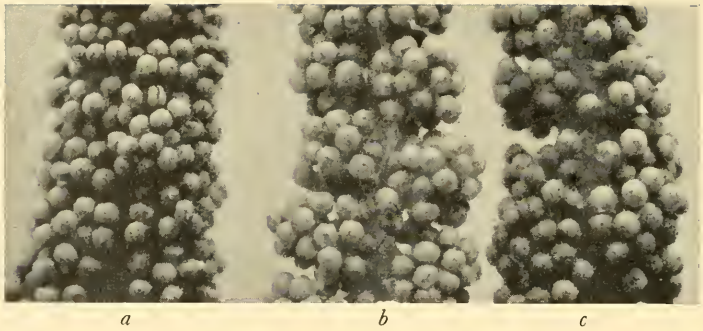
a *b*

Fig. 17. Comparative sizes of antheridia and spermatozoids in tetraploid (*a*) and haploid (*b*) prothalli of *Osmunda*. × 1000.

the diploid though not quite so regular in appearance, but the tetraploids are smaller even than the haploids, and the tetraploid sporophytes are depauperate in a similar degree.

The high-numbered forms are not only depauperate but show structural irregularities. Thus the tetraploid prothalli display a highly characteristic fimbriation of the margin, which is foreshadowed to a less extent in the triploids. The equivalent effect in the tetraploid sporophytes appears as an irregular lobing of the leaf.

These irregularities are likely, in part at least, to be the expression of increased cell size coupled with reduced growth rate. Cell size increases continuously, throughout the polyploid series. Fig. 16 shows the series of rhizoid diameters for the four types of prothalli and Fig. 17 gives the extreme spermatozoid sizes. The large spermatozoids of the tetraploid gametophytes have been seen to swim though with a slow and clumsy motion; the reason for the sterility of these prothalli is therefore not obvious. Similar



a *b* *c*

Fig. 18. Comparative sizes of sporangia in *Osmunda*. ×5. *a*, diploid; *b*, triploid; *c*, tetraploid.

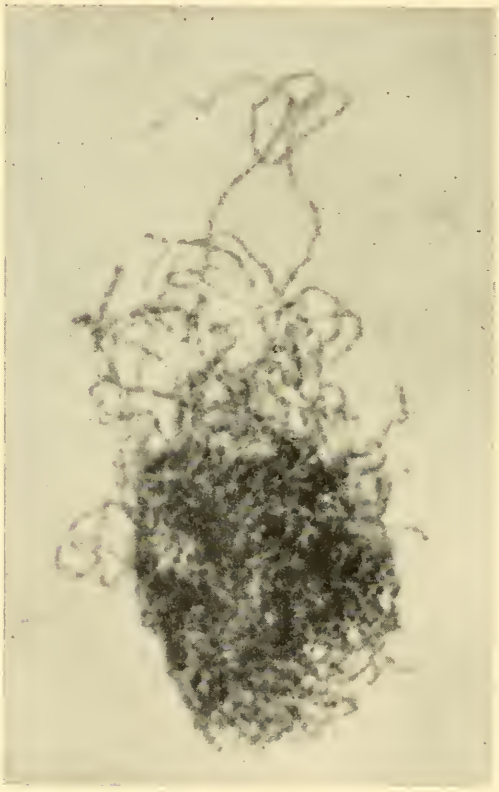


Fig. 19. A cell at the beginning of meiosis at the stage known as leptotene (literally the thin thread stage) before chromosome pairing has occurred. One complete, unpaired, chromosome has been separated from the tangled mass of the others at the top of the field. Permanent acetocarmine preparation of triploid *Osmunda*. ×2000. After Manton (1939).

size relations can be demonstrated for the sporophytes by using the spore mother cells or the epidermis from comparable regions of the leaf.

Increasing cell size is naturally reflected in increased size of particular organs wherever these depend for their structure on a rather precise cellular arrangement. This is particularly true of the reproductive organs, and gigantism continues to be expressed in the antheridia and sporangia long after it has ceased to appear in the total stature of the plant. This has already been seen in Fig. 17 for the antheridia, and a comparable set of sporangia is shown in Fig. 18.

Table 1. *Comparative measurements of cells and organs in the autopolyploid series of* Osmunda

Tissue	Observation	*n*	2*n*	3*n*	4*n*
Gametophyte:					
Spore	Diameter, alive (μ)	80	90	.	.
Rhizoid	Diameter, alive (μ)	18	26	33	42
Spermatocytes	Diameter, in section (μ)	5·5	7	.	9
Antheridia	Diameter, alive (μ)	60	80	.	160
Sporophyte:					
Sporangia	Diameter, alive (mm.)	.	0·55	0·7	0·8
Leaf	Maximum length (cm.) (under comparable conditions, 1946)	.	114	130	91

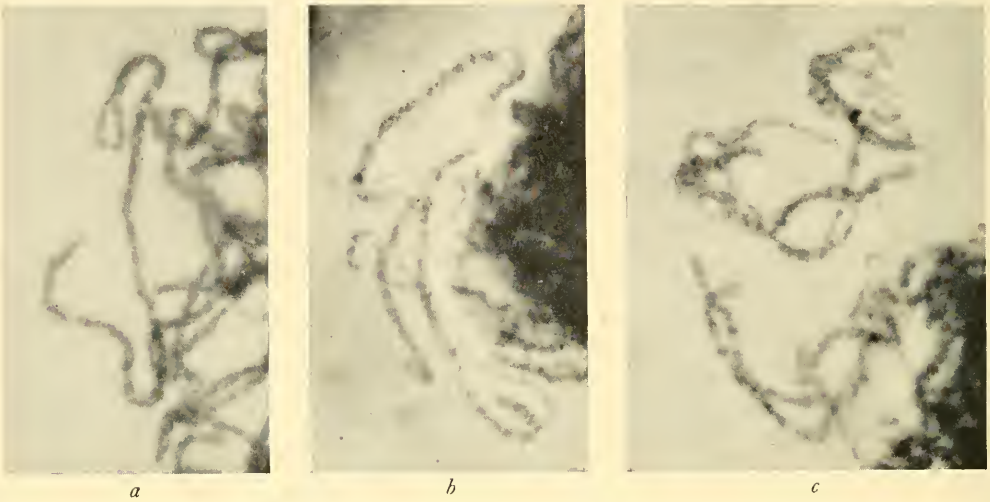
Reduction of growth rate is less easy to express in precise terms than are structural characters. An ocular demonstration of it is, however, provided by Fig. 13. This shows the relative speed of development of sexually produced offspring from haploid and from diploid prothalli which were fertilized on the same day and grown on together in one pot. The normals have far outstripped the polyploids. Another feature determined by growth rate is the date of shedding of spores in spring. Under identical conditions of culture, dehiscence of sporangia in diploids and triploids occurs almost simultaneously, with the diploids not more than a day or two in advance of the triploids; the tetraploids, in contrast, are always about a fortnight later.

For convenience of reference some of these observations are summarized in Table 1, and while it is obvious that many more could be made, especially with regard to the comparative study of physiological processes, enough has perhaps been given to provide a background to the cytological behaviour which, from the present point of view, is the centre of interest.

The chromosomes of *Osmunda* are fortunately sufficiently large to provide an almost diagrammatic demonstration of all the more important cytological manifestations of autopolyploidy. The most important of these, for reasons which have already been partly explained in Chapter 1, is multivalent pairing at meiosis. In a normal diploid, where only two sets of homologous chromosomes are present, the early stages of the reduction process (prophases* of the first meiotic division) consist in the pairing

* To those unfamiliar with cytological nomenclature it may be helpful to explain that the words *prophase*, *metaphase*, *anaphase* and *telophase* are the names given to successive stages of all types of cell division whether mitotic or meiotic. *Prophases* are the early stages, *metaphase* is the equatorial plate stage when

together laterally of every chromosome with the exactly equivalent homologous partner. Whilst this is occurring the chromosomes are very long and thin, as may be seen by a glance at Figs. 19 and 20, but when pairing is complete they appear to shorten and thicken in a most remarkable way (cf. Fig. 22). This change of shape is partly due to genuine shrinkage but is chiefly caused by the fact that each chromosome becomes coiled into a spiral configuration (Fig. 21) which is commonly referred to as spiral



a *b* *c*

Fig. 20. Paired chromosomes at the stage known as pachytene (literally the fat thread stage). *a*, a complete bivalent in the diploid; *b*, a trivalent in the triploid; *c*, a quadrivalent in the tetraploid. Permanent acetocarmine preparations. × 2000. *a* after Manton (1939); *b* after Manton (1945).

structure but which can only be clearly seen after special treatment and which was therefore for long disregarded. It will not often be necessary to refer to spiral structure in the course of this book, though it is perhaps worth mention at this point, since otherwise the changes of shape which affect a chromosome during prophase appear unnecessarily mysterious.

the chromosomes are assembled on the spindle, *anaphase* is the stage at which movement to the poles takes place, and *telophase* denotes the stage at which resting daughter nuclei are being reformed. It is not customary to subdivide these stages further for mitotic divisions, but the prophases of meiosis are so complex that a whole sequence of substages have been recognized and given separate names. The most important of these are *leptotene*, *zygotene*, *pachytene*, *diplotene* and *diakinesis*, after which metaphase of the first meiotic division sets in. Detailed description of these stages will be found in many elementary textbooks of cytology or genetics, but for the purpose of this book the descriptive notes given in this chapter for *leptotene*, *pachytene* and *diakinesis* should suffice. The only other cytological nomenclature which might perhaps cause confusion to a non-cytological reader concerns the appellations of the two meiotic divisions. In the older literature the names *heterotype* and *homotype* were applied to the two nuclear divisions which constitute meiosis; these words are, however, becoming superseded by the rather simpler designation of 'first meiotic division' and 'second meiotic division', and the latter practice will be adhered to throughout this book. The numerical chromosome reduction by means of chromosome pairing occurs at the first division. The second division involves the longitudinal separation of half-chromosomes as in a somatic mitosis. The result of the two divisions is a tetrad of four nuclei each with the reduced chromosome number.

Since the spiral forms independently in each chromosome the paired partners lose contact with one another as prophase advances, except at a few places at which an especially intimate relation has been established. Such places are termed chiasmata and one, two or three may occur in any chromosome pair (in other organisms with longer chromosomes the number may be greater) in positions which are to some extent determined at random. The shapes of paired chromosomes are affected by this, although the nature of chiasmata are not otherwise important for our present purpose and need not therefore be discussed. If a pair of chromosomes remains joined by a single chiasma it will look like a rod, V or X, according to whether the chiasma is median in position or terminal. If there are two chiasmata a pair of

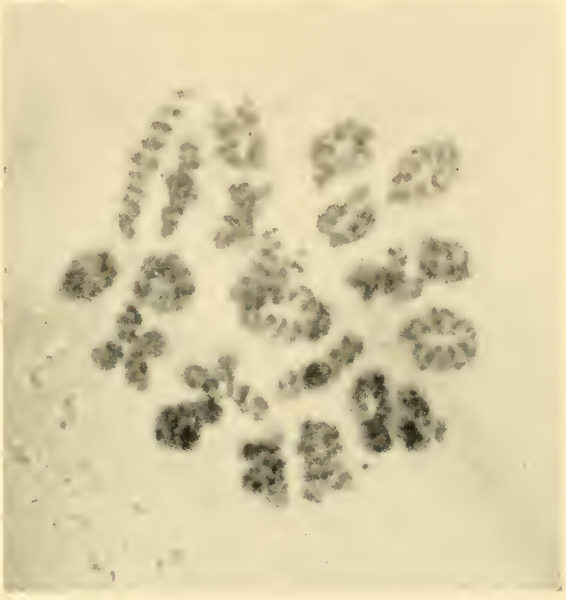


Fig. 21. The equatorial plate stage of the first meiotic division in diploid *Osmunda* showing 22 pairs of chromosomes in which spiral structure has been revealed by ammonia treatment before fixation. The formation of the spiral explains most, though not all, of the apparent changes of size and shape which a chromosome experiences during prophase. Fresh acetocarmine. × 2000.

chromosomes will resemble an O or an α; if there are three it will look like a figure of eight. Several of these shapes can be seen in Fig. 22 *a*, which represents the end of the first meiotic prophase at the stage known as diakinesis. After this the paired chromosomes assemble on the spindle and then separate to opposite poles.

The details of the later stages of meiosis need not at the moment concern us, and it is perhaps sufficient to refer to the anaphase of the second division (Fig. 22 *b*) in which the reduced (haploid or monoploid) number of 22 single chromosomes is very clearly displayed.

When more than two homologous sets of chromosomes are present both meiotic divisions are affected in a characteristic way. If there are three homologues of every chromosome they will attempt to unite in threes, an attempt which is not always

successful, in which case a potential trivalent will be represented by a pair and a univalent. The reason for this may partly be seen by examining pachytene. A potential trivalent in the fully extended condition is shown in Fig. 20*b*, and it looks rather like a

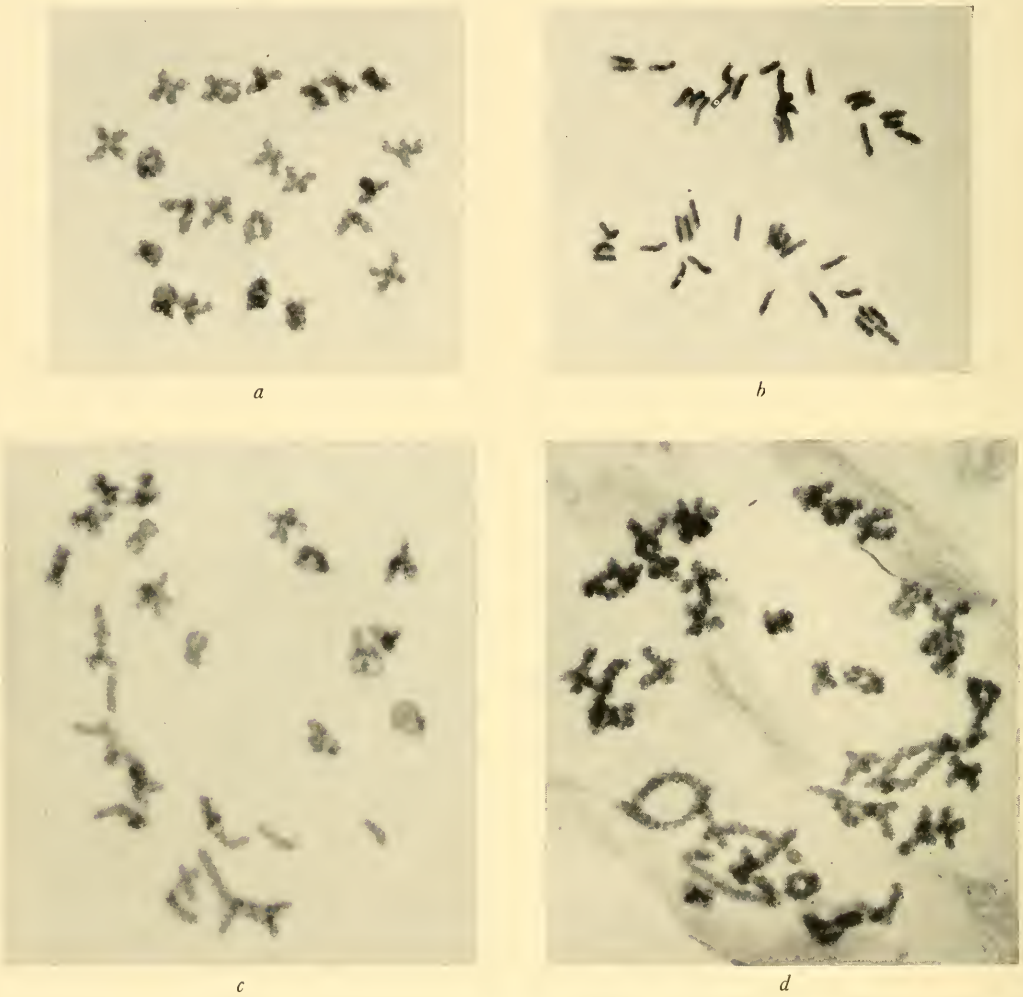


Fig. 22. Some other stages of meiosis in diploid and polyploid *Osmunda*. Permanent acetocarmine. × 1000. *a*. Diakinesis (late prophase) in the diploid showing 22 pairs resembling those of Fig. 21 except that the spiral is not visible. *b*. The end of the second meiotic division showing two of the daughter nuclei each with 22 single chromosomes, after Manton (1939). *c*. Diakinesis in triploid *Osmunda* showing trivalents, pairs and univalents. For explanatory diagram see Fig. 23. *d*. Diakinesis in tetraploid *Osmunda* showing quadrivalents and pairs. For explanatory diagram see Fig. 24.

pair with the third chromosome wound round it. Pairing is, in fact, only possible between two chromosomes at any one point, no matter how many homologues may be present, and the only effect of the presence of additional potential partners is that the identity of those in contact changes from time to time. Such changes of partner,

if they occur in suitable positions for chiasmata to form in relation to each of the homologues, will result in the establishment of a union between all three which is retained until metaphase with the production of the characteristic shapes associated with trivalents (Fig. 22*c*). If the chiasmata are unsuitably placed the group will fall apart.

The analysis of the triploid nucleus of Fig. 22*c* is given diagrammatically in Fig. 23, in which trivalents are shown in black and pairs and univalents in outline. Similar analyses for 109 cells are summarized in Table 2, and it is important to notice that although every chromosome is known to be present in triplicate, a circumstance which might lead one to expect that 22 trivalents would always be formed, the actual numbers

Fig. 23. Explanatory diagram to Fig. 22*c* with trivalents in black but pairs and univalents in outline.

Fig. 24. Explanatory diagram to Fig. 22*d* showing quadrivalents in black and pairs in outline.

found show a random distribution round an ill-defined mean which is considerably less than this (i.e. between 14 and 19). The observations recorded in the table are from several plants in different years, a fact to which the absence of a well-defined peak in the distribution is probably due, since the average almost certainly varies slightly with external conditions from year to year. Had a larger sample been analysed, the range of actual numbers would probably have been slightly wider, and it is possible that in extremely rare cases the maximum of 22 trivalents are actually formed. Such further possibilities are, however, of no importance from the present standpoint, for the main purpose of Table 2 is to indicate that, had the origin and nature of the plants been unknown, it would have been safe to infer autopolyploidy if the average number of trivalents found could be shown to involve more than half the chromosomes present. This fact has, of course, already been used for the analysis of the *Biscutella* series noted in Chapter 1.

Table 2. *Frequency of trivalents in* 109 *cells of autotriploid* Osmunda ($n=22$)

	No. of trivalents per cell															
	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
No. of cells	.	1	1	1	5	7	9	11	13	12	12	17	12	8	.	.

Comparable information for the tetraploid is contained in Figs. 20*c*, 22*d*, 24 and in Table 3. Where four homologous sets of chromosomes are seeking partners at meiosis, some quadrivalents will inevitably result, though in a proportion of cases, the exact numbers varying from cell to cell, a quadrivalent will be represented by two pairs or, in a smaller proportion of cases, by a trivalent and a single. In the figured cell (Figs. 22*d*, 24) only pairs and quadrivalents are contained; this is a fairly common condition, but examples with one or two trivalents and singles in addition to quadrivalents are almost equally so. Table 4 gives the actual frequency of trivalents in the analysed cells of Table 3.

Table 3. *Analysis of multivalent pairing in 101 cells of autotetraploid* Osmunda

	No. per cell															
	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Quadrivalents only	3	1	4	10	11	12	14	13	13	9	8	1	1	.	1	.
Total multivalents (quadri- + trivalents)	.	.	2	1	12	8	16	15	15	15	12	2	1	1	1	.

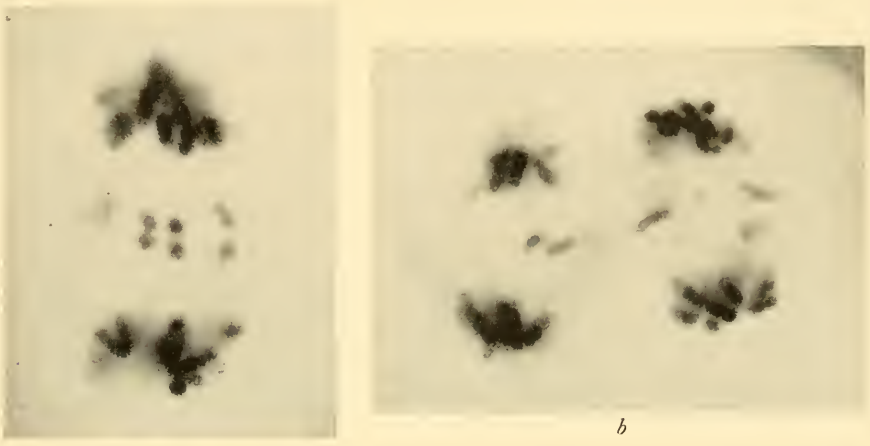
Table 4. *Frequency of trivalents in 101 cells of autotetraploid* Osmunda

	No. per cell							
	0	1	2	3	4	5	6	7
101 cells of Table 3	34	36	24	4	1	2	.	.

These numerical facts have been given, in what at first sight may seem to be rather pedantic detail, for two reasons. In the first place they are needed to illustrate the type of observation which, when it can be carried out, is more informative than any other for cytogenetic analysis. Secondly, they are required to explain the breeding behaviour of the polyploids, consideration of which will conclude this chapter.

The fertility and subsequent behaviour of the spores produced by any plant are very closely connected with their nuclear content which, in its turn, is largely controlled by the details of chromosome pairing at meiosis. Quadrivalents and bivalents can disjoin regularly with equal ease, and if only these were produced, meiosis in a tetraploid would be as uniform and effective as in a diploid. Trivalents and univalents cannot, however, disjoin equally. From a trivalent two chromosomes will generally go to one pole and one to the other at anaphase of the first meiotic division, so that the resulting nuclei will at once be dissimilar. A univalent cannot disjoin at all, but instead it lags on the spindle until it splits longitudinally. The half-chromosomes pass to each pole at the close of the first meiotic division, but they lag again at the second, being unable to split a second time. Finally, they either pass at random to one pole or the other or are lost. Two views of the behaviour of univalents are given in Fig. 25.

While the presence of both trivalents and univalents thus entails an element of random variation in chromosome numbers at the close of meiosis, this is of only limited extent in the tetraploid. In the triploid, on the other hand, variation will be extreme, since random segregation here concerns not one or two chromosomes only but the whole of the third haploid set. The probability that these will distribute themselves to the poles to give exactly balanced spores with n or $2n$ chromosomes is only one in about two million. All other types of spore will be unbalanced and possess chromosome numbers ranging from haploid to diploid but with the majority midway between.

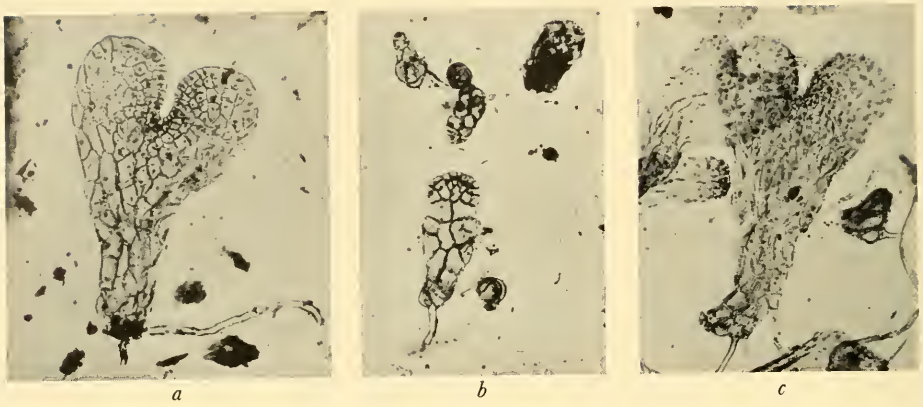


a *b*

Fig. 25. The behaviour of univalents at the two meiotic divisions in triploid *Osmunda*. *a*. Anaphase of the first meiotic division showing lagging univalents in the act of splitting longitudinally. *b*. Anaphase of the second meiotic division showing the half chromosomes produced from univalents at the previous division lagging again on the spindle in both dividing cells since they are unable to split longitudinally a second time. Lagging chromosomes at either of these divisions are therefore very clear indication that unpaired chromosomes have been present earlier. From a section stained in gentian violet. × 2000.

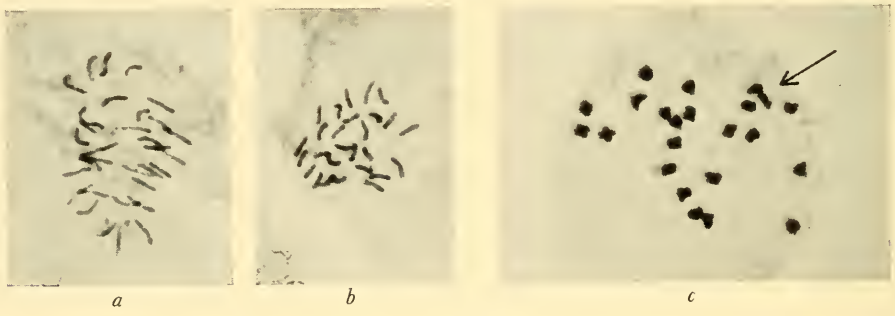
That the expected types of spore are, indeed, produced is readily ascertained either by counting chromosome numbers at the close of meiosis or by examining the early mitoses in the germinating spores. The latter is the less laborious task, for observations can be made within a week of sowing the spores. Two sample chromosome counts showing 33 and 27 chromosomes respectively in spores from the triploid are given in Fig. 27*a* and *b*.

Table 5 summarizes chromosome counts obtained in several successive years among spore sowings from the triploid and the preponderance of grossly unbalanced types is exactly as expected. Comparable figures for spores from the tetraploid are given in Table 6 and again expectation is realized. Gross unbalance is this time absent, but a high proportion (approximately two-thirds) of the spores which begin to germinate have one, or a few, chromosomes too few or too many. This proportion, it may be noted, resembles very closely the relative proportion of mother cells containing trivalents (Table 4).



a *b* *c*

Fig. 26. Comparison of germination in diploid, triploid and tetraploid *Osmunda*. ×40. *a*. Diploid prothalli from the tetraploid. *b*. Prothalli derived from the triploid, almost all abnormal and many non-viable. *c*. Normal haploid prothalli from the diploid.



a *b* *c*

Fig. 27. Chromosomes in descendants of triploid *Osmunda*. Permanent acetocarmine. ×500. *a*, *b*. Mitosis in two different germinating spores showing 33 chromosomes (*a*) and 27 chromosomes (*b*). *c*. Meiosis in a sporophyte produced from a spore sowing from the triploid showing presence of one extra chromosome which makes a single trivalent among the otherwise normal pairs. For further description see text.

The subsequent fate of these young prothalli is a matter of considerable interest. The progeny from the tetraploid have not been closely followed, though it would be expected that some unbalanced sporophytes must certainly be represented in the next generation. The situation in the triploid has, however, been studied as closely as circumstances would permit, because it might be expected that unbalance on the scale shown might lead to the production of fundamentally new types of plant if a prothallial culture of the type obtained could be self-fertilized. That this expectation was not fulfilled provides a highly instructive example of the power and mode of operation of natural selection.

The uneven appearance of a culture of spores from the triploid at the age when chromosome counts can most easily be made is shown in Fig. 26*b*, and this unevenness is never effaced. Many of the spores which start to grow proceed no further,

Table 5. *Chromosome numbers of germinating spores derived from autotriploid* Osmunda ($3n=66$)

	Chromosome number																
No. of spores	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
15 spores, 1942	.	.	.	.	.	.	.	1	3	1	3	5	.	.	1	.	1
10 spores, 1943	.	.	.	1	1	1	1	.	1	.	1	1	2	.	.	1	.
3 spores, 1944	.	.	.	.	.	1	.	.	.	.	1	1	.	.	.	.	.
Total 28 spores	.	.	.	1	1	2	1	1	4	1	5	7	2	.	1	1	1

Table 6. *Chromosome numbers of germinating fresh spores of autotetraploid* Osmunda ($4n=88$)

	Chromosome number									
No. of spores	39	40	41	42	43	44	45	46	47	48
14 spores, 1943	.	.	.	1	4	5	1	2	1	.
10 spores, 1944	1	1	3	1	1	3	.	.	.	.
Total 24 spores	1	1	3	2	5	8	1	2	1	.

while others reach adult stature but are misshapen and imperfect, and though they may live for years are unable to complete their life cycle. This is, indeed, the fate of the majority, and offspring can only be obtained at all if spores are sown on a very large scale.

As a result of repeated large-scale sowings and much patience, twenty-four descendants of a triploid plant have been accumulated. They are very varied in appearance as Fig. 28 will show; some died young and others seem unable to develop fertile fronds although of an age to do so; a few are perfectly normal. The distribution of chromosome numbers among them is highly significant. Had the average type of spore reached sexual maturity it would be expected that the triploid chromosome number would predominate in the progeny, although of course not the original triploid constitution. This is, however, not the case. As summarized in Table 7, the majority of such plants approximate to the diploid condition as regards chromosome number, though the prevalence of structural abnormalities (Fig. 28) is sufficient evidence that they are not all strict diploids of the normal kind.

Table 7. *Chromosome numbers of young sporophytes raised by sexual reproduction from spores derived from autotriploid* Osmunda ($3n = 66$)

	Chromosome number		
No. of plants	*c.* diploid	*c.* triploid	*c.* tetraploid
24	21	2	1

The degree of approximation to diploidy has only been exactly determined in those plants which had become fertile up till 1944 when the observations had to be discontinued. These amounted to six plants, of which two were exactly diploid, one

was a diploid plus 1, i.e. with 45 chromosomes instead of 44 (see Fig. 27*c*), and two were diploid plus 2, i.e. with 46 chromosomes. From the root-tip counts there is no reason to think that any of the remaining diploids which had not yet become fertile would show a greater degree of cytological unbalance than this, and none was deficient in chromosomes. It therefore seems certain that of all the prothalli formed, only those deviating in not more than two chromosomes from the haploid or diploid state were able to complete their life history. In other words natural selection, acting on the single character of fitness to reproduce, had at one blow eliminated all but a tiny minority of the prothalli brought under its influence.

Fig. 28. Leaves from two of the progeny from triploid *Osmunda* showing leaf aberrations. Both plants were three years old at the time the leaves were taken, but the left-hand plant remained permanently in the juvenile stage. For further description see text. Natural size.

Natural selection is evidently acting in this case in a capacity which is the exact opposite of a progressive evolutionary influence; it is clearly a powerful force tending to eliminate aberrations and to maintain the stability of the species unchanged. Had the autopolyploid series in *Osmunda* originated in nature, as it could possibly have done, it therefore seems probable that it would not appreciably have affected the evolution of the species, for the tetraploid seems unlikely to be able to compete with the diploid in the wild state, and the triploid would be eliminated quite rapidly by its sterility.

Disappointing as this conclusion may perhaps appear, the facts described have a very considerable comparative value which can at once be utilized. Looking downwards to the Bryophyta it is clear that the *Osmunda* series provides a very close parallel to the situation induced in many of the mosses by the Marchals, F. von Wettstein and others. In both groups polyploidy can be obtained at will as an inevitable consequence of induced apospory, and in both the series is cut short by sterility when a tetraploid gametophyte has been reached. Only in the exceptional case of species hybrids, notably that between *Funaria* and *Physcomitrium*, has an octoploid gametophyte been achieved, but to this there is as yet no parallel in the ferns.

Looking upwards to the Flowering Plants it is clear that the cytological manifestations of polyploidy in *Osmunda* are of exactly the same type as in those Dicotyledons and Monocotyledons in which they were first elucidated; multivalents are as well displayed as in *Datura*, pachytene pairing closely resembles *Lilium* or *Tulipa*. There is therefore direct evidence to justify the extension to the Pteridophyta of a type of inquiry first founded on experience in the higher plants. This in itself is an encouraging beginning.

SUMMARY

A brief account of the salient facts in the origin, morphology, cytology and reproductive effects of the autopolyploid series in *Osmunda regalis* has been given with essential details illustrated photographically. The series consists of haploid, diploid, triploid and tetraploid gametophytes and diploid, triploid and tetraploid fern plants, together with a limited number of their descendants. The information so obtained is intended in part as a standard of reference to aid in the interpretation of the facts to be elucidated elsewhere in the Pteridophyta. The evolutionary importance of the autopolyploid series itself is believed to be nil.

CHAPTER 4

THE MALE FERN *DRYOPTERIS FILIX-MAS*

A very natural starting point to any inquiry involving observation of our native ferns in the field is the Common Male Fern *Dryopteris* (= *Lastrea* = *Nephrodium* = *Aspidium*) *Filix-mas* (L.) Schott. To quote a popular handbook (Step, 1908), it is said to be the 'commonest and best known British fern', and the inevitability of its recurrent use in elementary classrooms may even at times have engendered that extreme measure of superficial familiarity which breeds contempt.

Little might one suspect that beneath this apparently familiar surface lies a welter of so much concealed complexity that a beginner in cytological matters might well be engulfed like Christian in the Slough of Despond and be tempted to abandon the whole project in despair. *D. Filix-mas* (L.) Schott is in fact not a species in any legitimate sense of the word, except perhaps the 'coenospecies' of Turesson.* It is an assemblage of forms differing in morphology, genetical constitution and life history, and there seems little doubt that, among the aggregate of forms found wild in Great Britain, at least three taxonomic species should be separately distinguished, and more may be expected to be found in other parts of the world.

In order to unravel the position even in outline, cytological and other observations have been made on about a hundred selected plants from the British Isles with a smaller quantity of material from the continent of Europe. The scope of this inquiry had to be somewhat restricted owing to the war, but enough of the necessary breeding work was completed to reveal a position of unusual interest to the evolutionist, partly because some of the larger problems raised have hope of solution.

The form of the species to which the name *D. Filix-mas* should still adhere when all necessary subdivision has been carried out is a large plant, common in hedgerows, woods and ditches throughout Great Britain, though probably not quite so abundant as the almost ubiquitous species, *D. dilatata*, which will be discussed in the next chapter. Any large population will show numerous small variations in form, an index no doubt of slight internal genetical diversity, but in addition to the broad morphological features listed below, which all of them share, must be added a sexual reproduction of the usual type (Fig. 42*c*, *e*), and chromosome numbers of 164 for the sporophyte and 82 for the gametophyte (Fig. 30). Forbidding as such numbers may perhaps at first sight appear, they are established with complete numerical accuracy and will be met with repeatedly on later occasions.

The principal morphological characters by means of which *D. Filix-mas* in the restricted sense can be distinguished in the field from the other species previously amalgamated with it are listed on the next two pages:

* A definition of this will be found on p. 71.

Fig. 29. Mature fertile pinnae of the three taxonomic species formerly included in the Male Fern (*Dryopteris Filix-mas* sens.lat.). Natural size. From left to right *D. abbreviata* (Lam. & DC.) Newm. from the Mourne Mountains (east Ireland), *D. Filix-mas* sens.strict.emend., and triploid *D. Borreri* Newm. Further description in text.

(1) The leaf texture is softly herbaceous* as opposed to coriaceous,* and decay in autumn is fairly rapid in consequence.

(2) When viewed from above the leaf is flat or convex but not concave (as in *D. abbreviata*).

* The words 'papery' and 'leathery' have been frequently used for these characters in the literature for amateurs.

(3) The pinnules are completely separated, toothed and tapering (unlike *D. Borreri*). Cf. Fig. 29.

(4) The margin of the indusium in the young state lies flat on the surface of the leaf and is not tucked under the sorus.

(5) The sori are fairly large, the average diameter being 1·5 mm. (contrast with *D. abbreviata*).

(6) The ramenta on the rachis are sparse.

Fig. 30. Diagram to explain Fig. 35. × 1500.

In contrast with this hedgerow type, in which it is not at the moment profitable to distinguish varieties, though with fuller genetical knowledge this could perhaps be done, it is important to separate a smaller mountain form with half the chromosome number (i.e. $2n=82$, $n=41$, cf. Fig. 31), which is, nevertheless, also sexually reproduced (Fig. 42*b*) and can be made to hybridize with the other (see p. 49 below). In many Floras it goes by the name of 'var. *abbreviata* Newman', and has been recorded from most of the mountainous regions of England, Scotland and Wales, and from two localities in Ireland; it is also said to have been found in central France.

The principal characters by which *D. abbreviata** can be distinguished in the field are as follows:

(1) The habitat is that of a mountain plant with a marked preference for the shallow soil of rock crevices or scree; it is only to be found under trees where these have invaded a previously exposed site.

(2) Its stature is smaller than that of *D. Filix-mas* and the form of the stock is much more tufted owing to frequent branching. A stock with a single crown is only usual among young plants.

* Some other details are enumerated by Wollaston (1875).

(3) The leaves are stiffer in texture than those of *D. Filix-mas*, though they become more like that species under shade conditions. Under normal conditions of exposure their relatively slow rate of decay results in an unusually conspicuous hanging mass of russet-coloured dead leaves, visible below the functional crown at all seasons of the year.

(4) One of the best characters to observe in the field, though, unfortunately, one which is lost in a herbarium specimen, is the concavity of the frond when viewed from above. The tips and edges of the leaves curl upwards conspicuously in a young frond and never quite flatten out in an old one (cf. Fig. 29). In all other forms of the *Filix-mas* complex the pinnae are either flat or have recurved edges.

(5) Average size of the sori does not as a rule exceed 1 mm. and is therefore smaller than in *D. Filix-mas*. Oddly enough the spores are almost identical in size with those of *D. Filix-mas* (cf. Figs. 39–40).

Fig. 31. Diagram to explain Fig. 36. × 1500.

(6) There are numerous pale scales (ramenta) on the rachis.

Though the existence of *D. abbreviata* as a distinct form has been known for over a hundred years, there has been much controversy about its status. This illustrates so well the way in which a question of taxonomy may be insoluble without cytogenetic information that it may be instructive to quote the literature in some detail as an example of a type of situation which will meet us repeatedly in later pages.

In 1815, Lamarck and de Candolle described in their *Flore Française* a small fern from south-west France as '*Polystichum abbreviatum*' in the following words:

'On pourrait, au premier coup-d'œil, prendre cette espèce pour une simple variété de la fougère mâle, mais elle est de moitié au moins plus petite; ses pinnules sont plus courtes, plus obtuses, et presque d'égale largeur dans toutes leurs étendues: leurs lobes sont plus larges, plus courts et moins nombreux, et chacun d'eux ne porte ordinairement à sa base qu'un seul groupe de fructifications, tandis qu'on en trouve plusieurs à la base de chaque lobe dans la fougère mâle.

'Cette plante a été trouvée dans les Landes, par les C. Dufour et Thore.' (*Fl. Fr.* II, 560.)

In Great Britain it is generally considered to have been Newman who first equated de Candolle's 'species' with a British plant from Ingleborough in Yorkshire (1844, *History of British Ferns*, 2nd ed., p. 202), while expressing doubts regarding its specific distinctness. Moore, in 1848 (*Handbook of British Ferns*, 1st ed., p. 43), regarded the Ingleborough plant as definitely a variety of *Filix-mas* and called it accordingly *Lastrea Filix-mas* var. *abbreviata*. Newman accepted varietal status for it in the third edition of his *History* (1854) and called it 'de Candolle's Male Fern, *Dryopteris Filix-mas* var. *abbreviata*'. In 1855, however, G. B. Wollaston, whose knowledge of British ferns, in Newman's words, 'infinitely exceeds that of any other botanist with whom I have ever

enjoyed the opportunity of conversing', again advocated very strongly that *D. Filix-mas* and *D. abbreviata* should be regarded as distinct species and proposed the name of *Lastrea propinqua* for the latter.

It is perhaps unnecessary to add further details, since the position to-day is substantially the same as that reached in 1855. There is slight doubt whether the British form is really the exact equivalent of Lamarck and de Candolle's French plant, since their description of a single sorus only, at the base of each pinnule, certainly does not apply in Britain, but 'var. *abbreviata* Newman' is a commonplace of British as of continental floras, and Wollaston's preference for specific distinctness has either not been known or has been generally disregarded by botanists. Among amateur fern collectors it is otherwise. Wollaston's name '*propinqua*' was accepted at once and is still in common use by members of the British Pteridological Society, and it figures in many collector's handbooks, including one as recent as that by Druery (1912). By the modern International Rules of Nomenclature* the name itself is illegitimate, since, among other things, it had been utilized for other plants on at least two previous occasions, namely, in 1841 by J. Smith and in 1849 by Presl. Wollaston's view of specific distinctness has, however, been fully borne out by the cytogenetical facts to be recorded below, and the only necessary modification is to admit the prior claim of the name *abbreviata* and to designate the species, if it is to be a species, as *Dryopteris abbreviata* (Lam. & DC.) Newman.

The cytogenetic confirmation of the correctness of the separation of *D. abbreviata* from *D. Filix-mas* is based on several lines of evidence. In the first place the difference of chromosome number, one being half the other, is very constantly displayed (cf. Figs. 34*a*, 36). The haploid complement of 41 chromosomes has been found in plants bearing the morphology of *D. abbreviata* from the following localities:

Wales: near Bala and near Bettwys-y-Coed; Lake District: Kentmere Valley and Borrowdale; Scotland: Greenhill Dod near Glasgow; Ireland: Mourne Mountains (east coast) and Brandon Mountain (west coast).

An even stronger argument for the specific distinctions of *D. abbreviata* is that when crossed with *D. Filix-mas* it forms a highly sterile hybrid.

The setting up of species hybrids is not an easy matter in ferns and it is always slow. The presence of both sexes on the same prothallus makes the risk of accidental self-fertilization greater than is usual, though it can be minimized by using only old prothalli which have been watered from below, to serve as females. The success or otherwise of the cross cannot easily be determined for some months after an insemination has been made owing to the morphological similarity of all related young ferns in the early stages. If a hybrid can be detected as such by its possession of a chromosome number different from that of the female parent, it can be detected by a root-tip count in about a year from its inception. Meiosis cannot be hoped for till the plant is ab out 3 years old.

* English-speaking botanists who are not professional taxonomists will find a very helpful introduction to the International Rules of Nomenclature in Bisby (1945), in which the Rules and Recommendations including those added at the 1935 International Congress at Amsterdam are reproduced verbatim. I am much indebted to F. Ballard of Kew for drawing my attention to this very useful little book.

Hybrids between *D. Filix-mas* in the restricted sense and *D. abbreviata* were successfully produced in 1939 using spermatozoids of a *D. abbreviata* from Greenhill Dod near Glasgow (Fig. 32*c*) and archegonia of a *D. Filix-mas* from Ingleborough, Yorkshire (Fig. 32*a*). Two hybrids were obtained out of six inseminations, the other four remaining without offspring. The hybrids were attested as such by showing a chromosome number intermediate between that of the two parent sporophytes ($2n = c.\ 120$), and

Fig. 32. Comparable pinnae, natural size, of the triploid hybrid (*b*) between *Dryopteris Filix-mas* sens.strict.emend. from Ingleborough (*a*) and *D. abbreviata* (Lam. & DC.) Newm. from Greenhill Dod near Glasgow (*c*).

meiosis has since been seen in both. The first sporangia were produced on one of the plants in 1943, though, unfortunately, it died in the following winter; the other was first fertile in 1944, and had become a large plant by 1948 when the silhouettes of Figs. 32*b* and 33 were taken. In pressed condition it is so like *D. Filix-mas* that it would probably be mistaken for that species but for its abortive spores and abundant ramenta. It is, however, definitely intermediate between its two parents in most characters as Fig. 32 may perhaps suggest. The frond is slightly concave when viewed from above, though less so than is *D. abbreviata.* The stock has, however, become much

Fig. 33. The triploid hybrid between *Dryopteris Filix-mas* sens.strict.emend. and *D. abbreviata* (Lam. & DC.) Newm.; a larger portion of the frond from which the pinna of Fig. 32*b* is taken, to show general morphology and ramenta on the rachis. Natural size.

branched as in that species. No viable spores have so far been obtained from it, though sowings have been made several times.

It may be mentioned in passing at this point that almost all attempts at defining the species concept include recognition of the necessity of some measure of morphological

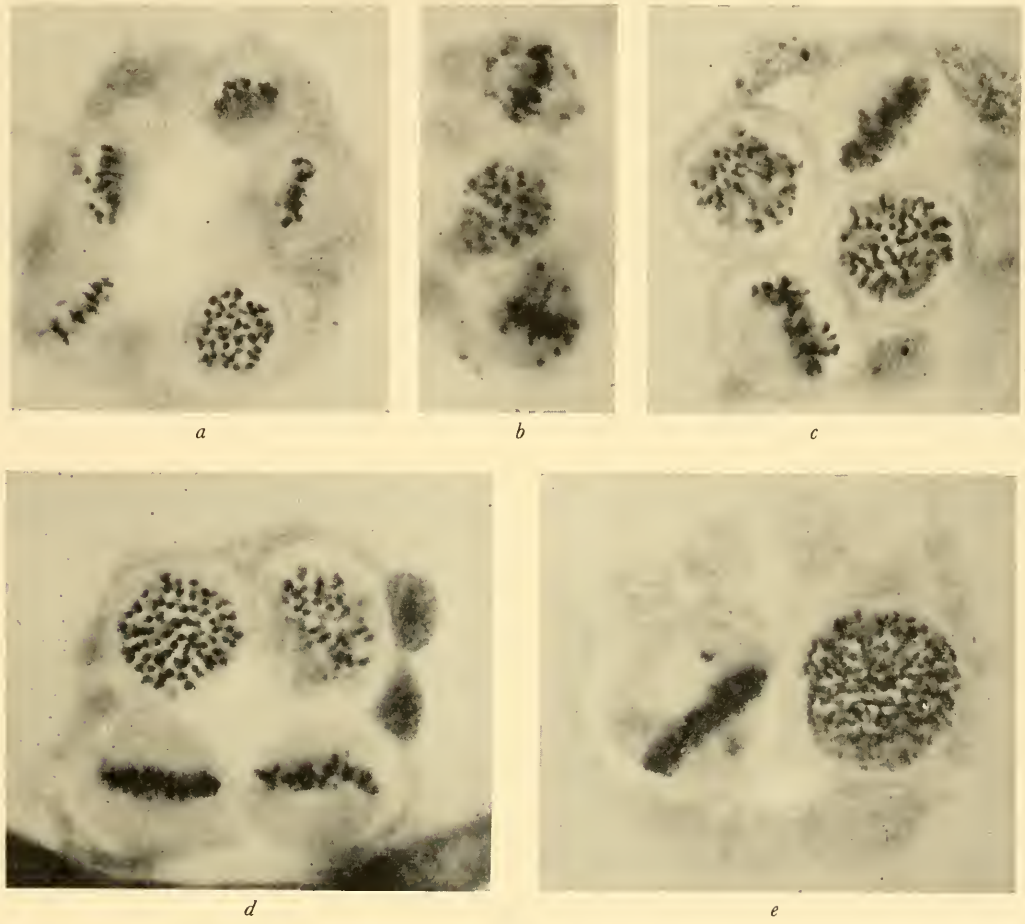


Fig. 34. Samples of meiosis in the Male Fern complex from sections stained in haematoxylin. × 1000. *a*. *Dryopteris abbreviata* Newm. Compare with Fig. 36 for details. *b*. Triploid hybrid between *D. abbreviata* and *D. Filix-mas* showing lagging unpaired chromosomes, but also numerous pairs. Compare with Fig. 37 for details. *c*. *D. Filix-mas* sens.strict.emend. Compare with Fig. 35 for details. *d*. Diploid *D. Borreri* Newm. Note the large size of the spore mother cells which compares with that of *D. Filix-mas* rather than with *D. abbreviata*. For explanation of this see p. 58. *e*. Pentaploid *D. Borreri* × *D. Filix-mas*, a wild hybrid. Note much larger cells than in *d* and very densely crowded metaphase plate.

distinctness in the species and, usually, of some element of sterility in crosses with other, even though related, species. On the evidence already presented therefore *D. abbreviata* is almost certainly a species.

Additional information is obtained by study of meiosis in this hybrid. As already made clear in previous chapters, the numerical details of chromosome pairing at

meiosis can be a very valuable guide to the genetical make-up of a plant. In this way the only condition under which *D. abbreviata* and *D. Filix-mas* could *not* legiti-

Fig. 35. Acetocarmine squash of *Dryopteris Filix-mas* sens.strict. emend. showing 82 pairs of chromosomes. For explanatory diagram see Fig. 30. × 1000.

Fig. 36. Acetocarmine squash of *Dryopteris abbreviata* Newm. showing 41 pairs of chromosomes. For explanatory diagram see Fig. 31. × 1000.

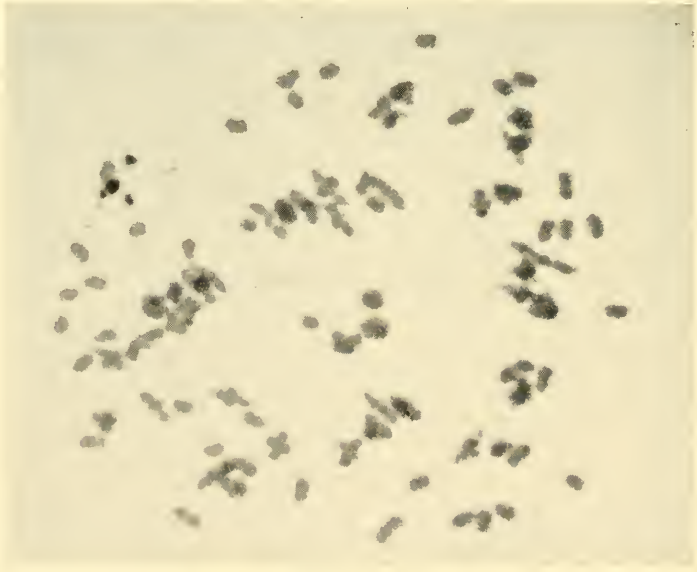


Fig. 37. Triploid hybrid between *Dryopteris Filix-mas* and *D. abbreviata*. Acetocarmine squash showing 40 univalents, 40 pairs and a trivalent. × 1500. For explanatory diagram see Fig. 38.

mately be separated taxonomically would be if it could be shown that one was merely an autotetraploid form of the other. In that case, however, one would expect to have found quadrivalent groups in *D. Filix-mas*, but these are certainly absent.

One would also expect numerous trivalents in the triploid hybrid, which, however, also do not occur.

Meiosis in the triploid hybrid between *D. Filix-mas* and *D. abbreviata* is shown in Fig. 34*b*, in which some cells at the first meiotic division are put between comparable views of the two parent species. The irregularity produced by lagging unpaired chromosomes is very conspicuous in the hybrid. The details of chromosome pairing are better displayed in a 'squash' preparation, and comparable cells of the three plants are given again in this technique in Figs. 35–37. Fig. 37 is reproduced at a higher magnification than Figs. 35 and 36 to facilitate observation, and a diagram of the

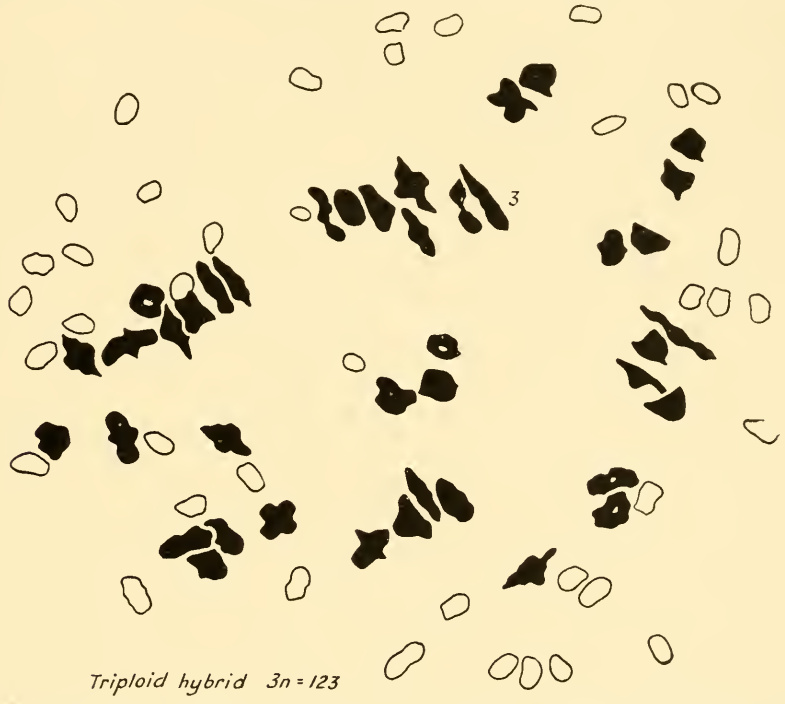


Fig. 38. Explanatory diagram to Fig. 37. ×2000. Pairs in black, univalents in outline.

complete analysis of it is given in Fig. 38. The 123 chromosomes contained in it are represented by 40 pairs, 40 univalents and 1 trivalent. This is not the type of pairing found in autotriploid *Osmunda* but is closely comparable to that of allotriploid watercress, allowance being made for the different monoploid number ($n=41$ in *Dryopteris*). The single trivalent is most easily explained by postulating one segmental interchange between two otherwise non-homologous chromosomes in the *D. Filix-mas* nucleus. Except for this the pairing bears a very close numerical relation to the basic haploid number, and the interpretation would appear to be, as in the case of the watercress, that all the chromosomes of the diploid species can find partners in the tetraploid species but that these represent only half the nucleus of the latter, the other half apparently appertaining to some different species with different homologies in its chromosomes.

The 'commonest and best known British fern' would therefore seem to be the first case of an allopolyploid to be detected among ferns and to have owed its origin in the first place to a cross between a species identical with or closely resembling *D. abbreviata* and some other diploid species which has not yet been identified, followed in the second place by chromosome doubling in the hybrid.

If this diagnosis is correct it should be possible at some future time to resynthesize the Male Fern from its component species. The identification of the second parent is, however, likely to be a matter of some difficulty. *D. Filix-mas* in the narrow sense is common all over Europe, extending east at least as far as the Caucasus. It must therefore have been in existence for some time, and the second parent may in fact be extinct. At least it may be expected to be non-British, since otherwise it seems likely to have been detected in some way by the sharp eyes of the early collectors. This problem may therefore be recommended to the interest of continental botanists.

Fig. 39. Spores of *Dryopteris Filix-mas* sens.strict.emend. × 100.

The third species that should be separated from the old *Filix-mas* complex cannot be fully dealt with until the cytological details accompanying apogamy have been described (see Chapters 10 and 11). It is *D. Borreri* Newman, and its principal diagnostic characters known to me in Great Britain are as follows:*

(1) Usually a large and handsome fern (except for certain local strains) and in both respects more striking than *D. Filix-mas*.

(2) Fronds tough and 'leathery', persisting long in the autumn and sometimes surviving the winter, often of a more yellowish green than in the other two species. The vernation of the unfolding fronds is usually more lax than in the other species.

(3) Surface of the pinnae glossy when living.

(4) Ramenta very abundant and more so than in either of the other two species. Often, though by no means always, of a bright orange yellow, sometimes with some darker cells at the base of the scale.

(5) Indusium in most strains tucking under the sorus.

(6) Shape of the pinnules very characteristic; they are not completely separated at the base; the sides of the pinnules are rather straight and the tips abruptly truncate and not tapering (cf. Fig. 29).

(7) A very characteristic detail always present, even in putative hybrids, though unfortunately lost in herbarium specimens, is the presence of a patch of dark pigment on

* For further amplification, see Wollaston (1875).

the base of the pinna rachises near where these join the main rachis. This character was first pointed out by Newman, and I have also found it very reliable even in hybrids with *D. Filix-mas*.

(8) The spores are consistently larger than in either of the other species, though sometimes much admixed with abortives for reasons which will appear. Figures will be found in Chapter 11. As a rule only 8 spore mother cells instead of 16 are produced in one sporangium.

(9) Prothalli consistently apogamous (cf. Fig. 42*a*, *d*).

(10) Habitat closely resembling *D. Filix-mas*, though with local strains accompanying

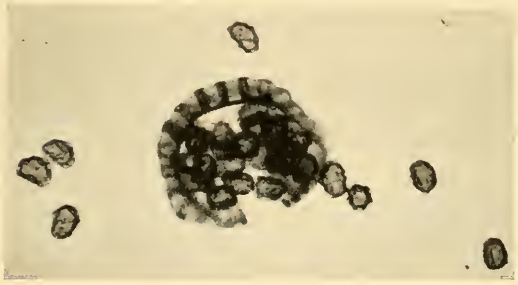


Fig. 40. Spores of *Dryopteris abbreviata* Newm. × 100.

Fig. 41. Spores of diploid *Dryopteris Borreri* Newm. × 100.

D. abbreviata. Common all over Great Britain, often forming almost pure stands covering large areas. In these cases the appearance of a local population is very uniform, and the feeling of individual differences so easily detected in *D. Filix-mas* is quite absent. Populations characteristic of different localities may show differences of detail (see below).

The history of *D. Borreri*, like that of *D. abbreviata*, is a record of a century or more of dispute. Many synonyms were already pointed out by Newman in 1854, the plant having been generally recognized both in Great Britain and on the Continent as a very distinct 'variety' (cf. Babington's *Manual of British Botany*). Its claim to specific rank has been repeatedly made, notably by Wollaston, who proposed the name of *Lastrea pseudo-mas* for it in 1855. This name is illegitimate, but as an index of the amount of attention that the idea of the species has received from continental botanists (unlike *Dryopteris abbreviata*) it may not be without interest to append a translation of a short note which appeared in the *Proceedings of the Swiss Natural History Society*, published in Geneva in 1937 (*Verh. schweiz. naturf. Ges.* 1937, p. 153):

'THE RANGE OF FORM IN *DRYOPTERIS BORRERI* NEWM.'

By Franz von Tavel (*Bern*)

'The author, in collaboration with E. Oberholzer of Zürich, has attempted to summarize the wealth of forms within *Dryopteris Borreri*, a group of ferns which is generally included in floras under the now obsolete names of *D. Filix-mas* var. *paleacea* (Moore) Druce, and var. *subintegra* (Döll) Briquet. The results are as follows:

I. *D. Borreri* Newm. s.str. Indusium stiff, leathery, turned inwards at the edge, sometimes splitting in a few sori.

(1) var. *atlantica* v. Tavel n.var. Sori small, up to 1 mm. wide, frond stiff and very tough—Madeira and Spain. Near this is f. *Merinoi Christ* (*Bull. Acad. Int. Geogr. bot. Le Mans*, no. 172, 1904) in Spanish Galicia.

(2) var. *Duriaei* Milde (*Fil. Europ. et Atlant.* 1867, 123) in Asturias.

(3) var. *insubrica* v. Tavel n.var. with large reddish brown indusia, touching each other, fronds a normal green, somewhat hairy—Tessin, south side of the Simplon; Unterwallis near Salvan (Coquoz, Farquet). In addition, conspicuous in the Bergamo Alps (Chenevard), Liguria (*Erb. Crittog. Ital.* 605) and Corsica (Aellen). With smaller sori also in the Black Forest; Baden-Baden (M. Lange) and Zastlertal (Lösch).

(4) var. *disjuncta* Fomin (Moniteur, *Jard. Bot. Tiflis*, xx, 27, 1911). The most highly developed form which has split indusia the most frequently—Tessin; Upper Rhone (Oberholzer); Black Forest (Christ, Lösch); Vosges (Walter); Caucasus (Fomin). f. *paleaceolobata* (Moore) (*Oct. Nat.-print. Ferns*, 1, 195, pl. 33C). Segments of the lower pinnae incised—Tessin; Upper Rhone (Oberholzer); England; Channel Islands; Scotland.

(5) var. *pumila* (Moore) (*Ferns of Great Britain and Ireland*, pl. 17 B, 1855). Alpine dwarf form with glandular indusium. Sori in a single row—Wales. South side of the Simplon; Tessin, in part in transition forms to var. *insubrica*.

(6) var. *rubiginosa* Fomin, loc. cit. 29. Not known with certainty beyond the Caucasus.

(7) var. *melanothrix* v. Tavel n.var. Leaf texture soft, with a dense indumentum composed of long patent black filamentous scales mixed with colourless lanceolate ones. Indusia small, black. Dillingen in the Saar (W. Freiburg).

II. Related forms with the habit of the first group, but with the deciduous flat indusium found in *Dryopteris Filix-mas*.

(8) var. *ursina* (W. Zimmermann) (*Allg. Bot. Z.* 22, 1916). Parallel form to var. *insubrica*, differing in the indusium. This is the form which is generally referred to as var. *subintegra*, but this name also includes var. *disjuncta* and other forms when used by Döll and Christ.—Widely distributed in the Alps in woods up to 1700 m. (Davos) also in the Black Forest and the Vosges. Near to this is f. *aurea* v. Tavel f.n. apparently a subalpine handsome form of yellow-green colour—Upper Rhone (Oberholzer); Bernese Oberland; Pont de Nant (Wirtgen).

(9) var. *pseudodisjuncta* v. Tavel n.var. Habit of var. *disjuncta*, indusia of *D. Filix-mas* —central Switzerland (Oberholzer); Bernese Oberland.

(10) var. *tenuis* v. Tavel n.nom. (syn. var. *subintegra* Fomin, loc. cit. 29. *Aspidium Filix-mas* var. *subintegrum* Döll. p.p. Christ p.p.). Spores in Swiss material partly aborted (Oberholzer).—Upper Rhone (Oberholzer); neighbourhood of Bern; Schaffhausen (Kummer); neighbourhood of St Gallen.

(11) var. *robusta* v. Tavel n.var. In various ways intermediate between *D. Borreri* and the different varieties of *D. Filix-mas*, with the leaf texture and hairs of the first and the pinnule shape and indusium of the second. Possibly hybrids between the two but fertile.—With the two parent species in mountain woods. Upper Rhone (Oberholzer); Bernese Oberland; Unterwallis (Coquoz); Black Forest, Hirschsprung (Lösch).'

One of the principal reasons for the very large number of apparently true-breeding varieties and forms within *D. Borreri* undoubtedly is the persistent apogamy of the species. Any morphological variant produced by mutation, hybridization or other

means, can persist and multiply if not adversely affected by selection. Local populations may therefore virtually be single clones, and their appearance of extreme uniformity already commented upon is almost certainly an expression of this.

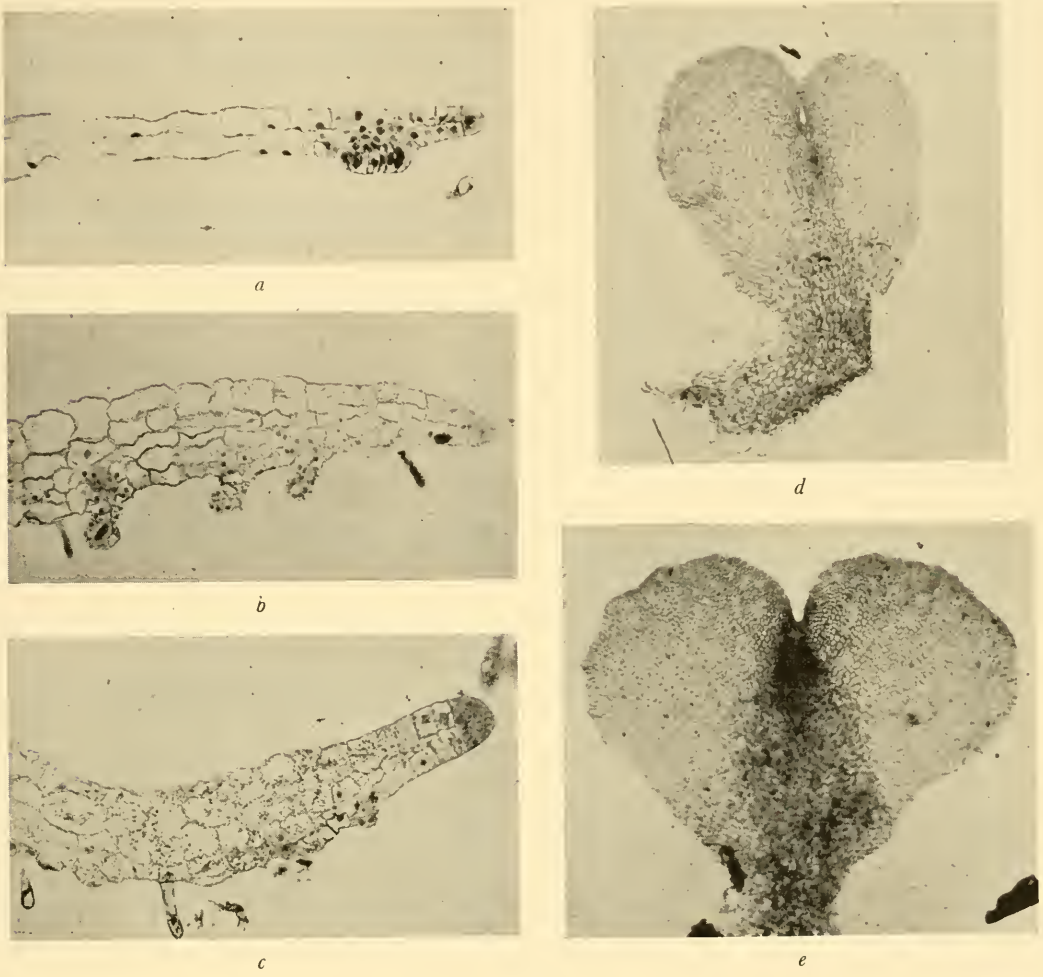


Fig. 42. Sexual and apogamous prothalli. *a*. Median longitudinal section through a prothallus of diploid *Dryopteris Borreri* showing apical cell of prothallus and an early stage of an apogamous embryo behind. × 100. *b*. The same through a sexual prothallus of *D. abbreviata* Newm. showing central cushion bearing archegonia and glandular hairs on the lower surface. × 100. *c*. The same through a sexual prothallus of *D. Filix-mas* sens-strict.emend. showing glandular hairs and young archegonia on the central cushion near the apex. × 100. *d*. Whole mount in glycerine jelly of a prothallus of diploid *D. Borreri* Newm. at the stage sectioned in *a*. The apogamous embryo appears like a dark spot. × 10. *e*. The same of a sexual prothallus of *D. Filix-mas* with archegonia.

The principal characteristics by which apogamy in these ferns can be diagnosed are as follows:

(1) The chromosome number of the prothalli is the same as that of the parent plant, no matter what this may be.

(2) Sex organs are imperfect or absent, the antheridia being usually functional but the archegonia absent.

(3) The central cushion of the prothallus remains thin, and at a very early age (compared with the time needed for a sexual prothallus) the various organs of a new sporophyte are produced directly from it (cf. Fig. 42*a* and *d*).

(4) The first leaf of an apogamously produced plant resembles the second or third leaf of a sexual one, the very simple first leaf of the normal sporophyte being unrepresented (cf. Fig. 43*b*).*

a *b*

Fig. 43.

Fig. 44.

Fig. 43. Prothalli bearing young plants each with a leaf and a root expanded. *a. Dryopteris Filix-mas* produced sexually. *b.* Triploid *D. Borreri* produced apogamously. Note the difference in the form of the first leaf. Twice natural size.

Fig. 44. A pinna of *Dryopteris Borreri* var. *polydactyla* Wills. Natural size. Formerly known as *D.* '*pseudo-mas*' var. *polydactyla* Wills, a classic horticultural monstrosity originally found wild as a single specimen but preserved in cultivation on account of the curious forked apices. A very similar specimen known in horticulture as var. *polydactyla* Dadds is illustrated in Fig. 195, p. 187; var. Wills is diploid (see below), var. Dadds is triploid (see Chapter 11).

(5) The good sporangia generally liberate only 32 large spores instead of the 64 of normal ferns. In addition to 'good' sporangia, others with aborted spores in various numbers can generally be found (see Chapter 8 for further details).

(6) In the ripening of the sporangia there are some characteristic aberrations affecting the last (premeiotic) mitosis (for further details see Chapter 10).

Apogamy, detected by one or all of these various criteria, has been found in every plant examined bearing any of the *D. Borreri* morphological characteristics from over twenty different localities in the British Isles. In addition, I was fortunate in receiving

* This character was first noticed by Stange (1887) at a very early stage in the history of apogamy.

from Dr von Tavel in 1939 spore samples of seven of his Swiss varieties and forms. These were var. *insubrica*, var. *disjuncta*, var. *ursina*, var. *ursina* f. *aurea*, var. *tenuis*, 'var. *tenuis nigricans*', var. *robusta*, 'var. *punctata*'. All of these were apogamous, and apogamy had previously been recorded by Döpp (1939) in some plants from Germany.

It would be a matter of considerable interest to know if sexual forms of this species exist, but from their obvious scarcity the suspicion is aroused that they do not. If this were known with certainty to be the case, a very interesting problem would be raised as to the possible origin of these plants. Apart from undoubted hybrids with *Filix-mas* (and perhaps with *D. abbreviata*), which will be referred to below (see also Chapter 11), the morphological characteristics of *D. Borreri* concern a number of organs and both morphological generations. A simple mutant origin of all these characters at once seems somewhat improbable. An alternative explanation might be that both apogamy and morphological characters were produced simultaneously by an act of hybridization, the incidence of apogamy making the hybrid stable from the outset without other cytological change (for further discussion of this, see Chapter 11).

Be that as it may, it is certain that some cytological changes have taken place subsequently. Within the *D. Borreri* complex a whole range of chromosome numbers, in polyploid series, can be found. In the Swiss material var. *disjuncta* and 'var. *punctata*' were diploid and the remainder triploid. In Great Britain the majority of plants are triploid (Figs. 46, 48) but extensive local diploid populations have been met with in Ireland, Wales and the Lake District. Diploids and triploids are so much alike that I have not succeeded in detecting any constant morphological differences that will serve for their identification in the field in a new locality, except perhaps spore size which is greater in the triploid than in the diploid, and it is significant that when pressed fronds of British plants were submitted to Dr von Tavel, a diploid from north Wales was identified as var. *insubrica* which in Switzerland was triploid, with only the remark that the sori in the Welsh specimen were smaller.

Without at the moment discussing the possible origin of polyploidy within *D. Borreri* which will more easily be dealt with at a later stage (cf. Chapter 11), an extension of the polyploid series by subsequent hybridization with *D. Filix-mas* can nevertheless be detected. An artificially produced hybrid between *D. Filix-mas* as female parent and a horticultural strain of diploid *D. Borreri* with forked leaves, was described by Döpp in 1939, the hybrid being identified as such with complete certainty by having the forked fronds and apogamous reproduction derived from its male parent, but combined with the tetraploid and not the diploid chromosome number. The output of 'good' spores was also much reduced. I have not myself repeated Döpp's experiment for the reasons explained in the Preface, but it is not unusual to find individual specimens of closely comparable type where populations of the two species are growing together in the wild state. Such plants will show the wealth of ramenta and the dark spot on the pinna rachises characteristic of *D. Borreri* combined with the pinna shape and some other characteristics of *D. Filix-mas*. The spore output is low, but such 'good' spores as may be formed, which are always very large, give rise to apogamously reproducing prothalli. In two cases found by myself the chromosome number of such plants proved to be tetraploid, as in Döpp's hybrid, but two other cases picked out on their

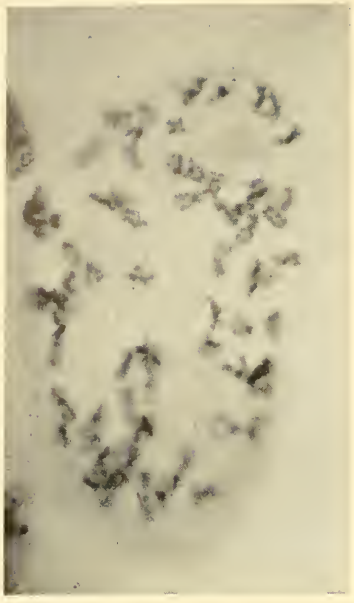


Fig. 45. *Dryopteris Borreri* var. *polydactyla* Wills, a diploid form of *D. Borreri* with 82 chromosomes, from an acetocarmine squash. × 1000. For explanatory diagram see Fig. 47.

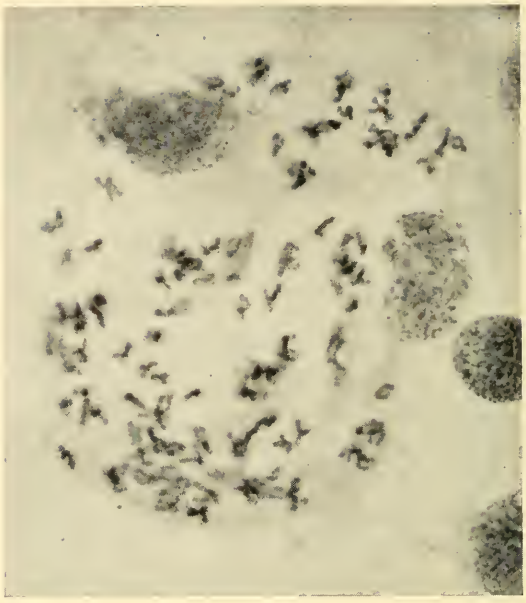


Fig. 46. Triploid *Dryopteris Borreri*, normal form. Acetocarmine squash. × 1000. For explanatory diagram see Fig. 48.

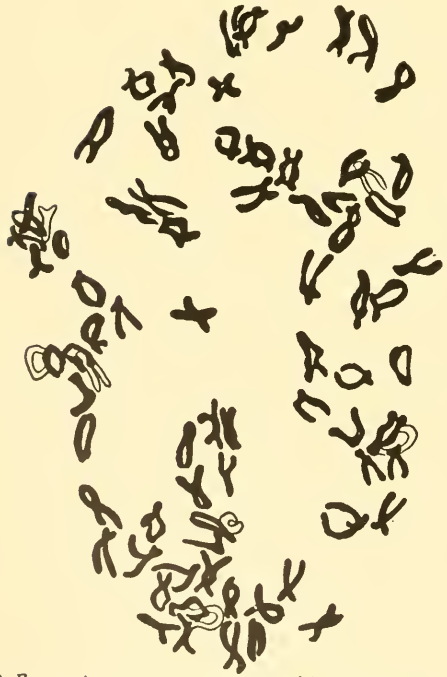


Fig. 47. Explanatory diagram to Fig. 45. × 1500.

Fig. 48. Explanatory diagram to Fig. 46. × 1500.

morphological characters by Dr L. Praeger were pentaploids. A meiotic metaphase plate of one of the latter is illustrated in Fig. 34*e* with a comparable figure of a diploid *D. Borreri* (Fig. 34*d*) added for comparison. Some photographs of the spores will be found in Fig. 201, p. 192, Chapter 11. Reduced fertility, mixed morphology and chromosome number all support a hybrid origin for these plants, the most probable parentage for the tetraploids being diploid *D. Borreri* (male) × *D. Filix-mas* (female), while that of the pentaploids seems necessarily to be triploid *D. Borreri* (male) × *D. Filix-mas* (female). The fact that both types of hybrid can to a limited extent breed true by means of the persistence of a few apogamously reproducing spores makes them somewhat more conspicuous elements in our flora than is usually the case with newly

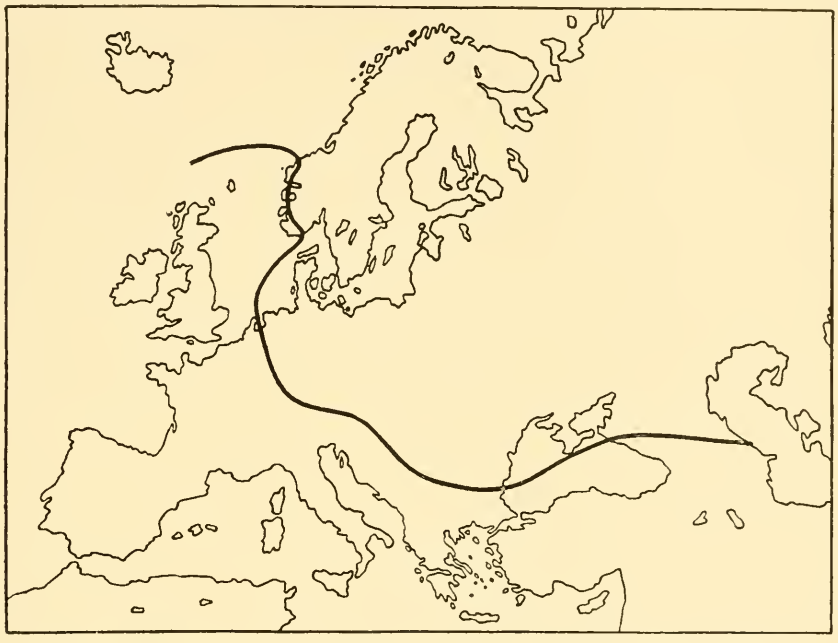


Fig. 49. Map of the northern limits of *Dryopteris Borreri* Newm. in Europe. After Nordhagen (1947).

formed species hybrids, and their existence is no doubt the chief reason why the specific distinction of *D. Borreri* has for so long been opposed by some taxonomists.

Summarizing these conclusions, without going further at present into the many interesting problems raised by *D. Borreri* Newman, it is sufficient for the moment to have shown that on the simple criteria of morphological distinctions and genetical discontinuity, three taxonomic species should be distinguished in the Male Fern populations of Great Britain. Two of these species show polyploidy and both of these can be proved, or suspected, to have arisen as a result of hybridity. All three species can probably cross with each other, though the subsequent fertility of such crosses is low.

Confusing as all this may perhaps appear to a beginner and especially to an amateur field collector, the choice of the Male Fern has turned out to be an unexpectedly fortunate one for the purpose of the present inquiry. The use of the methods of

cytogenetics has been fully justified, and while it may be left to the reader to decide whether or not 'the commonest and best known British fern' was really as simple as it seemed, an answer in no uncertain terms has been received to our first general question. By the time that we have diagnosed the existence of polyploid hybrids, of hybrids with hybrids, it has become almost impertinent to ask for further evidence that polyploidy and hybridity do at least exist in ferns.

SUMMARY

A brief morphological and cytological description is given of the three taxonomic species into which the Male Fern complex should be split. One of these species, *D. abbreviata* (Lam. & DC.) Newman, is a sexual diploid with a gametic chromosome number of 41; it has various distinctive morphological characters, including small size and an ecological preference for rocky localities in mountains. *D. Filix-mas* s.str. is a sexual type with twice this chromosome number (i.e. a sporophytic number of 164 and a gametic number of 82). It can be hybridized with *D. abbreviata*, and from the pairing behaviour in such a hybrid it is deduced that the *D. Filix-mas* is itself an allopolyploid with half its nucleus homologous with that of *D. abbreviata* and the other half of unknown origin. *D. Borreri* Newman owes its specific distinctness to its very characteristic morphology, in which it differs from both the others. It appears to be exclusively apogamous and diploid, triploid, tetraploid and pentaploid strains are known, the last two being almost certainly hybrids between the first two and *D. Filix-mas*. Further consideration of *D. Borreri* will be found in Chapters 10 and 11.

CHAPTER 5

THE GENUS *DRYOPTERIS** IN BRITAIN

Proceeding from the single species, or, as it has turned out species complex, to the wider background of the genus, the other British members of *Dryopteris* may next claim our attention. But here the diversity of forms is such, even in the limited flora of one small island, that a simple narrative will at times have to give place to something resembling a catalogue in which the logical coherence is supplied only by the fact that each of the species listed has, until quite recently, been regarded as sufficiently akin to all the others to be classified with them.

At first sight the dozen or so British species of *Dryopteris* (sensu lato) fall into two rather distinct groups. On the one hand there are about eight species of the 'Buckler Fern' type with kidney-shaped indusium, formerly placed in the genus *Lastrea* (or sometimes *Nephrodium* or *Aspidium*). On the other hand, there are the three species with naked sori and small creeping rhizomes popularly known as the Oak Fern, the Beech Fern, and the Limestone Polypody. These are very different in habit from the British species of '*Lastrea*' and were all formerly placed in the composite genus *Polypodium* owing to the absence of an indusium to their sori. The inadequacy of this negative feature as the basis of a genus has, however, been generally recognized for some time, and though it is not always easy to determine the affinities of non-indusiate species with certainty, it was Bower's (1935) opinion that the nearest indusiate relatives to the three species in question were in *Dryopteris*.

That this simple description of the genus is an incomplete picture has, however, been shown with surprising clarity by the cytological results. And since it will be necessary to call attention on several occasions to the need for taxonomic revision, not of species only but also of the genus, it may be well in the first instance to disregard all previous impressions and deal with each species categorically in the order that its chromosomes suggest.

Dryopteris aemula (Ait.) O. Kuntze (*Nephrodium foenisecii* Lowe) makes an appropriate starting point. This very characteristic and beautiful species (Fig. 50) has a markedly Atlantic distribution, being far more abundant in Ireland than in England, where it is commonest in the west and south-west. I have investigated plants from Cornwall and from Ireland with identical results.

Meiosis in *Dryopteris aemula* as seen in a section is reproduced in Fig. 71*b*, but the details of the chromosome count are better displayed in a 'squash' preparation as may be seen in Figs. 51 and 53*a*. The chromosome number in both is exactly $n=41$. This

* The classification adopted at the beginning of this chapter is that of Druce's *Comital Flora* (1932), which follows Christensen's *Index Filicum*. Recommendations on generic subdivisions to suit the cytology will be found at the end, and it is perhaps only fair to state that some of these have already been introduced on other grounds by systematists (cf. Clapham's List 1946).

Fig. 50. *Dryopteris aemula* (Ait.) O. Kuntze from County Wicklow, Ireland. $\frac{3}{4}$ natural size.

is of importance first in showing that *D. aemula* fits well into the cytological scheme of the genus *Dryopteris* outlined already by the Male Fern story. The exact identity of chromosome number between *D. aemula* and the diploid species *D. abbreviata* is, however, also of interest in showing that this somewhat unusual prime number must be far older than the Male Fern complex itself, and it will be found, indeed, to characterize not only these species but also several other related genera as the next chapter will show.

A second somewhat isolated species is *D. Villarsii* (Bell.) Woynar (*D. rigida* (Hoffm.) Underw.). This plant inhabits the deep cracks in the limestone pavement of parts of the northern Pennines, but elsewhere in Britain it seems to have been recorded only from one doubtful station in north Wales. In Switzerland it occurs as an alpine, and therefore its British station may be suspected to be that of a glacial relict. In Great Britain *D. Villarsii* is a tetraploid species having $n=82$ (Fig. 53*b*). As in *D. Filix-mas* quadrivalents are absent. A somewhat unexpected observation made as a result of a personal visit to Switzerland in the summer of 1947 is, however, that the species in that country is diploid. Fixations were taken both in the alpine garden of Pont-de-Nant sur Bex and on the wild plants in their natural habitat some kilometres away. The number is unquestionably half that of British material. This observation was obtained too late to be given more than a passing mention before this manuscript is completed for the press, but it raises some very interesting problems concerning the past history and nature of *D. Villarsii* in Britain, which will perhaps be further pursued elsewhere.

Fig. 51. Explanatory diagram to Fig. 53*a*. × 1500.

The next group of species can best be treated together. They are the species complex once united under the now obsolete name of '*Nephrodium spinulosum*',* but long since resolved, by the common consent of taxonomists, into three good species and the hybrids between them. The three good species are *D. cristata* (L.) A. Gray, *D. spinulosa* (Müll.) Watt and *D. dilatata* (Hoffm.) A. Gray. They differ from each other in morphology, habitat and frequency of occurrence. *D. cristata*, the rarest of the three, is a plant of very wet bogs, so rare as to be in danger of extinction in this country, no doubt in part owing to drainage. It was formerly recorded from a few isolated stations in a number of counties scattered from Dorset to the Glasgow area, but it appears to have died out in most of these except Norfolk. It has, however, recently been discovered in Surrey (Payne, 1939) and is still to be found near Glasgow. *D. spinulosa*, in contrast, is found in most parts of the British Isles and is often abundant, though it is limited

* Ignorance of the biology and taxonomy of this group has led some recent writers on evolution, notably Huxley (1942), to some very erroneous conclusions.

Fig. 52. *Dryopteris Villarsii* (Bell.) Woynar (*D. rigida* (Hoffm.) Underw.) from the northern Pennines. Natural size.

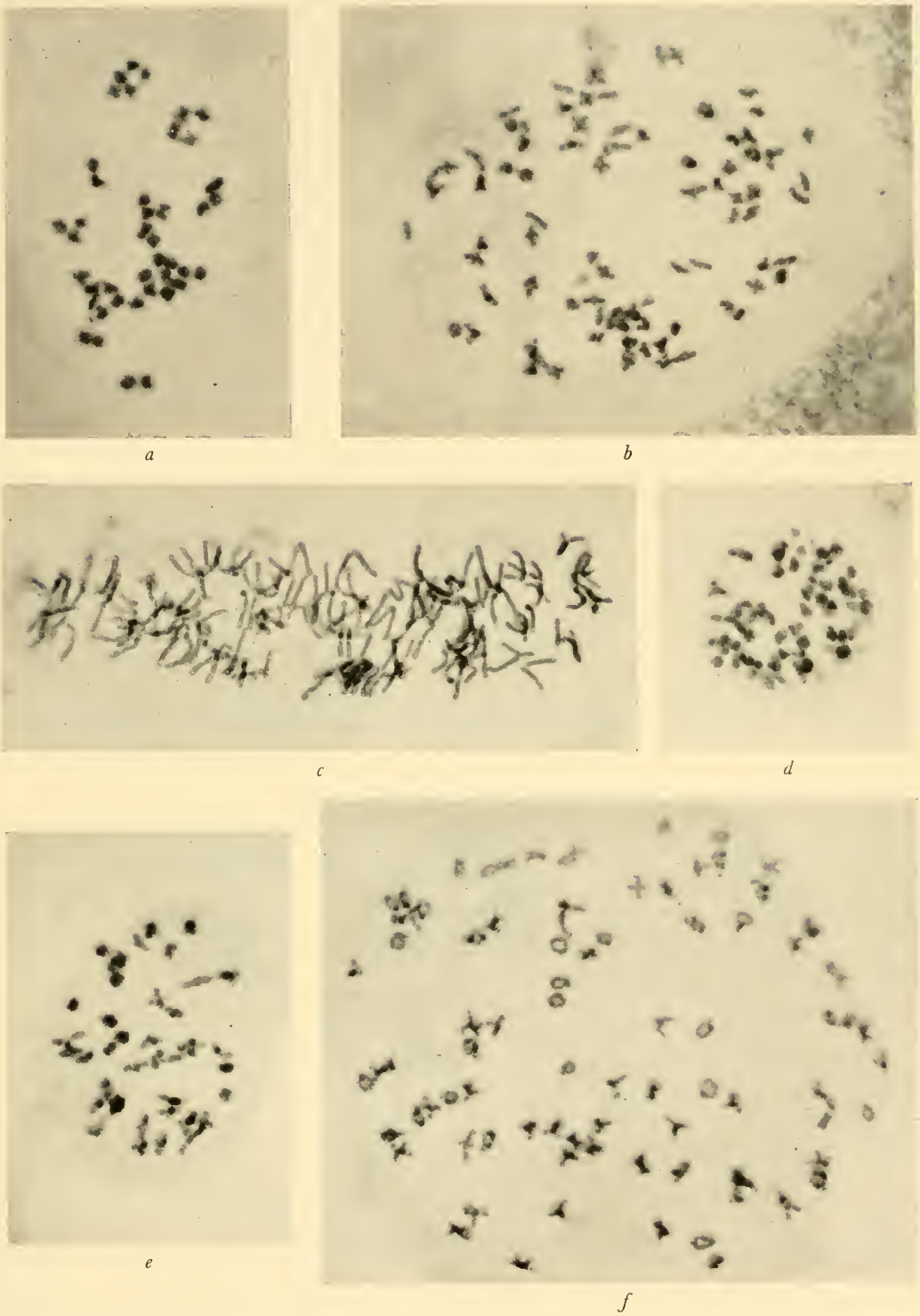


Fig. 53. Acetocarmine preparations of the British species of *Dryopteris* sens.strict. × 1000. *a*. *D. aemula* (Ait.) O. Kuntze. $n=41$. For explanatory diagram see Fig. 51. *b*. *D. Villarsii* (Bell.) Woynar from Britain. $n=82$. *c*. *D. spinulosa* (Müll.) Watt, somatic metaphase in a tapetal cell. $2n=164$. For explanatory diagram see Fig. 55. *d*. *D. cristata* (L.) A. Gray from Surrey. $n=82$. For explanatory diagram see Fig. 56. *e*. Diploid *D. dilatata* from Switzerland. For explanation see text. $n=41$. *f*. Normal British form of *D. dilatata* (Hoffm.) A. Gray. $n=82$.

Fig. 54. The lowest pinna from fully mature fertile fronds of comparable stature from the various species and hybrids hitherto recognized in the *Dryopteris spinulosa* complex. The two hybrids are *b*, '*D. uliginosa*' (= putative *D. spinulosa* × *D. cristata*) and *c*, putative *D. spinulosa* × *D. dilatata*.

ecologically to marshy woods. *D. dilatata* is relatively little bound to water, and in many parts of Britain is both commoner and hardier than the Male Fern, and like that species it has been recorded from every county and vice-county in the British Isles.

Some idea of the range of form exemplified by these three species and the hybrids between them can perhaps be obtained from Fig. 54, which shows, not indeed the whole plant, but the bottom pinna from a fully adult frond of the cytologically worked specimens. The sources of material were as follows: *D. spinulosa* and *D. dilatata* have been available in abundance from various localities in England, Scotland and Ireland,

Fig. 55. Diagram to Fig. 53*c*. × 1500.

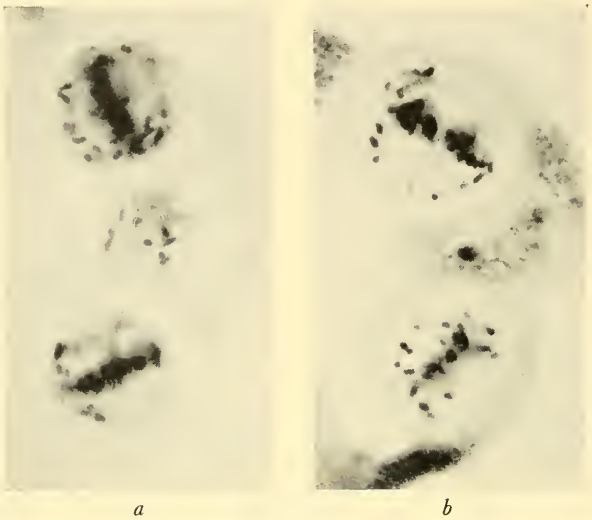
Fig. 56. Diagram to Fig. 53*d*. × 1500.

the particular specimens represented in Fig. 54*a* and *e* being from central Ireland. *D. cristata* was at first only available in cultivation from a plant supplied to the late Dr F. W. Stansfield by a dealer and believed to have come originally from Switzerland; this has, however, latterly been supplemented by material from the Surrey plant kindly supplied by the discoverer (Payne, 1939). Of the two hybrids the first (Fig. 54*c*) came from central Ireland, where it was found by Dr Praeger in company with the two putative parent species. The second hybrid, *D. uliginosa* (Newman) Druce (Fig. 54*b*), was supplied by the late Dr F. W. Stansfield from a continental specimen which, owing to the excessive rarity of this type in Great Britain, has not yet been supplemented by one of British origin.

The cytological uniformity which prevails throughout most of the '*spinulosa*' complex makes it at first sight easy to deal with all the species collectively. All three taxonomic

species (*D. dilatata*, *D. spinulosa* and *D. cristata*), in British and in continental specimens, have a regular meiotic process and identical chromosome numbers which are $n=82$ for the reduced number and $2n=164$ for the sporophytic somatic tissues. Sample photographs to illustrate these facts will be found in Figs. 53, 55, 56 and 71, of which Figs. 53*c* and 55 give the unreduced number for *D. spinulosa*, Figs. 53*d* and 56 show an aceto-carmine preparation of the British *D. cristata*, and Fig. 53*f* shows meiosis in *D. dilatata*, so perfectly that a diagram is not required. A view of *D. dilatata* in a section will be found in Fig. 71*c*.

With regard to the hybrids, Fig. 57*b* shows two spore mother cells from the hybrid between *D. dilatata* and *D. spinulosa* at the first meiotic division in side view. The large number of lagging unpaired chromosomes makes a striking contrast with the neat



a *b*

Fig. 57. Meiosis in *Dryopteris spinulosa* hybrids from sections. × 1000.
a, *D. uliginosa*; *b*, *D. spinulosa* × *D. dilatata*, wild hybrid.

appearance of the parent species (Fig. 71*c*), and is sufficient confirmation of hybridity. Since this particular hybrid is by no means rare, having been described from time to time from many European countries and, in my experience, being not difficult to find in Great Britain (I have examined specimens from England, Scotland and Ireland), it may perhaps be of interest to field botanists to append a brief description of it.

The hybrid between *D. spinulosa* and *D. dilatata* is met with not uncommonly in mixed populations of the two parent species such as may occur, characteristically, where an old and previously swampy *D. spinulosa* habitat is beginning to dry out. The hybrid (Fig. 54*c*) shows a mixture of the characters of the two parents very clearly. It has the semi-erect rhizome and dark coloured scales of *D. dilatata*, but a relatively narrow frond with the bottom pinna shorter than the one above it as in *D. spinulosa*. It will often show hybrid vigour by growing to a very great size, and the profuse stoloniferous branching of the stock will, in an old plant, cause it to lack the neat shuttlecock-like form of its parents and to present instead a rather shapeless but compact jumble of fronds. The

spores are commonly shrivelled, though it is not known whether a few might be viable. The general appearance of mixed populations suggests strongly that this is probably the case, and it is much to be desired that breeding work should be undertaken to test this point, preferably after resynthesis of the hybrid from known parents.

The hybrid between *D. spinulosa* and *D. cristata* is better known than the one last mentioned perhaps, paradoxically, in part because of its greater rarity, its frequency of occurrence being necessarily limited by that of *D. cristata*, the scarcer of its two parents. The morphological differences between *D. cristata* and *D. spinulosa* are greater than those between the latter species and *D. dilatata*, so that it is not surprising to find that the intermediate characters of the hybrid are sufficient to endow it with a marked individuality of its own. This led at an early stage to its recognition in the field as a distinct morphological type to which the name *D. uliginosa* (Newman) Druce has been given.

Meiosis in *D. uliginosa* is shown in Fig. 57*a*, and the close similarity between this and the preceding hybrid is very evident.

The interpretation of the *spinulosa* complex on the basis of all this information would therefore appear to be that we have here an old polyploid 'coenospecies'* which now contains three 'ecospecies'* adapted to different degrees of waterlogged soil. These have spread over a much larger geographical area than that so far colonized by the relatively young Male Fern, and *D. spinulosa*, *D. dilatata* and *D. cristata* are widespread in North America as well as in Europe and Asia, whereas the Male Fern itself seems to be a characteristic occupant of the Old World only. The three ecospecies are, however, still sufficiently akin to hybridize when they meet and such hybrids, though highly sterile, nevertheless show sufficient proportion of pairing chromosomes to demonstrate a fairly high degree of chromosome homology to be present among the three ecospecies. Whether these themselves arose suddenly or by gradual accumulation of mutational differences is, however, entirely unknown.

This is as far as the study of the *D. spinulosa* complex would have gone but for the evidence of '*D. remota*', to which problematical plant we may now turn. *D. remota* is a putative species hybrid, thought to combine the characters of *D. spinulosa* and *D. Filix-mas*. It is listed in the British Flora (e.g. Babington, 1922) on the basis of one plant which was once found (by Huddard in 1867) in Brathay Wood on the shore of Lake Windermere, and thereafter exterminated in the wild state though maintained and multiplied by vegetative means in cultivation. It was much prized by the amateur fern

* We can scarcely do better here than to quote the definitions of terms given by Clausen, Keck and Hiesey (1945), for the application to experimental taxonomy of the ecological concepts originally introduced by Turesson. The chief of these are:

'(1) *Ecotype* (Turesson, 1922*a*, 1922*b*): all the members of a species that are fitted to survive in a particular kind of environment within the total range of the species.

(2) *Ecospecies* (Turesson, 1922*b*, 1929): all the ecotypes genetically so related that they are able to exchange genes freely without loss of fertility or vigour in the offspring.

(3) *Coenospecies* (Turesson, 1922*b*, 1929): all the ecospecies so related that they may exchange genes among themselves to a limited extent through hybridization.'

It will probably already be apparent that while knowledge of the Pteridophyta is only rarely sufficiently advanced to deal in ecotypes, both of the other concepts will be found from time to time to coincide with the taxonomic species.

collectors of the last century, owing to which fortunate circumstance it is still possible to obtain portions of it in the living state in old collections, and I was most fortunate in being given access to a very large and ancient specimen of authentic Windermere origin in the garden of the late Dr F. W. Stansfield of Reading. In Babington's *Manual* (1922) '*L. remota* Moore' is listed as a species, with the possibility that it might be a hybrid indicated only in brackets. The complete sterility of the plant when spores are sown, however, has always been known to British pteridologists, who have been unanimous in their opinion that it is a hybrid, though the attribution of parentage has varied somewhat. *D. Filix-mas*, *D. spinulosa*, *D. dilatata* and *D. rigida* have all been named from time to time in this connexion, though the general consensus of opinion has been in favour of the combination *D. Filix-mas* × *D. spinulosa*. Some of the reasons for this diagnosis may perhaps be judged by examination of the pinna shown in Fig. 58.

Fig. 58. Lowest pinna from a large frond of an offset from the original '*Lastrea remota* Moore' from Windermere. Natural size.

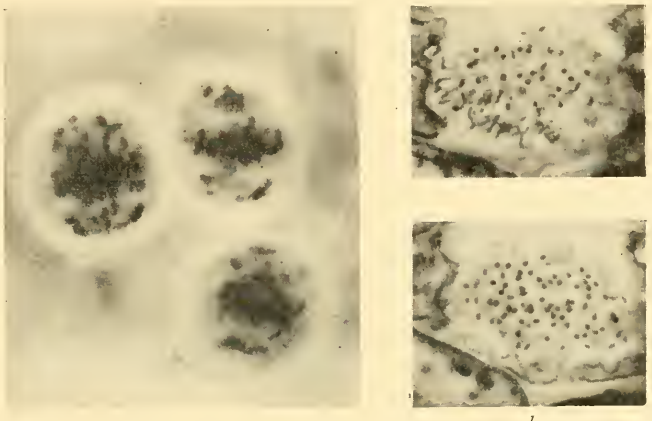
Cytological examination of the Windermere plant has strongly confirmed the diagnosis of hybridity without thereby throwing any further light on the nature of the parental species, a fact which need cause no surprise, since all those listed have identical chromosome numbers and could therefore not be distinguished cytologically. As expected, the chromosome number found in the roots of the Windermere plant is of the order of 160 (Fig. 59*b*) (that of both putative parents being of the same order, i.e. $2n = 164$). Meiosis in the Windermere plant is extremely irregular (Fig. 59*a*), a circumstance which fully explains the sterility of the spores.

Since a single plant incapable of reproduction and demonstrably a hybrid cannot possibly be accepted as a species on any definition of that word, these findings at once indicate that '*Lastrea remota* Moore' is not the same thing as the continental material to which the specific epithet *remotum* was first applied. This* was a plant first found by A. Braun near Geroldsau in Baden and described by him in 1843 as *Aspidium rigidum β remotum*, though subsequently raised to the rank of a species in 1850 under the name of *A. remotum* A. Braun. This 'species' is fertile from spores but apogamous, and when investigated by Döpp in 1932 a chromosome number was found which approximates to the triploid condition in this circle of affinity (Döpp's actual number was reported as *c.* 130; the triploid condition itself is now known to be 123). Morphologically *A. remotum* A. Braun resembles the Windermere plant in that it looks like a hybrid between the same two parent species, namely, *Dryopteris Filix-mas* and *D. spinulosa*, though its triploid nature makes its exact derivation more problematical, since

* Cf. Praeger (1909).

both its suggested parents are now known to be tetraploids. By virtue of its apogamous reproduction it is not a single plant but forms local populations in several parts of Germany, Alsace and Silesia, while a variant which has spread to Switzerland has there been designated *Aspidium remotum* var. *subalpina* Borbas,* or sometimes *Dryopteris Borbasii* Litard.

I have not seen any material of the original *Aspidium remotum* A. Braun, but a plant purporting to be var. *subalpina* from Switzerland was kindly presented to me by the late Dr F. W. Stansfield, the lowermost pinna of which is represented in Fig. 60. This agrees closely with Luerssen's Fig. 145 (Rabenhorst's *Flora*, 1889) of *A. remotum* A.Br., and the importance of giving varietal status to the Swiss material may perhaps be doubted. Be that as it may, my specimen of '*subalpina*' agrees with Döpp's *remota* in being apogamous and approximately triploid.



a *b*

Fig. 59. The Windermere '*Lastrea remota*', from sections. × 1000. *a*. Meiosis. *b*. Two extreme focal levels through a metaphase plate in a root with approximately 164 chromosomes, for comparison with Fig. 61.

Though *Dryopteris remota* in the continental sense cannot be claimed for Britain on the evidence of the Windermere plant discussed above, there are nevertheless two other records of single plants each found once and exterminated in the wild state by the act of collection though maintained in cultivation, which place the matter in a rather different light. These are '*Lastrea Boydii*', collected on the shore of Loch Lomond at the end of the last century (see Stansfield, 1934; von Tavel, 1934) and at first identified as *L. remota* from a general resemblance to the Windermere plant; and another specimen found in central Ireland by Praeger in 1898 (see Praeger, 1909) and also identified as *L. remota*. Both these finds have been available to me as spore descendants of the original plants, '*Dryopteris Boydii*' being presented to me by the late Dr Stansfield and the Irish *remota* having been obtained from Dr Praeger in 1935. Pinnae of both these plants are shown in Fig. 60, and their general resemblance to the continental material is at once apparent. Cytologically (Fig. 61) these plants also resemble the continental material in being apogamous and triploid.

* Cf. Luerssen (1889).

It therefore seems probable that *D. remota* in the continental sense, or at least plants very like it, do occur sporadically in Britain, though the evidence suggests that they

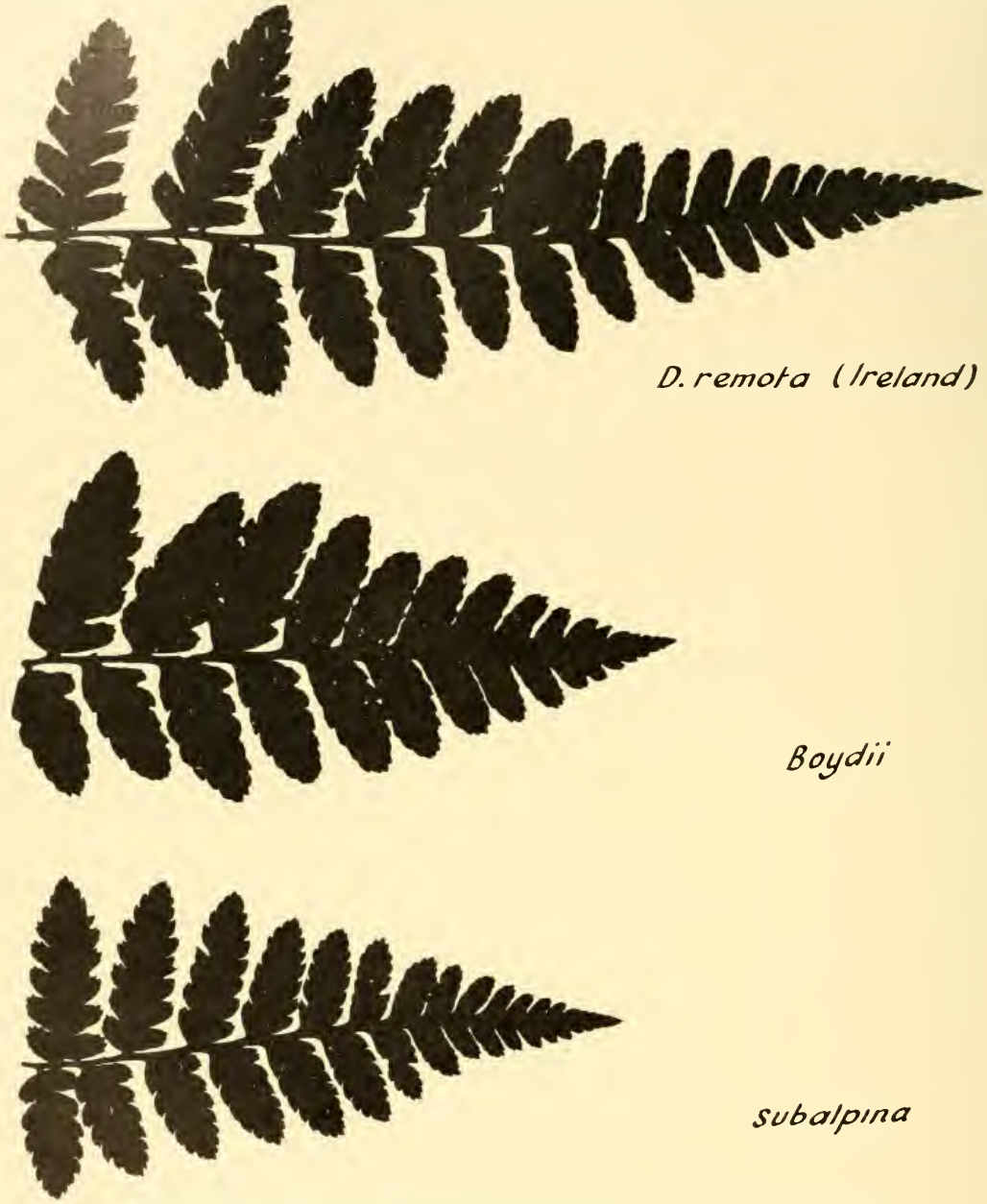
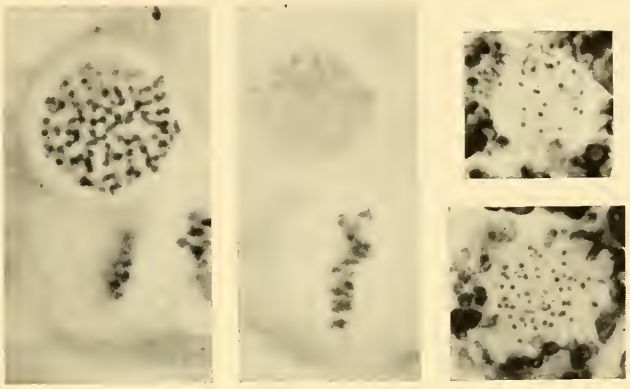


Fig. 60. The lowest pinna from plants of different age of apogamous triploids referable to *Dryopteris remota* (A.Br.) in the continental sense. Natural size.

have not yet become established as local populations. The question of their origin is therefore a matter of interest. They could perhaps arrive in Britain as stray spores from the Continent. If this is so, however, it is perhaps remarkable that they should be

found first in relatively remote parts of the British Isles (central Ireland and Scotland) and not, so far, in the more accessible parts of England or Wales. The other alternative is that they have been formed *de novo* in each locality by an act of hybridization, but here we are confronted with the dilemma that all the species with which a parental relation has been suspected (*D. spinulosa*, *D. dilatata*, *D. rigida*, *D. Filix-mas*) are tetraploids and could therefore not give rise at once to a triploid by a simple cross.

A possible solution to this problem was detected in the summer of 1948 by the discovery that a diploid form of *D. dilatata* exists on the Continent and, from its morphological characters, probably also in Great Britain, though a plant of British origin has not yet been confirmed cytologically. Fig. 62 shows a complete frond, slightly reduced but otherwise characteristic of a population of small individuals found growing in profusion on the north face of a sheltered gully above the tree line on the frontier



a *b* *c*

Fig. 61. The cytology of '*Dryopteris remota*' from Ireland, for comparison with that of the Windermere specimen. For description see text. From sections. × 1000. *a*, *b*. Two different focal levels through the same group of mother cells showing a polar and a side view. Note the extreme regularity and the large number of the chromosomes. *c*. Two focal levels of a mitotic metaphase plate in a root. Note the lower chromosome number in comparison with Fig. 59*b*.

between Norway and Sweden at Storlien in Jämtland. Its oval outline is distinctly narrower than is normally shown by lowland forms of *D. dilatata* in Britain, and the shape of the lowest pinna bears some resemblance to *D. spinulosa* (cf. Fig. 54*e*); it has, however, quite unmistakably the dark central streak to the scales (*ramenta*) which is otherwise diagnostic of *D. dilatata*. Fig. 63 shows the central portion, natural size, of a larger specimen found in a wood at sea-level at Trondheim in Norway, and Fig. 64 shows the lowest pinna only of a still larger plant of the same general character which came from a high altitude at Arolla in Switzerland. All these plants agree in having 41 chromosomes instead of 82 in their spores, a feature which is perhaps sufficiently demonstrated by a comparison between Fig. 53*e*, p. 67, from the Swiss plant with Fig. 53*f* of normal British *D. dilatata* placed immediately beside it. All agree also in their more finely cut pinnation, as may be seen by comparing any of the three Figs. 62–64 with Fig. 54. This character is mentioned from time to time in the early British literature in relation to various rather ill-specified 'varieties' of *D. dilatata*, which is one

Fig. 62. Whole frond of a small, high mountain form, of the new diploid of *Dryopteris dilatata* (see text) from Storlien, Jämtland, Sweden. $\frac{3}{4}$ natural size.

Fig. 63. Base of the leaf blade of a lowland form of the new diploid of *Dryopteris dilatata* from Trondheim, Norway. Natural size.

reason for believing that the plant is likely to be found here though not yet diagnosed. Further information about diagnostic characters, genetical affinities, geographical distribution and appropriate name must, however, await further study. Enough has

Fig. 64. Lowest pinna of a very large plant of the new diploid of *Dryopteris dilatata* from the Alps. Natural size.

nevertheless perhaps been said to explain why taxonomists working on the alpine and subarctic forms of the *spinulosa* complex have been less satisfied with the simple subdivision into *D. spinulosa*, *D. dilatata* and *D. cristata* than have botanists working on the lowland populations of more equable latitudes.

We have also now a new possibility for the parentage of *D. remota*. If the new diploid were to hybridize with *D. Filix-mas*, or another of the suggested species of appropriate morphology, a triploid would be formed at once which might or might not be apogamous but which would be expected to show much the same mixture of characters that we find in *D. remota*. Attempts to synthesize such a hybrid have already been begun though they will take several years to mature. If they are successful a very long-standing problem in the European flora will have been solved.

Leaving these problems aside, we come next to two very distinct and well-known species, *D. Oreopteris* Ehrh., the Sweet Scented Mountain Fern, and the Marsh Fern, *D. Thelypteris* (L.) A. Gray. In habitat and in habit these two species are about as far removed from each other as any in the genus. The Mountain Fern is never found far from hills; it is of the ordinary *Lastrea* type, growing abundantly in exposed positions

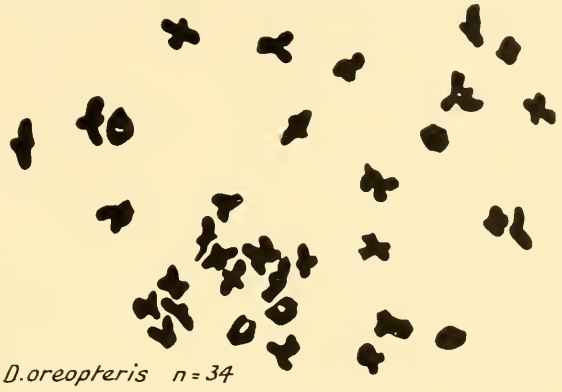


Fig. 65. Explanatory diagram to Fig. 66. × 1500.

up to 1680 ft. in England, 2000 ft. in Ireland and 2900 ft. in Scotland, sometimes mixed with *Dryopteris abbreviata*, *D. Borreri* or *D. dilatata* at the lower levels but eventually outstripping these as it ascends. The Marsh Fern has a creeping habit and grows submerged in the wettest of lowland bogs to which it is absolutely confined. In nature these two species probably never meet under present conditions, and that they are ancient is indicated by a geographical distribution which includes America as well as Europe. To a cytologist they show, however, several striking points of resemblance. Although the Marsh Fern has a curiously inverted periodicity in putting up its fertile leaves after its sterile leaves, at the end of July, the sori share with those of the Mountain Fern the peculiarity that they are placed very near to the edge of the pinnules, and though an indusium is present, the main protection for the sporangia at the stage of full meiosis is the recurved margin of the leaf. They also differ very markedly in chromosome number from all other British species of the genus.

Fig. 71*a* represents meiosis in *D. Oreopteris* as it appears in sections, and Figs. 65 and 66 show a similar stage in a squash preparation in which the number $n = 34$ is very clearly displayed.

Figs. 67 and 68 represent a squash preparation of the Marsh Fern, *D. Thelypteris*. The number appears not to be 34 but still less is it 41. In this species, as nearly as can be determined, $n = 35$.

The interest of finding an entirely new chromosome number in each of these two species is enhanced by two further circumstances. It has already been seen that the gametic number of 41 is fundamental to the *D. Filix-mas* complex, the *D. spinulosa* complex, to *D. aemula* and *D. rigida*, but as will be shown in detail in the next chapter,

Fig. 66. Meiosis in *Dryopteris Oreopteris* (Ehrh.) Max. showing $n=34$, permanent acetocarmine. × 1000. For explanatory diagram see Fig. 65.

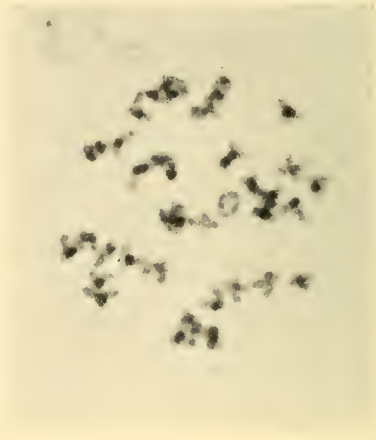


Fig. 67. Meiosis in *Dryopteris Thelypteris* (L.) A. Gray showing $n=35$, permanent acetocarmine. × 1000. For explanatory diagram see Fig. 68.

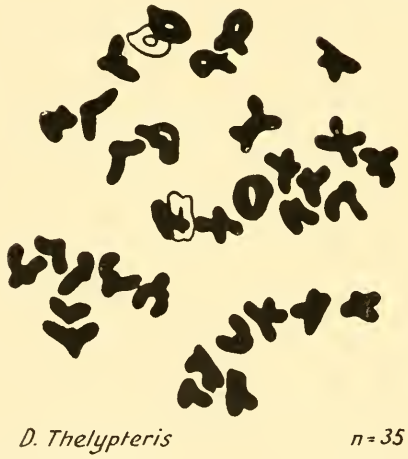


Fig. 68. Explanatory diagram to Fig. 67. × 1500.

it is equally so to a number of other related genera, notably *Polystichum*, *Cyrtomium* and perhaps *Woodsia*. It must therefore be an ancient character which may even antedate the evolutionary separation of *Dryopteris*. It would, however, be a mistake to suppose on this account that the numbers 34 and 35 are merely recent developments from this. The inference should probably be that the generic boundaries have here been wrongly drawn, and some very instructive comments suggesting this have been made from time to time by taxonomists. Thus in 1920 Christensen wrote: 'As I have tried

to prove in my earlier papers on *Dryopteris*, that large genus of ferns may be divided into a number of well-defined subgenera, and in the first part of the present monograph I have referred the tropical American species . . . to ten subgenera. Since that part was published I have examined about 3000 specimens of species with more divided leaves, and these later studies have confirmed that my classification is a natural one. I have no doubt that those species which are grouped together within the same subgenus belong together genetically, while, on the other hand, they are very remotely related to species belonging to other subgenera. I am convinced that most, if not all, of the defined subgenera really are good genera such as genera are commonly understood.'

A further stage in the subdivision of *Dryopteris* on these lines is contained in Verdoorn's *Manual of Pteridology* published in 1938, in which Christensen himself separates a much-reduced genus *Dryopteris*, with *D. Filix-mas* as its type species, into a separate tribe from the genus *Thelypteris* with the Marsh Fern (*T. palustris*) as its type species. With the Marsh Fern is placed the Mountain Fern under the name *T. Oreopteris*, and also the Beech Fern, which will be dealt with below. In Christensen's opinion (Verdoorn's *Manual*, p. 543) the old genus contains 'at least two phyletic lines which have arrived at, or perhaps preserved the same soral condition, but as to anatomical structure . . . have followed different lines'. A still more extreme view is expressed by Holttum, whose *Revised Classification of the Leptosporangiate Ferns* (1947) appeared during the writing of this chapter. In this he remarks that 'it seemed to me that too little stress had been laid on the differences of the *Thelypteris* group of genera from the *Dryopteris* group'. In Holttum's view *Thelypteris* is likely to have been descended from a Cyatheaceous or Gleicheniaceous ancestor, as Bower believed to be the case with the whole 'genus', but *Dryopteris* in the narrow sense he would relate to *Dennstaedtia*. Copeland's views (1947) are not dissimilar though his nomenclature is somewhat different.

These expressions of opinion are perhaps enough to warn us against wasting time at this stage in premature discussions as to what nuclear condition can have been primitive in '*Dryopteris*' as a whole, and we need only record the fact that the Marsh Fern and the Mountain Fern are as different from all the species previously considered in their chromosome numbers as they appear to be in their other characters.

Christensen's mention of the Beech Fern as a possible relative of *Thelypteris* rather than of *Dryopteris* in the narrow sense may serve to introduce the last three species with which this chapter will be concerned. These are, as explained at the beginning of the chapter, the Beech Fern, the Oak Fern and the Limestone Polypody. So confused is the Latin nomenclature of these very distinct and well-known species that for once the English names may be preferred. The naked sorus, which they all share, has not confused the specific identity of any of them, but it has led to considerable doubt regarding the generic affinities which are by no means yet resolved. The old solution, to put them all in the genus *Polypodium* along with the common Polypody itself (*P. vulgare*), has by common consent now been abandoned. To assimilate them all into *Dryopteris* is, however, also unsatisfactory now that the polyphyletic nature of that 'genus' has been made clear. The other alternatives can perhaps better be discussed after the cytology has been examined.

The chromosomes of the Beech Fern (*Phegopteris*) are shown in some detail in Figs. 69–71. In Fig. 71*f* meiosis is seen in a section, and the somewhat smaller size of the chromosomes than in the species previously examined may be seen. The chromosome number in the metaphase plate shown is of the order of 90 pairs, and this number can

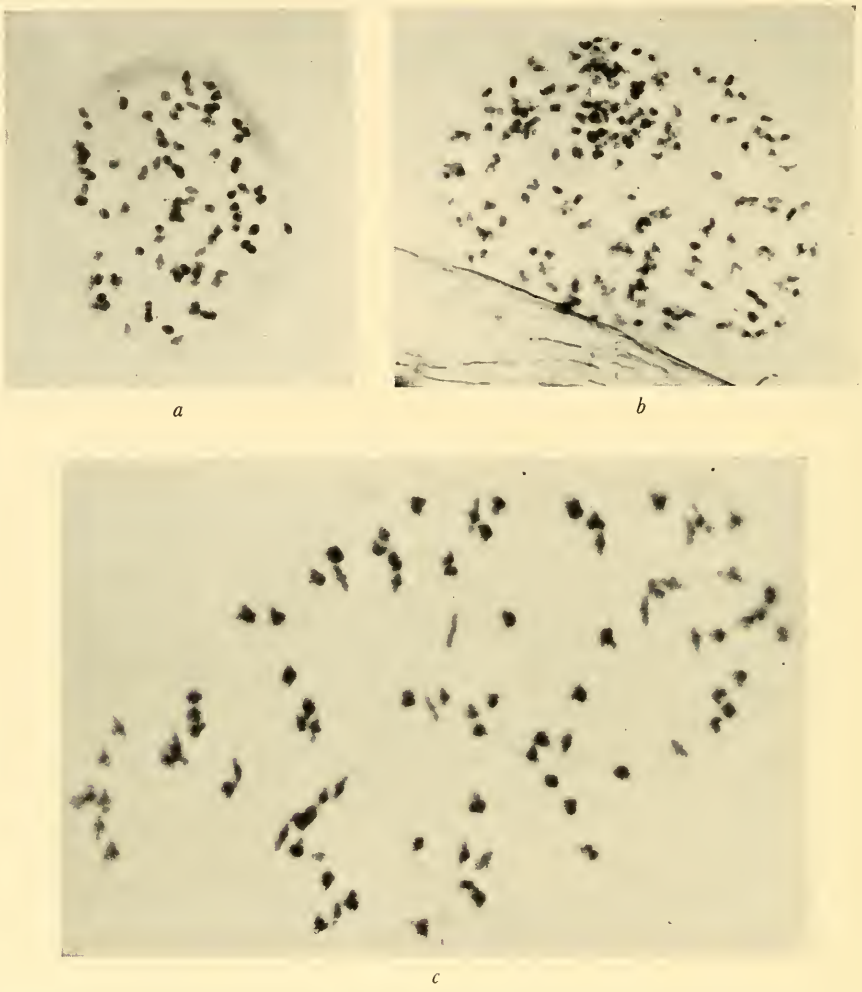


Fig. 69. Meiosis in the Beech Fern (*Phegopteris*). Permanent acetocarmine preparations. *a*, *b*. From a British specimen, two cells in the same preparation. × 500. For explanatory diagram of *a* see Fig. 70. *c*. A Scandinavian specimen from Storlien, Jämtland, Sweden, showing 90 chromosome pairs with exceptional clarity.

be seen again in the squash preparations of Fig. 69, with one explanatory diagram in Fig. 70*a*. The large cell of Fig. 69*b* is a giant mother cell from the same sorus as Fig. 69*a* showing twice this number, an occurrence which will be discussed in greater detail in Chapter 9.

A section through a sorus of the Beech Fern, however, shows at once that the majority of the sporangia have only 8 spore mother cells, instead of the customary 16, a condition

which was noticed in *Dryopteris Borreri* (Chapter 4) as indicative of obligate apogamy. In the Beech Fern its significance is no less and a chromosome count in a root, as indicated by Figs. 70*b* and 71*e*, also shows about 90 chromosomes. The meiotic process, for all its apparent regularity, is, therefore, ineffectual as a means of changing the nuclear content, and both morphological generations possess the same number of chromosomes. By sowing the spores it is not difficult to demonstrate that the resulting prothalli are indeed apogamous as anticipated.

Fig. 70. Explanatory diagram to Figs. 69*a* and 71*e*.

The detection of obligate apogamy yet once more is a matter of some interest. We have met it so far in *D. Borreri* and *D. remota*, but that there can be any close relationship between either of these and the Beech Fern is quite out of the question, and the polyphyletic origin of apogamy must be accepted as irrefutable. With regard to the possible parentage or phyletic affinities of the Beech Fern we are wholly in doubt. The chromosome number is so unlike that of any other known species of either *Dryopteris* or *Thelypteris* that pending further information it seems better to suspend judgement by placing the Beech Fern in a separate genus *Phegopteris* as has sometimes been done, rather than to prejudge the position by following Christensen in uniting it with *Thelypteris*. Its name under this treatment should then be *Phegopteris polypodioides* Fée.

A similar procedure may also be advised for the remaining two species. The Oak Fern and the Limestone Polypody are much alike cytologically and have a normal

sexual life history. The chromosomes agree with those of *Phegopteris* in their small size, a fact which is demonstrated by comparing Fig. 71 *d* from a section of the Oak Fern with other photographs on this page. The number, however, is different. Owing to the

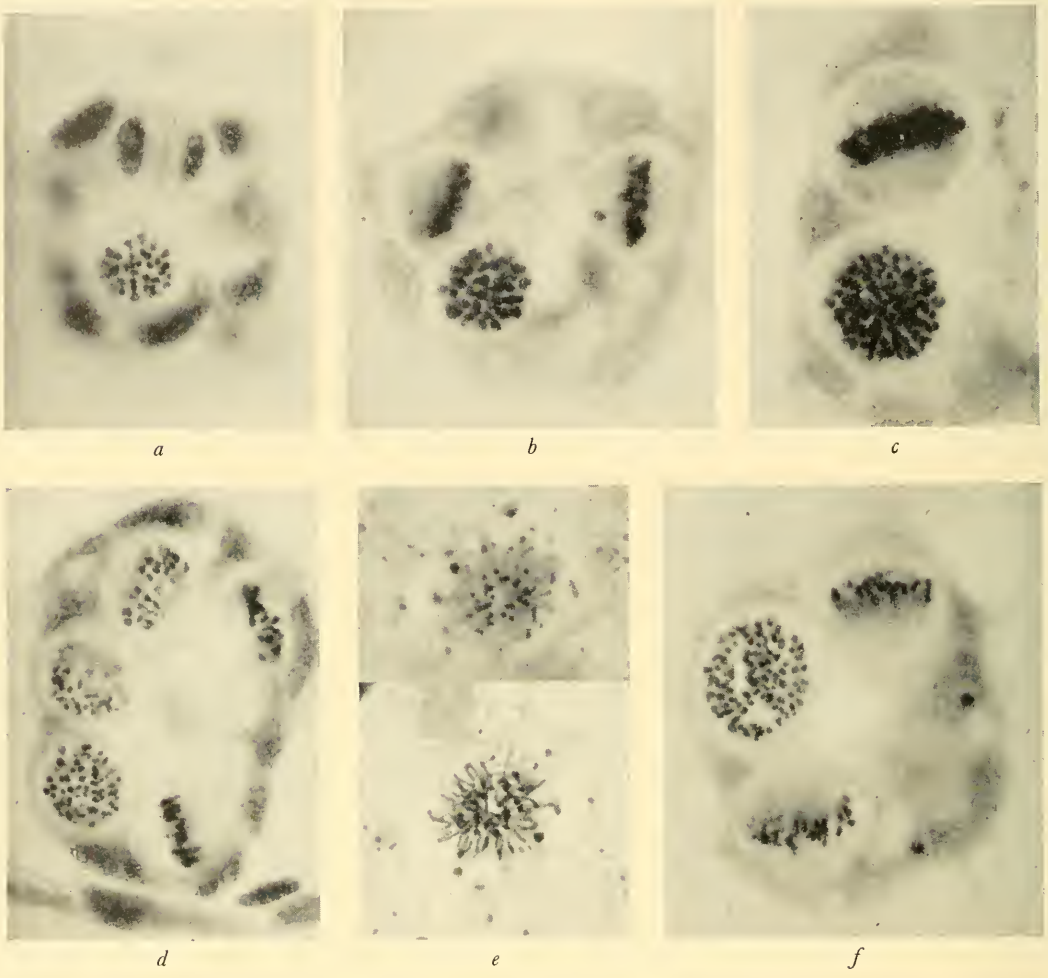


Fig. 71. Cytology of some species of *Dryopteris* in the wide sense seen in sections. × 1000. *a*. Meiosis in *D. Oreopteris* (Ehrh.) Max. $n=34$. For further details see Figs. 65 and 66. *b*. Meiosis in *D. aemula* (Ait.) O. Kuntze. $n=41$. For further details see Figs. 53*a* and 51. *c*. Meiosis in *D. dilatata* (Hoffm.) A. Gray. $n=82$. For further details see Fig. 53*f*. *d*. Meiosis in the Oak Fern (*Gymnocarpium*). $n=80$. For further details see Figs. 72 and 73. Note small size of the chromosomes. *e*. Mitosis in a root of the Beech Fern (*Phegopteris*). $2n=90$. For explanatory diagram see Fig. 70*b*. *f*. Meiosis in the Beech Fern (*Phegopteris*) with the same number of chromosome pairs as single chromosomes in the root. For explanation see text.

small size of the chromosome it has proved unusually difficult to determine their number with absolute accuracy. In both species n is of the order of 80, and in the Oak Fern it appears to be exactly this number (Figs. 72*c*, 73). It therefore seems possible that these two species when better known may prove not to be identical with other European

members of the old genus *Dryopteris*, and their separation, suggested first by Newman in 1851, into an independent genus *Gymnocarpium* is perhaps desirable. If this were done their names should be *G. Dryopteris* (L.) Newman and *G. Robertianum* (Hoffm.)

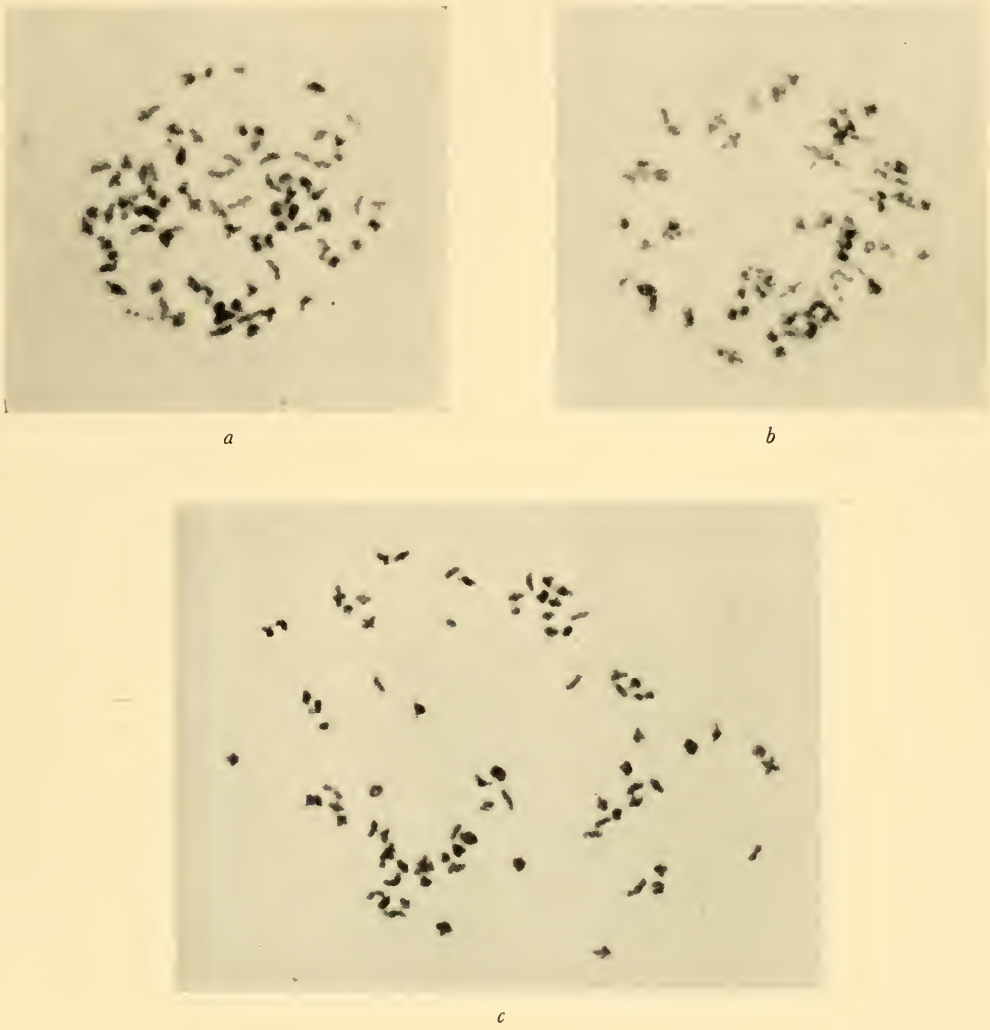


Fig. 72. Meiosis in *Gymnocarpium*, permanent acetocarmine. × 1000. *a*. The 'Oak Fern' in Britain. *b*. The 'Limestone Polypody' in Britain, not countable with certainty but not less than 80 or more than 84 pairs. *c*. The Oak Fern from Storlien, Jämtland, Sweden, showing 80 pairs of chromosomes with exceptional clarity. For explanatory diagram see Fig. 73.

Newman, and it may be said in passing that such a procedure has been advocated on morphological grounds by many recent writers, e.g. Ching, Christensen and Holttum.

Summing up the information for the British species of '*Dryopteris*' it may be stated that not one but probably at least four distinct genera are actually represented, some of which, notably *Dryopteris* in the narrow sense and *Thelypteris*, seem to have come from

widely different sources and to owe their present resemblance to parallel evolution. Within the curtailed genus *Dryopteris* polyploidy and hybridization are rife; the other three genera are still too few in analysed species for conclusions to be drawn. Apogamy has arisen repeatedly, both in the restricted genus *Dryopteris* and in the relatively unrelated Beech Fern, thereby adding a very clear example of the polyphyletic origin of a fairly complicated set of characters.

Fig. 73. Explanatory diagram to Fig. 72*c*. × 1500.

SUMMARY

Since the above statement recapitulates most of the points of general interest raised in the chapter a formal summary may perhaps be replaced by a simple list in which the cytological facts of this chapter and the last are assembled. The specific and generic subdivisions follow the conclusions given in the text of both chapters, but special attention should perhaps be drawn to the newly discovered diploid forms of *D. dilatata*, pp. 75–78, and *D. Villarsii* (*D. rigida*), p. 65, both of which deserve further study.

Cytology of the genus Dryopteris *sensu lato in Britain and West Europe*

Name	2*n*	*n*	Reproduction	Status
Dryopteris:				
D. Filix-mas (L.) Schott sens.strict.	164	82	Sexual	Allotetraploid species
D. abbreviata (Lam. & DC.) Newman	82	41	,,	Diploid species
D. Borreri Newman	82	82	Apogamous	Diploid form
	123	123	,,	Triploid form
	164	164	,,	Tetraploid hybrid
	205	205	,,	Pentaploid hybrid
D. aemula (Ait.) O. Kuntze	82	41	Sexual	Diploid species
D. Villarsii Woynar (=*D. rigida* (Hoffm.) Underw.):				
British	164	82	,,	Tetraploid } Status undecided
Swiss	82	41	,,	Diploid }
D. spinulosa (Müll.) Watt	164	82	,,	Tetraploid species
D. cristata (L.) A. Gray	164	82	,,	,, ,,
D. dilatata (Hoffm.) A. Gray	164	82	,,	Tetraploid }
'*D. dilatata*':				} Status undecided
Swiss	.	41	,,	}
Swedish	.	41	,,	Diploid }
Norwegian	82	.	,,	
D. uliginosa (Newm.) Druce	164	.	Sterile	Species hybrid
D. spinulosa × *D. dilatata*	164	.	,,	,, ,,
'*D. remota* Moore', Windermere	*c.* 164	.	,,	,, ,,
D. remota var. *subalpina* Borbas	*c.* 120 (=prob. 123)	*c.* 120	Apogamous	Triploid species-hybrid
'*D. remota Boydii*', Scotland	*c.* 120 (=prob. 123)	*c.* 120	,,	,, ,,
D. remota (A.Br.) Hayek, Ireland	*c.* 120 (=prob. 123)	*c.* 120	,,	,, ,,
Thelypteris:				
T. palustris Schott	70	35	Sexual	Diploid species
T. Oreopteris (Ehrh.) Sloss.	68	34	,,	,, ,,
Gymnocarpium:				
G. Dryopteris (L.) Newman	.	80	,,	Species
G. Robertianum (Hoffm.) Newman	.	*c.* 80 (=80–84)	,,	,,
Phegopteris:				
P. polypodioides Fée	90	90	Apogamous	,,

CHAPTER 6

THE OTHER BRITISH FERNS: *POLYSTICHUM, ATHYRIUM, ASPLENIUM, CETERACH*

The British fern flora, a quarter of which has now been passed in review, is less than a hundredth part of the ferns of the world, yet it is fortunately sufficiently varied to serve as a qualitative sample of ferns in general provided always that the whole of it is studied.* This can perhaps best be shown by a diagrammatic reproduction of Bower's phyletic views (Fig. 74), from which it will be seen that of the six major groups into which the leptosporangiate ferns are divided only one, the 'Davallioids', is wholly without a British representative. It should perhaps be pointed out that only the higher ferns are included in this scheme, the more primitive ones comprising the Eusporangiatae, Hymenophyllaceae and the Osmundaceae being more conveniently deferred to the end of the book. All the other British genera and every British species in each will, however, now be reviewed in this and the two following chapters, and if again the narrative at times resembles a catalogue apology can no longer be made for it is of the essence of a sample that it should be within its limits complete but composed of varied and, if necessary, unconnected elements.

It may, however, be helpful in maintaining a thread of continuity with the two preceding chapters if we begin our wider survey with the remaining British genera of the 'Dryopteroid' affinity. This, as a glance at the diagram will make clear, contains four other genera and two doubtful ones in addition to '*Dryopteris*', and to the first of these, namely *Polystichum*, we may now turn.

There are three British species of *Polystichum*, illustrated as fully as the size of the page permits in Figs. 75–77. The first of these, *P. Lonchitis* (L.) Roth, the Holly Fern, is rare in the British Isles except on some of the richer Scottish mountains. It is usually a plant of high altitudes save for a few localities, such as the limestone 'Pavement' areas of Yorkshire. Once seen in the fully mature condition it cannot be confused with anything else, though young plants of *P. aculeatum* are sometimes mistaken for it by those unfamiliar with the true Holly Fern. Though rarely abundant in Britain it is both widespread and often prolific in many other European countries, having a total range which

*The importance of this proviso will probably become more obvious at a later stage in the book, but it should perhaps be pointed out at once that completeness of a sample within the parameter chosen (in this case the geographical limits of the flora of Britain) is as necessary as any other consideration to its claim to be fairly representative. Any selection within the sample leading to deletion of certain elements for extraneous reasons such as horticultural or cytological convenience to the observer will necessarily falsify the picture by over-simplification.

The impossibility of obtaining truly random sampling of the world's vegetation in a botanic garden is one of the many reasons for believing that a complete study of a local small flora will be far more informative than the comparison of a greater number of miscellaneous species of unknown origin which might happen to be available from horticultural sources.

extends from the Himalayas on one side to Greenland and eastern North America on the other. Both the other British species are commoner than the Holly Fern in our flora. *P. angulare* Presl (*P. setiferum* (Forsk.) Woynar),* the Soft Prickly Shield Fern, is a woodland plant more frequent in Ireland and the south of Great Britain than in the north and lacking the preference for limestone shown by the other two species. When growing luxuriantly as it often does in parts of Devon it exceeds the Male Fern in size,

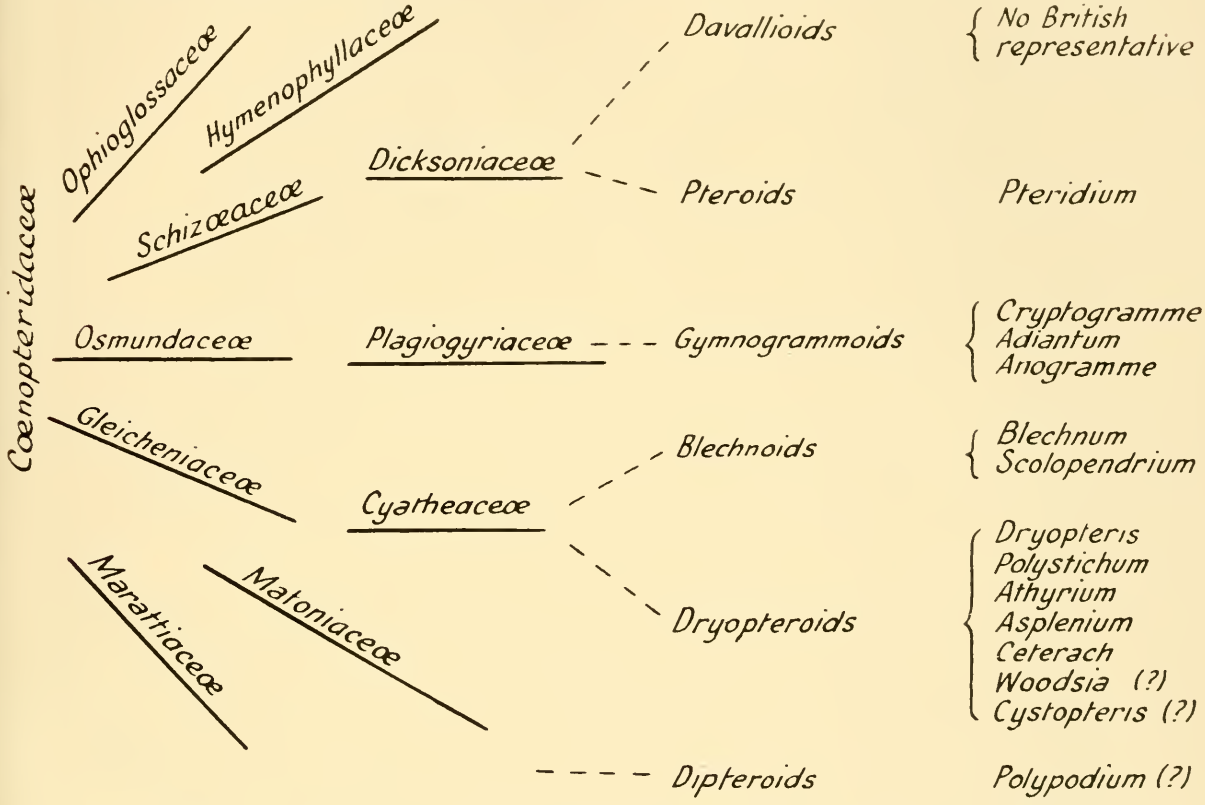


Fig. 74. Phylogenetic affinities of the principal genera of British ferns (right-hand column) redrawn after Bower (1929, 1935).

and as in that species the fronds die down in winter except in a very mild climate. *P. aculeatum* (L.) Roth, the Prickly Shield Fern, is of a much tougher texture and, like the Holly Fern, is normally evergreen in all climates in which it occurs. It is commoner in the north of Great Britain than the south, and is characteristically a plant of rocky ground though it does not usually occur as high on mountains as the Holly Fern. The geographical ranges of the last two species outside Great Britain and Europe are not clearly known owing to their confusion with each other and with non-European species.

*According to the International Rules the valid name for this species should now be *P. setiferum*. The retension of the older name of *P. angulare* for the purposes of this chapter and again in Chapter 9 is merely a temporary expedient for the sake of consistency with the principles of nomenclature explained in the Preface.

Fig. 75. Part of an adult fertile frond of the Holly Fern (*Polystichum Lonchitis* (L.) Roth) from Scotland. Natural size.

Fig. 76. Part of an adult fertile frond of *Polystichum angulare* Presl from Devon. Natural size.

Fig. 77. An adult fertile frond of *Polystichum aculeatum* (L.) Roth from Ingleborough, Yorks. Natural size.

That they are distinct and valid species is, however, now the prevailing opinion among systematists, although much discussion of this will be found in older Floras, owing probably to the fact that hybrids of intermediate morphology can sometimes be found.

Cytological observations have been made on British and on continental specimens of all three species. The Holly Fern, *P. Lonchitis*, has been examined from Scotland, Ireland and Switzerland with identical results; as shown in Figs. 78 and 79*c* the reduced chromosome number is $n = 41$. This number was found again in *P. angulare* from Devon, the Lake District and north Italy, and is very clearly demonstrated in Fig. 79*a*. On the other hand, *P. aculeatum* from the Lake District, Yorkshire and Switzerland has twice this number, as may be seen in Figs. 79*b*, 79*e* and 80.

Fig. 78. Explanatory diagram to Fig. 79*c*. × 2000.

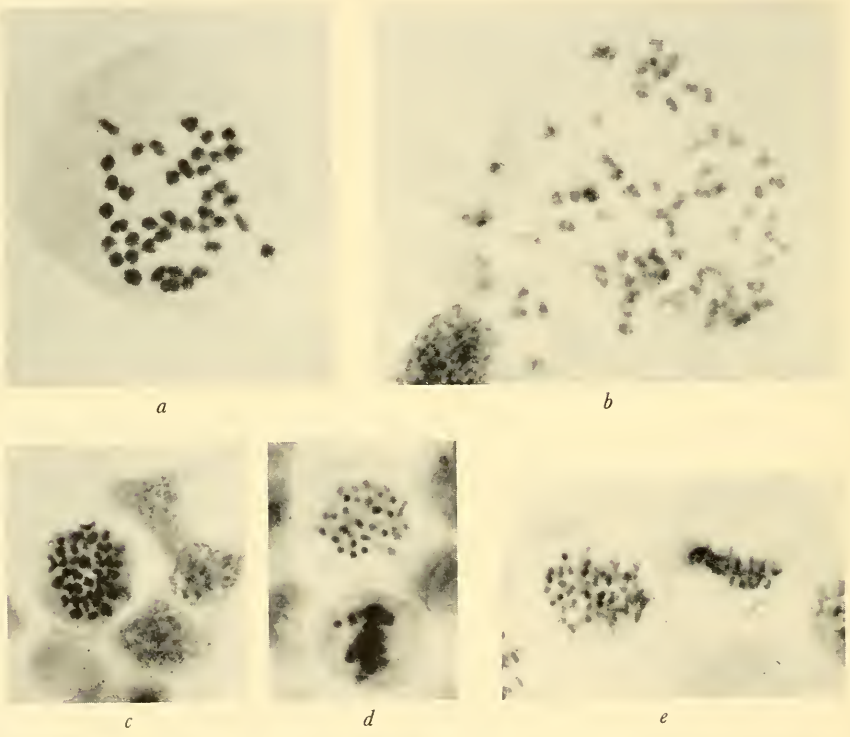


Fig. 79. Meiosis in British species of *Polystichum*. × 1000. *a*. Permanent acetocarmine squash of *P. angulare* Presl. $n=41$. *b*. Permanent acetocarmine squash of *P. aculeatum* (L.) Roth. $n=82$. For explanatory diagram see Fig. 80. *c*. Section of *P. Lonchitis* (L.) Roth. $n=41$. For explanatory diagram see Fig. 78. *d*. Section of *P. angulare*. $n=41$; cf. *a*. *e*. Section of *P. aculeatum*. $n=82$; cf. *b*.

Further information about this genus will be found in Chapter 9, and for the present the only conclusions which need be drawn from these facts are that the close affinity between *Polystichum* and the Male Fern section of *Dryopteris* is strongly confirmed by the

identity of chromosome numbers, as is the validity of the specific distinctness of *Polystichum aculeatum* from *P. angulare.*

The second genus thought to be closely related to the Male Fern by Bower is *Athyrium*, the Lady Fern. *A. Filix-femina* (L.) Roth, the Lady Fern itself, needs little if any introduction. As its name implies it resembles the Male Fern in a number of ways, notably in size and in general habit, but the more delicately cut up pinnules as well as the elongated indusium will at once distinguish it. It is hardy and abundant throughout the British Isles and has probably provided more of the monstrous and peculiar forms beloved of collectors than has any other British species. For this reason it is a commonplace of gardens, though often it must be admitted in a bizarre condition which bears little resemblance to the normal wild species, which is on the whole surprisingly con-

Fig. 80. Explanatory diagram to Fig. 79*b*. × 1500.

stant in appearance. A characteristic difference from the Male Fern is the very tender foliage which dies down sooner in autumn than is usual in any of our other large hardy ferns.

One of the more important taxonomic characters for the classification of the Lady Fern is the shape of the indusium which resembles so much that of the Spleenworts (*Asplenium*) that the Lady Fern was for long included in that genus. This has, however, often aroused protests in the handbooks for fern collectors, e.g. Newman. The justice of these views has at last been recognized by the separation of *Athyrium* as a genus distinct from *Asplenium* and with a closer connexion with *Dryopteris* (sens.lat.).

The cytology of the Lady Fern has been examined in specimens from Scotland, the Lake District and Yorkshire. At first the resemblance to some of the diploid species previously studied is so close that identity could readily be assumed. Such an assumption would, however, probably not be correct. After repeated study from which a decision has been difficult, my personal interpretation of *Athyrium Filix-femina* is that the haploid chromosome number (see Figs. 81 and 82) is not 41 but 40. This, if

confirmed, and confirmation from another source is very desirable, will not necessarily invalidate the suggestion of a derivation from *Dryopteris* sens.lat. (especially in view of the findings for *Gymnocarpium* in Chapter 5), but might merely indicate that the relationship to the Male Fern itself is less close than between that species and *Polystichum*, a conclusion which few systematists would deny. Of more importance than this is the strong confirmation which this chromosome number provides of a lack of close affinity with the next genus *Asplenium*. Before discussing this aspect, however, it will first be necessary to complete our study of the genus *Athyrium* by consideration of two other species which are generally, though not always, included in it.

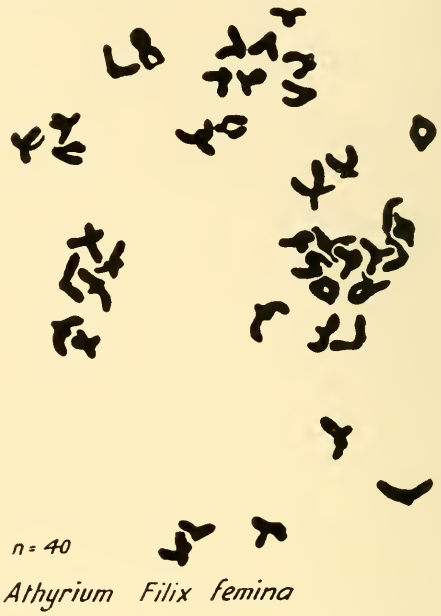


Fig. 81. Explanatory diagram to Fig. 82*a*. × 1500.

The other two species of *Athyrium* present in Britain are far less familiar than is the Lady Fern, since both are confined to the Scottish mountains and one is so very limited even in Scotland that few living botanists have ever collected it, and there is therefore still doubt as to whether it is really a species or only a local mutant. Assuming for the moment that they are both species, the two in question are *A. alpestre* (Hoppe) Rylands and *A. flexile* (Newman) Syme, both differing from the Lady Fern by the absence or early abortion of

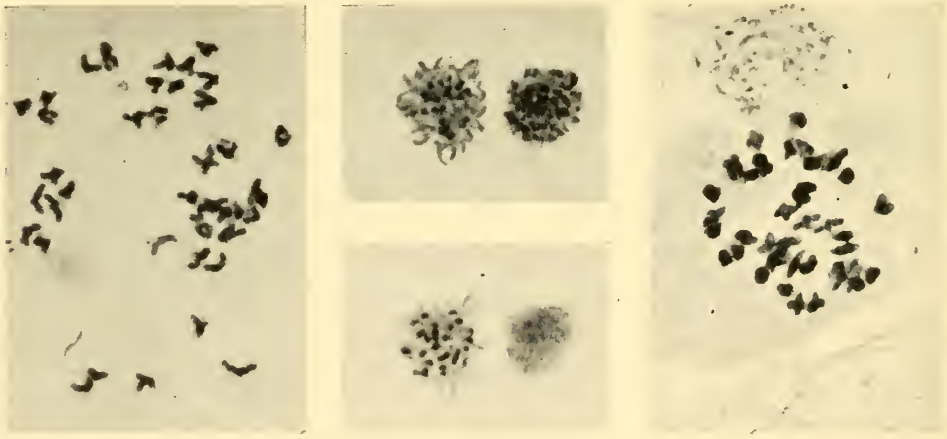


Fig. 82. The British species of *Athyrium*. × 1000. *a*. Meiosis in a permanent acetocarmine mount of *A. Filix-femina* (L.) Roth. $n=40$. For explanatory diagram see Fig. 81. *b*. Two focal levels of a root section showing a somatic chromosome count, in *A. alpestre* (Hoppe) Rylands. $2n=80$. For explanatory diagram see Fig. 84. *c*. Fresh acetocarmine preparation of *A. flexile* (Newman) Syme. $n=40$. For explanatory diagram see Fig. 88.

the indusium and therefore, as in the comparable case of *Gymnocarpium* and *Phegopteris* (Chapter 5), formerly classed as *Polypodium*.

Athyrium alpestre (Fig. 83) is superficially very similar to the Lady Fern proper when well developed, though it is generally somewhat smaller and in cultivation matures its fronds much earlier. In Scotland it occurs at an altitude shared by the Holly Fern, and like that species it is less restricted in range on the Continent than with us, being locally abundant, at suitable altitudes, in most of the mountain ranges of Europe and

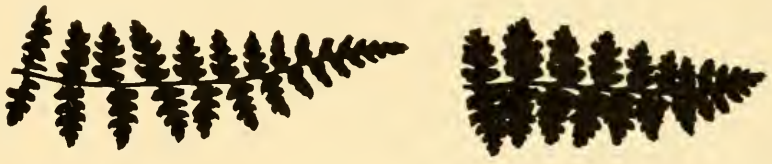


Fig. 83. Pinnae of *Athyrium alpestre* (Hoppe) Rylands, from two different plants to show range of morphology in this species on Ben Alder, locality of *A. flexile*. The left-hand plant is probably the variety *obtusatum* Syme. Natural size.

in parts of America. Cytologically it appears to resemble the Lady Fern, although my study of it is imperfect. I have had several Scottish plants in cultivation for many years, but they are fertile so infrequently that I have not yet seen meiosis. From a root-tip count represented by Figs. 82*b* and 84 the somatic number, however, appears to be $2n = 80$.

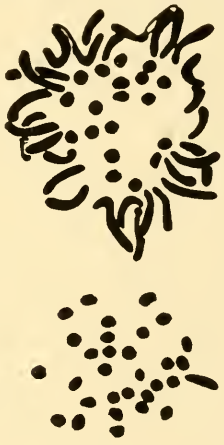


Fig. 84. Explanatory diagram to Fig. 82*b*. × 2000.

The third species of *Athyrium*, *A. flexile* (Newm.) Syme, is a very remarkable and interesting little plant which would well repay genetical investigation. Its claim to specific rather than to varietal status can only finally be settled after breeding experiments with its nearest relatives have been carried out, but on morphology alone it would probably long ago have been accepted as a species but for the circumstance that the only known locality in which it now occurs in any quantity is so remote and difficult of access that very few botanists have ever visited it. The locality in question is the great corrie on the north face of Ben Alder in west Inverness-shire, where it was said to be abundant by Professor J. H. Balfour in 1867 and where in 1946 it was still plentiful. The only other station in which it has ever been recorded in quantity is Glen Prosen, Forfarshire, where the type specimens (cf. Fig. 85) were first found by Backhouse in 1853. *A. flexile* may still exist in Glen Prosen, but it has not recently been seen there. The following notes have, however, been supplied from Ben Alder by my colleague, Dr Sledge, who in August 1946 camped at the foot of the mountain (which is 12 miles away from the nearest house) in order to search for the plant and who succeeded in bringing back a number of living specimens (cf. Fig. 86) in excellent condition which have since been maintained in cultivation.

'*Athyrium flexile* grows intermixed with *A. alpestre* amongst rocks in the north corrie of Ben Alder from 2750 to 3400 ft. Mature plants of *A. flexile* are readily distinguishable by their much smaller size—the fronds rarely exceed 6 in. in length—by the more distant and usually deflexed pinnae with narrow-based pinnules and the distinctly narrower

FLEXILE LADY FERN, (*natural size of a large plant*).

Fig. 85. Newman's original illustration of *Athyrium flexile* (1854).

and more tapering fronds. When the rhizome is not too deeply embedded amongst stones the very short stipes are bent over at their bases so that the fronds spread more or less horizontally, further accentuating the difference in appearance between this species and the invariably erect *A. alpestre*. These differences in habit and frond

Fig. 86. *Athyrium flexile* (Newm.) Syme. A pressed specimen collected on Ben Alder by Dr Sledge in August 1946. Natural size.

characters enable immature plants of *A. alpestre* of comparable size to be recognized without difficulty, and the sterility of such dwarf plants always affords confirmation of their distinctness. As described by Newman and others, the sori of *A. flexile* are usually restricted to the basal half of the frond. The sporangia in the sori are always few in number, not infrequently reduced to three or four or they may even be solitary. In cultivation the plant remains small and retains all its distinguishing characters.' This is perhaps demonstrated by Fig. 87.

Owing to the unusually small size of the sori and to the limited number even of these, it is a matter of some difficulty to obtain dividing mother cells in sufficient abundance for a satisfactory determination of chromosome number in *A. flexile*. Out of half a dozen plants kept in cultivation in Leeds, only one fertile frond was present in the first season after collection from the wild, and among three similar plants presented to Kew there

Fig. 87. Silhouette of a living frond of *Athyrium flexile* (Newm.) Syme after two years in cultivation. Natural size.

was likewise only one fertile frond. Fixings obtained from all available sporangia on both these fronds only showed some stages of the second meiotic division in sectioned material, but the second meiotic division is never suitable for determination of chromosome number in ferns, and these preparations therefore only serve to demonstrate that there are the normal sixteen mother cells present which are fairly small and the division not irregular. With squash preparations success was somewhat better, and about half a dozen individual cells at the first meiotic division were seen. One of these is shown in Figs. 82*c* and 88 and, although this would not in itself be quite conclusive in distinguishing $n = 40$ from $n = 41$, the most probable count is $n = 40$. It may therefore be stated with confidence that no detectable cytological differences appear to exist between the three British species of *Athyrium*, and further exploration of the differences between them would need to be carried out by genetical means.

We may now turn to *Asplenium*, and here we come to the largest group after *Dryopteris* as regards number of species represented in this country, though owing to their small size (Figs. 89 et seq.) and predilection for rock crevices they are less conspicuous. There are seven British species. The rarest is *Asplenium septentrionale* (L.) Hoffm., a plant of southern affinities only found in a few presumably relict localities in the mountains of England, Scotland and Wales. *A. marinum* L. is confined to maritime rocks. *A. viride* Huds. occurs both as an alpine and as a lowland plant somewhat resembling *Polystichum Lonchitis* in its preference for crevices in limestone rocks, though more widespread and abundant in individuals than that species. *Asplenium lanceolatum* Huds. is markedly Atlantic in its distribution, being found only in scattered localities from Cornwall to

Cumberland; it is absent from Scotland. The remaining species, *A. ruta-muraria* L., *A. Trichomanes* L. and *A. Adiantum-nigrum* L., are fairly generally distributed and often rich in individuals, the first two at least being familiar occupants of the cracks in masonry in all our moister districts.

Fig. 88. Explanatory diagram to Fig. 82*c*. × 1500.

Fig. 89. Two diploid British species of *Asplenium*. Natural size. *a*. *A. marinum* L. from a dried wild frond. *b*. *A. viride* Huds. from a living wild frond.

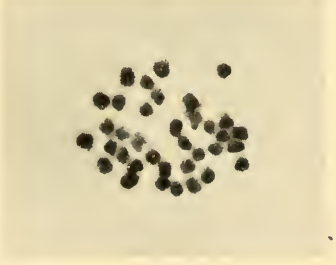


Fig. 90. Meiosis in *Asplenium viride* Huds., permanent acetocarmine. × 1000. $n = 36$.

Fig. 91. Explanatory diagram to Fig. 93*c*. × 2000.

The easiest species to investigate cytologically, though not perhaps the simplest to collect, are *A. viride* and *A. marinum* (Fig. 89). In their chromosomes these two resemble each other very closely, and both possess a haploid chromosome number of 36 with no uncertainty. The clearest demonstration of this number is contained in Fig. 90, which represents a squash preparation of *A. viride*, the root of which is represented by Figs. 93*a* and 94. A section of *A. marinum* is represented in Figs. 91 and 93*c*.

Exactly twice this chromosome number, namely, $n = 72$, has been found in all the other British species of *Asplenium*, namely, in *A. Trichomanes*, *A. ruta-muraria*, *A. Adiantum-nigrum*,* *A. lanceolatum* and *A. septentrionale*. Some particularly beautiful squashes showing the 72 pairs of chromosomes for *A. ruta-muraria* and *A. lanceolatum* are portrayed in Fig. 95*a* and *b*, while a section of *A. Trichomanes* mother cells and a root of *A. septentrionale* may be seen in Fig. 93*d* and *b*, each beside an appropriate diploid for comparison.

It is thus clear that the number 36 is as characteristic and deeply seated in the genus *Asplenium* as is the number 41 in *Polystichum* and *Dryopteris*, and if further demonstration were required it is perhaps appropriate to mention that these counts have been obtained not only on British but also, in a number of species, on continental specimens. In particular two non-British continental species, *A. fontanum* (L.) Bernh. from Switzerland and *A. Petrarchae* DC. from the south of France, have been collected alive as opportunity offered and grown on for cytological study. Since both are somewhat unfamiliar to British readers and one (*A. Petrarchae*) is also very rare in its native country, authenticating silhouettes of the actual living leaves from which fixations were taken are reproduced in Fig. 96*a* and *b*. The chromosome numbers found in the spore mother cells of these plants were $n = 36$ in *A. fontanum* and $n = 72$ in *A. Petrarchae*.

This is all that would now be known about the cytology of the genus *Asplenium* but for evidence supplied by a very well-known putative species-hybrid found sparingly both in Britain and on the Continent, to which the name of *A. germanicum* auct. non Weiss (or sometimes *A. alternifolium* Sm. or *A. Breynii* Retz) is commonly given. This plant

Fig. 92. A tetraploid British species of *Asplenium*, *A. Adiantum-nigrum* L. from Cornwall, from a living leaf of the plant used, grown in cultivation. Natural size.

* Since this was written *A. Adiantum-nigrum* var. *acutum* (Bory) Pollini forma *lineare* Praeger has been obtained from Madeira and found to be diploid. This may mean that '*A. Adiantum-nigrum*' will need to be split into two species both of which may actually be in the British flora (cf. Praeger 1934) although a British specimen of 'var. *acutum*' has not yet been examined cytologically.

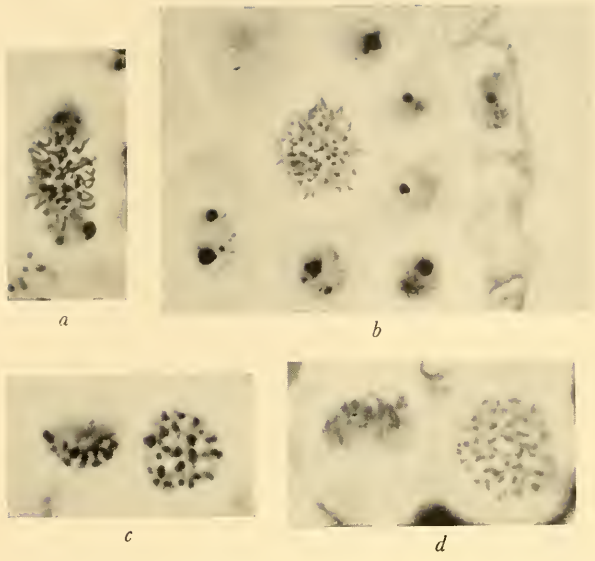


Fig. 93. Chromosomes of *Asplenium* in sections. × 1000. *a*. Mitosis in a root of *A. viride* Huds. For explanatory diagram see Fig. 94. *b*. The same in *A. septentrionale* (L.) Hoffm. to show higher chromosome number. For explanatory diagram see Fig. 94. *c*. Meiosis in *A. marinum* L. For explanatory diagram see Fig. 91. *d*. The same in *A. Trichomanes* L. to show higher chromosome number. For further details see Fig. 101.

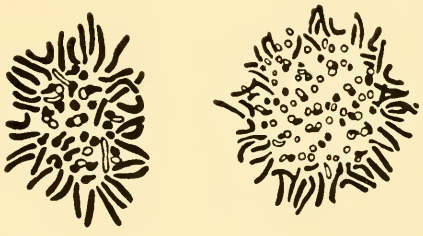


Fig. 94. Explanatory diagrams to Fig. 93 *a*, *b*. × 2000.

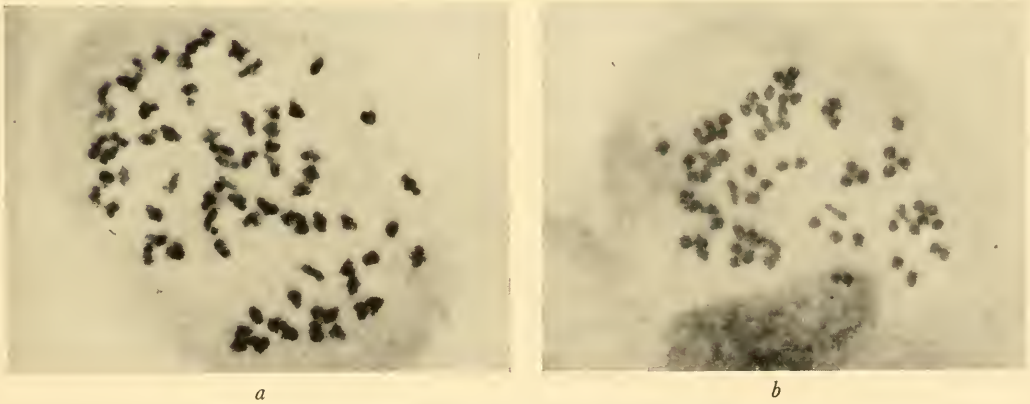


Fig. 95. Meiosis in tetraploid species of *Asplenium*, permanent acetocarmine. × 1000. $n = 72$. *a*. *A. ruta-muraria* L. *b*. *A. lanceolatum* Huds.

(Fig. 97*a*, *b*) has a very characteristic appearance which does not resemble closely any other species, least of all those with which it is habitually found. Its hybrid nature has, however, long been suspected both from its sporadic and solitary occurrence, single

Fig. 96. Two non-British species of *Asplenium*, from living leaves, grown in cultivation. Natural size. *a*. *A. Petrarchae* DC. from southern France. *b*. *A. fontanum* (L.) Bernh. from Switzerland.

Fig. 97. *Asplenium germanicum* auct. non Weiss and its supposed parents, from living fronds grown in cultivation. Natural size. *a*. *A. germanicum* from Wales. Cf. Fig. 98. *b*. The same from Runmarö, Sweden. *c*. *A. Trichomanes* L. from southern France, a very depauperate specimen, but shown to be tetraploid. Cf. Fig. 103*a*. *d*. *A. septentrionale* (L.) Hoffm. from Arthur's Seat, Scotland.

plants only but never populations of similar plants being found, and also from an apparently invariable association with *A. septentrionale* (Fig. 97*d*) and *A. Trichomanes* (Fig. 97*c*). From this circumstance, added to the fact that the spores are always completely or almost completely abortive, it is generally thought to be a hybrid between

these two species. Attempts to synthesize it have, however, been curiously unsatisfactory. Only one author is known to me to have succeeded in carrying out a cross between *A. septentrionale* and another species of *Asplenium*, namely Heilbronn (1910), but even he was forced to point out a dissimilarity between the hybrid he produced and the natural one.* It is true that a surprisingly contradictory statement was made by a distinguished amateur, P. Kestner, who remarks in the *British Fern Gazette* of 1935 that *A. germanicum* is the only fern hybrid that can be synthesized with ease. This statement,

Fig. 98. The Welsh plant of *Asplenium germanicum* auct. non Weiss in its original habitat. Natural size. From a photograph kindly supplied by the finder, Dr H. F. Dovaston.

though perhaps true as will be seen below, cannot unfortunately be used as evidence, since Kestner failed to authenticate it by keeping a record, photographic or otherwise, of the plants he refers to.

I am fortunate in having had access to living specimens of *A. germanicum* from several different countries. A British specimen from south Wales, a leaf of which is shown in Fig. 97*a*, was made available to me by the finder, Dr Dovaston, whose photograph of it in its original habitat is also reproduced (Fig. 98). Two continental specimens were

* Heilbronn claims to have crossed *A. septentrionale* with *A. ruta-muraria*. This should have yielded *A. Murbeckii* Dörfl. and it is therefore not quite clear why the author was looking for *A. germanicum*, although his photograph does show some points of resemblance to *A. germanicum*.

found by me on separate occasions in the summer of 1937 in the North Italian Alps and brought alive to England, where they unfortunately perished during the war, but not before successful fixations had been made. To replace them additional material

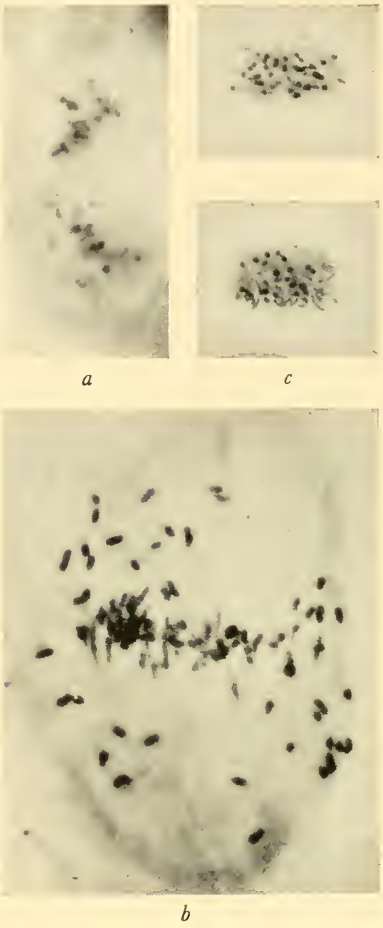


Fig. 99.

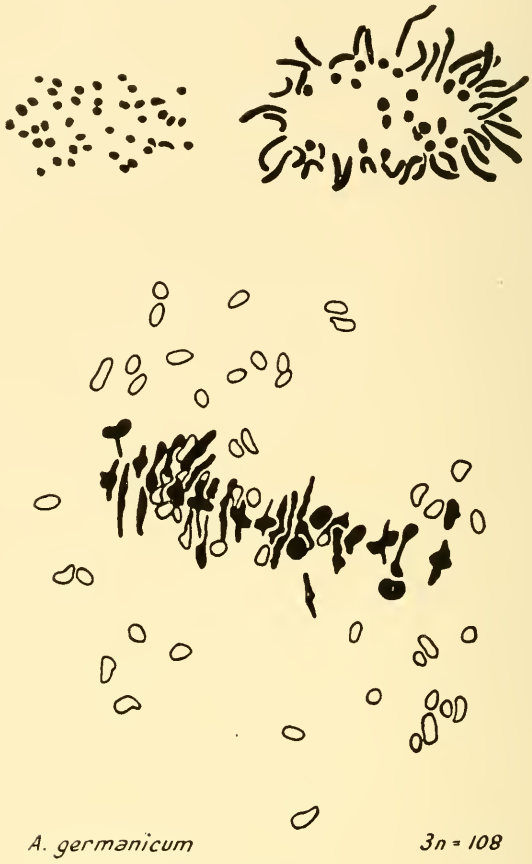


Fig. 100.

Fig. 99. The cytology of *Asplenium germanicum* auct.non Weiss. × 1000. For explanatory diagrams see Fig. 100. *a*. Meiosis in a section of an Italian specimen showing lagging univalents. *b*. Meiosis in the Welsh specimen in balsam after acetocarmine showing pairs and univalents. *c*. Two focal levels through a mitotic figure in a root of the Welsh plant showing the triploid chromosome number.

Fig. 100. Explanatory diagrams to Fig. 99*b*, *c*. × 2000.

was obtained in the summer of 1948 consisting of one plant from the island of Runmarö near Stockholm, Sweden, kindly procured for me by Professor Halle and posted to the Royal Botanic Gardens, Kew; and a Swiss plant found near Sion by my colleague, Miss Davies, and brought back by her to Leeds. A leaf of the Swedish specimen is reproduced in Fig. 97*b*.

All these plants agree very closely in their cytology. They show unmistakable signs of hybridity in the irregular appearance of the first meiotic division, which may perhaps be sufficiently demonstrated by Fig. 99*a* and *b*. The first of these shows a section of one of the Italian specimens with numerous unpaired chromosomes; the second (Fig. 99*b*) is a single mother cell in an acetocarmine preparation of the Welsh specimen in which fuller details of pairs and univalents can be made out.

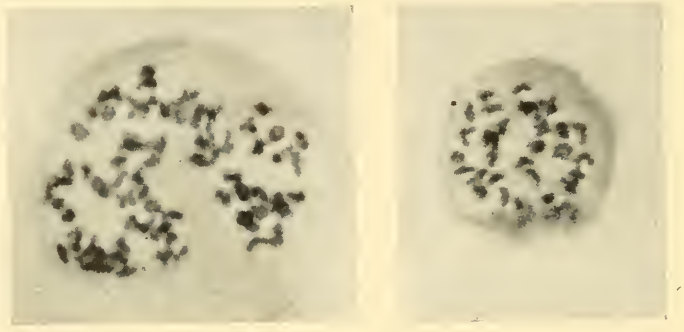
In all cases, however, the chromosome number was anomalous. The first detailed count had been made on a root of one of the Italian plants in which about 100, and not the expected 144, chromosomes had been found. This seemed at the time so inexplicable that the record was at first discarded as a possible case of mis-identity since the plant had died during the war without a herbarium record of it having been preserved. The same result, however, was obtained with the British plant, as may be seen from the photographs and diagrams of Figs. 99 and 100. When, as a result of this discovery, the two additional continental specimens (from Sweden and Switzerland) had been examined and an exactly similar result obtained in each, the facts could no longer be doubted; *A. germanicum* in four European countries and therefore probably always, is not a tetraploid as its putative parentage would suggest but a triploid ($3n = 108$).

The discovery of a triploid hybrid where a tetraploid was expected has already occurred in the case of *Dryopteris remota* (see Chapter 5), but since *Asplenium germanicum* differs from *Dryopteris remota* in not being apogamous, the possible explanations of its origin are less numerous. Since the plants are sterile they cannot have been disseminated by stray spores from a common source, and it must be assumed that each has arisen *de novo* wherever it is found. Since they are all very similar morphologically a complex origin by a process of segregation from a previous hybrid of different chromosome number seems impossible. It remains, therefore, to be seen whether a diploid form of one or other of the parent species can be found from which a triploid could be produced directly as a simple hybrid.

In searching for this it was felt at once that *Asplenium Trichomanes* was the more probable species to be involved, since *A. septentrionale* had already been examined cytologically several times from *A. germanicum* localities; moreover, it is so uncommon in Great Britain that the probability of finding more than one form of it in this country at any rate seemed remote. With *A. Trichomanes*, on the other hand, the position is different. It is so abundant and familiar that it had not previously been felt necessary to take special precautions to collect it from the immediate vicinity of the hybrid, and reliance had been placed on the wealth of wild specimens nearer home. This meant that while chromosome counts, invariably tetraploid, had been made on specimens of *A. Trichomanes* from Devon, Derbyshire, Yorkshire and the south of France, none of these plants had actually been associated with either *A. septentrionale* or *A. germanicum*, and it was conceivable that had such an association been insisted upon a different result might have been obtained. Attention was therefore directed towards sampling *A. Trichomanes* populations from these precise habitats.

At once what was looked for was found. A form of *A. Trichomanes* obtained from Snowdon (Wales), and transferred to Kew in 1946 was found to be diploid. The two chromosome numbers are demonstrated in Figs. 101–102, and a leaf of each type

is shown in Fig. 103. The most obvious morphological difference is the crenate edge of the diploid, but it is still too soon to say exactly what diagnostic criteria will best serve to distinguish it in the field. It is also at present uncertain whether the tetraploid in this case is an autopolyploid or an allopolyploid. The most that can be said is that quadrivalents are not obvious and seem to be absent; the very close morphological similarity of the two forms raises the question, however, which must also be answered for the comparable cases of *Dryopteris dilatata* and *D. Villarsii*, as to whether



a *b*

Fig. 101. The two forms of *Asplenium Trichomanes* L. in Britain, permanent acetocarmine. × 1000. For explanatory diagrams see Fig. 102. *a*. Tetraploid plant from Ashburton (Devon) with leaves as in Fig. 103*a*. *b*. Diploid plant from Snowdon (Wales). Cf. Fig. 103*b*.

Fig. 102. Explanatory diagrams to Fig. 101. × 2000.

an autopolyploid can perhaps lose its power of multivalent formation after a sufficient lapse of time. Only further experiment can elucidate this matter, but in the meantime the results so far make it extremely probable that *Asplenium germanicum* is indeed a species-hybrid as originally postulated but that the diploid form of *A. Trichomanes* and not the tetraploid must be used. With this information it should only be a matter of time before this hitherto perplexing hybrid has been synthesized.

So close to *Asplenium* that it must certainly be discussed with it is *Ceterach*, the 'Rusty Back' (Fig. 104). This small genus of only 3–5 species in all has just one representative in Europe, *C. officinarum* Lam. & DC. This is locally abundant in many parts of Great Britain, especially in the west, and it shows a marked preference for somewhat calcareous

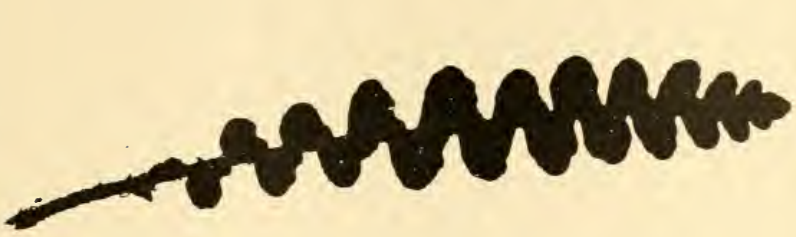


Fig. 104. *Ceterach officinarum* Lam. & DC. a fresh frond of a British specimen. Natural size.

Fig. 105. Meiosis in *Ceterach officinarum* Lam. & DC., permanent acetocarmine. × 1000.

Fig. 106. Explanatory diagram to Fig. 105. × 1500.

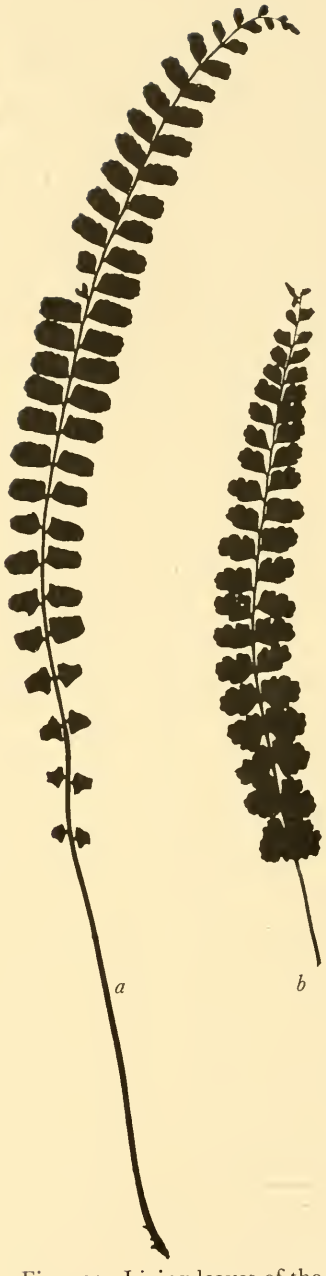


Fig. 103. Living leaves of the two forms of *Asplenium Trichomanes*.

a. Tetraploid, cf. Fig. 101 *a*.

b. Diploid, cf. Fig. 101 *b*. Kew reference no. 157.46.

habitats. This preference is even more marked on the Continent, where *Ceterach* shows its remarkable power of withstanding seasonal drying up by being one of the most characteristic ferns in the cracks of glaring white limestone on the north Mediterranean coast. Taxonomically it differs from *Asplenium* chiefly by the profusion of papery scales on the backs of the fronds; apart from this and the somewhat fugaceous indusium, the soral characters of the two genera are very similar.

That *Ceterach* is closely related to *Asplenium* is further indicated by the cytology. In both English and French specimens I have found $2n = 144$ and $n = 72$,* as in the majority of *Aspleniums*. One photograph (Fig. 105) and a diagram (Fig. 106) are appended in illustration.

The cytological confirmation of the suspected close affinity between *Ceterach* and *Asplenium* brings into strong relief the contrasting case of *Athyrium*, and we may therefore fittingly close this chapter by drawing attention to a not unimportant conclusion which both *Athyrium* and *Dryopteris* have brought out. This is the unreliability of soral characters when taken alone as a guide to phylogeny. In the previous chapter we were forced to accept the conclusion that the apparent resemblance of *Dryopteris* in the narrow sense to *Thelypteris* was due to parallel evolution from different ancestral stocks, and we are now confronted with the same situation in *Asplenium* and *Athyrium*. The sum of anatomical characters, and chromosome number, are both more reliable than are the details of the sorus when taken alone, as an index of affinity. This fact is perhaps of some importance for taxonomists to know.

SUMMARY

As in the previous chapter, the most suitable factual summary is perhaps merely a list of the chromosome numbers recorded in it, and this is appended. As points of special interest attention may perhaps be directed to the new observations on the very rare and little known *Athyrium flexile* (pp. 95–98), to the unexpected facts regarding the hybrid *Asplenium germanicum* (pp. 100–106), and to the existence of a diploid as well as a tetraploid form of *Asplenium Trichomanes*. The diploid form of the last species has only recently been discovered and requires further study. It is present in Britain and probably on the Continent, but in Britain at least is less common than the tetraploid.

* This number has since been found also in *Ceterach aureum* (Cav.) v. Buch from Teneriffe.

List of chromosome numbers recorded in this chapter

Name	Source	Roots	Meiosis	Remarks
Polystichum:				
P. Lonchitis (L.) Roth	Ireland	82	41	Diploid species
	Scotland	.	41	,, ,,
	Switzerland	.	41	,, ,,
P. setiferum (Forsk.) Woynar	England	.	41	,, ,,
(*P. angulare* Presl)	Switzerland	82	41	,, ,,
P. aculeatum (L.) Roth (*P. lobatum* (Huds.) Woynar)	England	164	82	Tetraploid species
	Switzerland	164	82	,, ,,
Athyrium:				
A. Filix-femina (L.) Roth	England	.	40	Diploid species
	Scotland	.	40	,, ,,
A. alpestre (Hoppe) Rylands	,,	80	.	,, ,,
A. flexile (Newman) Syme	,,	80	40	,, ,,
Asplenium:				
A. fontanum (L.) Bernh.	Switzerland	72	36	,, ,,
A. viride Huds.	England	72	36	,, ,,
A. marinum L.	England (Devon)	.	36	,, ,,
	Ireland	.	36	,, ,,
A. Adiantum-nigrum var. *acutum* (Bory) Pollini	Madeira	72	.	,, ,, (?)
A. ruta-muraria L.	Devon	.	72	Tetraploid species
A. lanceolatum Huds.	,,	.	72	,, ,,
A. Adiantum-nigrum L.	,,	.	72	,, ,,
A. septentrionale (L.) Hoffm.	Scotland (Arthur's Seat)	.	72	,, ,,
	Switzerland	144	.	,, ,,
A. Petrarchae DC.	Southern France	.	72	,, ,,
A. Trichomanes L.	Devon, Yorkshire, Southern France	144	72	,, ,, Perhaps auto
	Wales	72	36	Diploid species (?)
A. germanicum auct. non Weiss	Sweden, Italy, Wales	*c.* 100 (probably 108)	Irregular	Triploid hybrid
Ceterach:				
C. officinarum Lam. & DC.	Devon	.	72	Tetraploid species
	Southern France	.	72	
C. aureum (Cav.) v. Buch	Teneriffe	.	72	,, ,,

CHAPTER 7

THE OTHER BRITISH FERNS (CONTINUED)

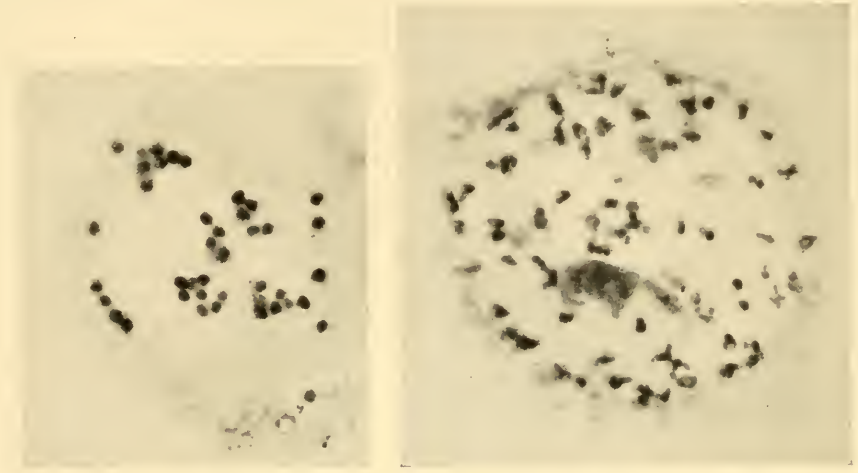
Two genera which should perhaps be considered with the Dryopteroids are *Woodsia* and *Cystopteris*, but both are somewhat more doubtful in position than those dealt with in the previous chapter, and for this reason discussion of them was deferred. As will be seen by reference back to the diagram on p. 89, the phyletic affinities of both are marked as questionable by Bower. With regard to *Woodsia* the general consensus of opinion seems to be that it is probably a primitive genus related to *Dryopteris*, either directly by way of certain tree ferns known as the Cyatheaceae (Bower's view) or indirectly (view of Christensen, Copeland, etc.). *Cystopteris*, according to Bower, is frankly 'incertae sedis', but other authors (e.g. Newman, 1854) place it with confidence near *Woodsia*.

Fig. 107. British *Woodsia* from living leaves grown in cultivation. Natural size. *a*. *W. ilvensis* (L.) R.Br. from Wales. *b*. *W. alpina* (Bolton) S. F. Gray from Scotland.

Taking *Woodsia* first, this is represented in Europe by three species, only two of which are British. *W. ilvensis* (L.) R.Br. and *W. alpina* (Bolton) S. F. Gray (*W. hyperborea* R.Br.) are among our rarest ferns, being confined to a few localities in the mountains of Scotland and Wales, in most of which their numbers, unhappily, appear to be diminishing. *W. ilvensis*, originally the commoner and somewhat the larger of the two, is now quite extinct in many of its classic haunts, though fortunately it still lingers on a few unfrequented and inaccessible crags. For precise instructions as to how to reach one of these I am greatly indebted to my friend, Dr H. F. Dovaston. Thanks to this, and with the help of Professor D. Thoday of Bangor, I was able in July 1945 to collect a living fertile frond from an authentic wild Welsh plant and to bring one small offset, from a group proliferating from the base of a dead stock, into cultivation. Both the frond and the offset yielded cytological information. For the other species I am indebted to Mr G. Roger of Manchester Museum and to Dr B. T. Cromwell of Hull, each of whom has lent me one living plant collected wild in Scotland. Silhouettes of fronds of the plants examined are given in Fig. 107*a* and *b*.

The chromosomes of the two species are shown in Fig. 108*a* and *b* (explanatory diagrams in Fig. 109*a* and *b*). It is obvious at a glance that they are not identical and that one species (*W. alpina*, Fig. 108*b*) has about twice as many chromosomes as the other (*W. ilvensis*, Fig. 108*a*). As nearly as can be determined the exact numbers are $n = 41$

for *W. ilvensis* and $n = 82$ for *W. alpina*, and the maximum possible error is in both species no more than one chromosome in the monoploid count. This uncertainty arises from the presence of what appears to be a foreign body accidentally superimposed on the cell of Fig. 108*a* and indicated as such in the diagram. It gives, however, a super-



a *b*

Fig. 108. Meiosis in British *Woodsia*, from permanent acetocarmine preparations. × 1000. For explanatory diagrams see Fig. 109. *a*. *W. ilvensis* (L.) R.Br. $n=41$. *b*. *W. alpina* (Bolton) S. F. Gray. $n=82$.

a *b*

Fig. 109. Explanatory diagrams to Fig. 108. × 1500.

ficial resemblance to an extra chromosome. As long as slight uncertainty affects the monoploid count it would be unwise to depend on the higher number of the other species (Fig. 108*b*) for greater precision. These numbers should therefore for the moment be accepted as probable, though not quite certain. The fact that in *Woodsia* we must for the moment be content with less than the usual precision is due to the very delicate

nature and small size of these plants, coupled with their extreme rarity in Britain, which makes a large-scale multiplication of preparations unusually difficult. Other cells of both species have, of course, been seen, but none of better quality.

Turning now to *Cystopteris* we come to what is in some ways the most remarkable cytological problem yet raised in the ferns. *C. fragilis* (L.) Bernh. is a small rock plant of world-wide distribution, being met with even in the southern hemisphere. It is a variable plant, and it has often puzzled systematists to decide whether it should or should not be subdivided into several species. A closely cognate problem is to decide whether *C. alpina* Desv. (*C. regia* Desv.) from the central European mountains should be separated from it as a species, and, if so, whether this species is present in the British Isles. Comparable difficulties concern the status of other extreme foliar types, *C. Dickieana* Sim, once found near Aberdeen, being one of the most frequently debated. The difficulty

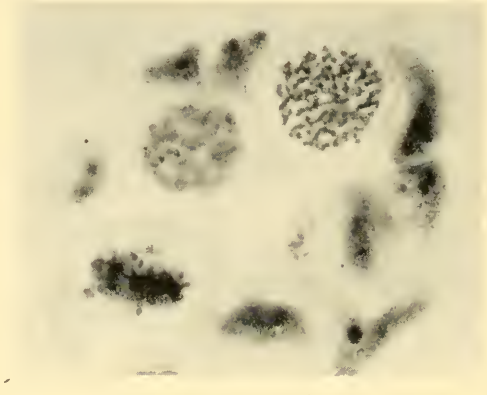


Fig. 110. Meiosis in *Cystopteris fragilis* (L.) Bernh. in section. × 1000.
The chromosomes uncountable, but see Figs. 112–115.

with all these plants is their extreme plasticity under different environmental conditions, so that without experimental cultures it may be quite impossible to obtain sufficient evidence from herbarium material alone, and even with experimental cultures it may require far more difficult types of observation than mere comparison of foliage.

In contrast to the host of problems raised by or round *C. fragilis* (L.) Bernh., the other British species, *C. montana* (Lam.) Desv., is simplicity itself. With its creeping rhizome and deltoid leaves it is as distinct from the *fragilis* complex as is the Oak Fern from the *Lastrea* type of *Dryopteris*. It is, however, excessively rare in the British Isles, being confined to central Scotland. Although I have had a specimen from this region in cultivation, I have not so far been able to obtain a cytological result from it. In the account which follows I have had to be content with a specimen of garden origin, previously collected in Switzerland.

Before presenting what can only be regarded as a preliminary report on the cytology of *Cystopteris*, a special note on technique is perhaps desirable. In my experience this genus is more than usually difficult to study effectively by any of the older cytological methods. In sections of roots the hundreds of thin and contorted chromosomes are virtually uncountable, and the same is true of meiosis, as may perhaps be seen from Fig.

Fig. 111. Forms of *Cystopteris fragilis* and *C. alpina* with spiny spores and the high chromosome number ($n = 126$), from living leaves grown in cultivation. Natural size. *a*. '*C. regia* forma *obtusa*' supplied by Dr Kestner from Switzerland. *b*. '*C. regia* forma *acuta*' collected by Waltham, Switzerland. *c*. '*C. fragilis*' from Cader Idris, Wales. *d*. '*C. fragilis*' from Teesdale.

110, in which the chromosomes at metaphase are present in layers. Only by the squash method, details of which are explained in Appendix 1, can success be achieved, and although this has been used with effect in the work described in several previous chapters the precise details of its application to the higher ferns were in the first instance devised to meet a complete technical deadlock which had descended on the genus with which we are now concerned. The introduction of the squash method resolved the technical impasse at once, but thereby revealed a cyto-

Fig. 112. Meiosis in '*Cystopteris alpina*' from the leaf of Fig. 111*b*. Fresh acetocarmine. × 1000. $n = 126$. For explanatory diagram see Fig. 113.

genetic problem of quite unusual complexity, a circumstance which was nevertheless to be expected in view of the well-known taxonomic confusion prevailing in the genus.

The first squash preparation to give a satisfactory result was that of Figs. 112 and 113. The leaf from which it was obtained is shown in Fig. 111*b*, and its very finely cut pinnation characteristic of '*C. alpina*' is clearly seen. This specimen, which was kindly given to me by Dr Rowlands of Doncaster, was described by him as 'the most authentic form of *C. alpina* (*C. regia*) to be found in Switzerland', since it retains fully its distinctive characters in cultivation.* This is by no means always the case with *alpina*-like forms

*The origin of this particular material is discussed in several letters included in volume VI of the *British Fern Gazette* (1931). The finely cut specimen of *C. regia* collected by Waltham is said to have come from 'limestone above Geneva at about 4600 ft.' in 1926 (loc. cit. 1931, p. 37). My plant agrees exactly with that figured on pp. 72 and 85 of this volume of the *Fern Gazette*.

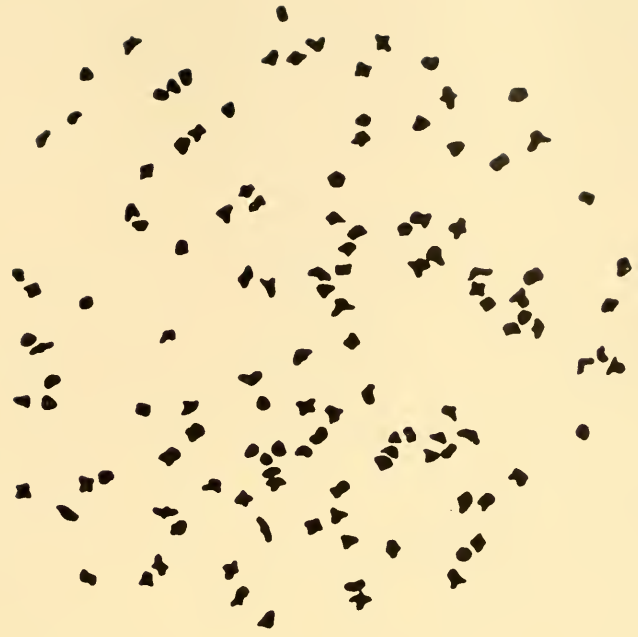


Fig. 113. Explanatory diagram to Fig. 112. × 1000.

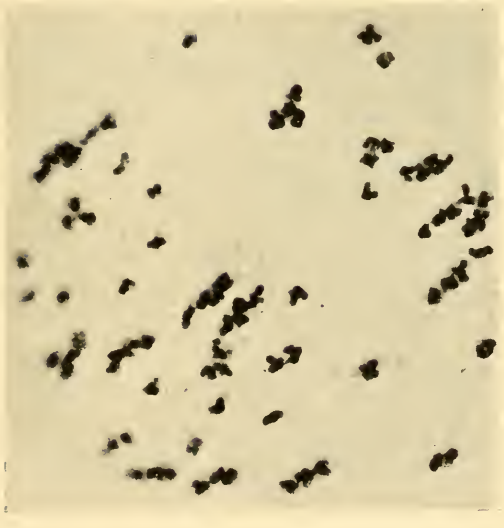


Fig. 114. Meiosis in *Cystopteris Dickieana* Sim, fresh acetocarmine. × 1000. From the leaf of Fig. 117*a*. For explanatory diagram see Fig. 115.

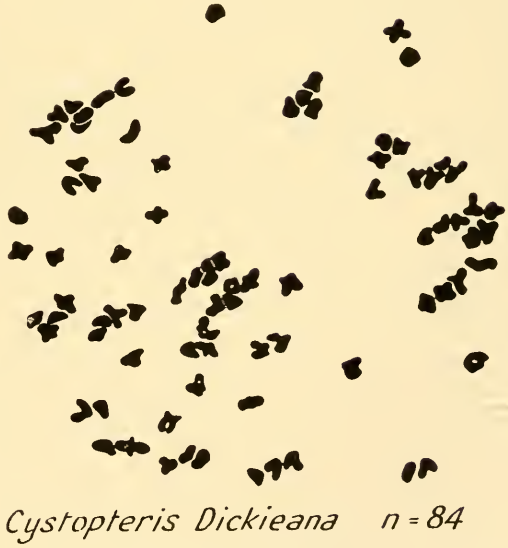
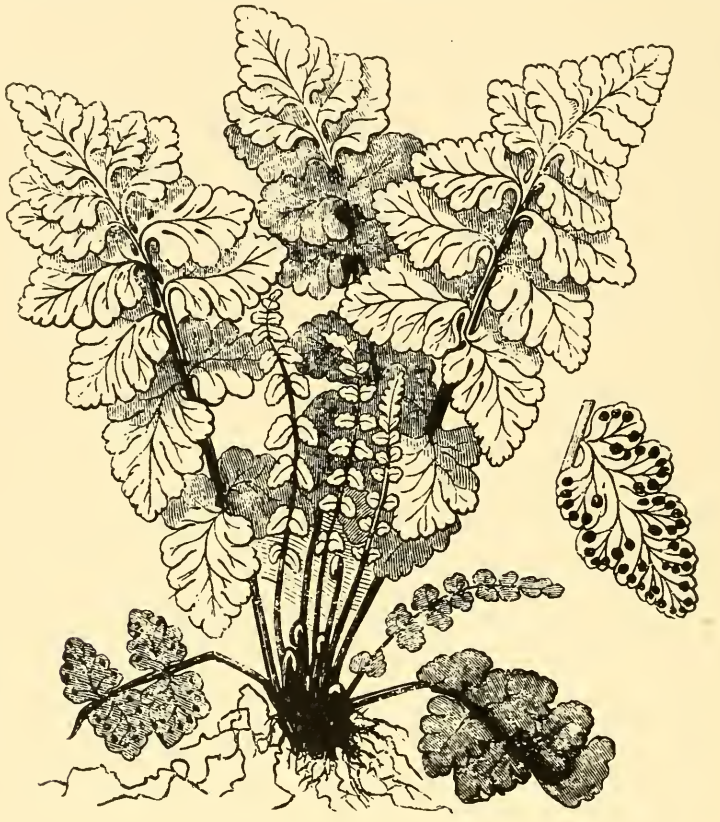


Fig. 115. Explanatory diagram to Fig. 114. × 1000.

from high altitudes which often tend to revert to the more usual form of *C. fragilis* when grown at sea-level. The chromosome number of this specimen is not in doubt. As comparison between the photograph and the explanatory diagram should prove there are exactly 126 pairs of chromosomes in this cell.

A gametic chromosome number of 126 was subsequently found many times in plants of very diverse origin and appearance. A small array of relevant leaves is contained in



DICKIE'S FERN, (*natural size*).

Fig. 116. Newman's original figure of *Cystopteris Dickieana* Sim (after Newman, 1854). Natural size.

Fig. 111, plants of both Swiss and British origin being represented, and two further samples of cytological preparations together with one additional explanatory diagram are placed later in the chapter in Figs. 120*a* and *b* and 121*b*. In spite of the considerable range of morphology there appears to be no detectable cytological difference between '*C. fragilis*' types and '*C. alpina*' or *alpina*-like types. The reality or otherwise of *C. alpina* as a justifiable species can therefore only be further investigated by genetical means.

An extended search through European populations of *Cystopteris* has, however, shown that some genuine cytological differences do exist within the *C. fragilis* complex, some of

which may perhaps be of taxonomic importance. The first preparation to give a chromosome number different from 126 was that of Figs. 114 and 115 which refer to *C. Dickieana* Sim, a leaf of which is shown in Fig. 117*a*. There are 84 chromosomes only.

C. Dickieana is a well-known horticultural 'variety' which was originally found wild on the coast near Aberdeen and subsequently in a very few other parts of Scotland

Fig. 117. Forms of *Cystopteris* with verrucose spores from living leaves grown in cultivation. Natural size. *a*. *C. Dickieana* Sim. *b*. Specimens from Greenland (see text). *c*. '*C. Baenitzii*' from the type locality in Norway.

(Druce, 1919) though probably now exterminated there except in cultivation.* It was described by Newman in 1854 as a probable species, and it has retained all its distinguishing marks with great constancy in cultivation, as comparison of Newman's figure (Fig. 116) with my specimen (Fig. 117*a*) will perhaps indicate. Newman's view that this might be a new species was not wholly based on the leaf morphology, in

*Notes on the origin of *C. Dickieana* will be found in vol. VI of the *British Fern Gazette*, notably pp. 18 and 19 (Rowlands, 1929). The original description will be found in Newman (1854).

which the congested pinnae are the most conspicuous feature, but was also based on the observation that the spores have a highly characteristic surface pattern when seen under the low power of the microscope. This pattern has been described as 'verrucose' (Fig. 118*c*) to distinguish it from the very spiny surface met with in other forms of *C. fragilis* (Fig. 118*a*, *b*) and *C. alpina*.

The first reaction to finding a distinctive spore pattern as well as a new chromosome number in *C. Dickieana* is perhaps to strengthen Newman's conclusion that here is a new species. This is probably the correct interpretation, but before redefining the specific characters it is desirable to make comparisons with material from other sources if possible, and unfortunately the moment this is attempted uncertainty of a different sort sets in.

The fronds reproduced on Fig. 117 have all spineless spores and the low chromosome number ($n = 84$). In leaf morphology they are, however, very diverse. The leaf of

Fig. 118. Spore forms in *Cystopteris*. × 250. *a*. The large spiny spores associated with the high chromosome number ($n = 126$) from the leaf of Fig. 111*b*. *b*. Slightly smaller spiny spores associated with the lower chromosome number ($n = 84$) from the leaf of Fig. 119*c*. *c*. *C. Dickieana* Sim with verrucose spores.

Fig. 117*c* is from Kongsvold, Dovrefjell, Norway, from a plant kindly sent to me alive in 1948 by Mrs Gunvor Knaben of Oslo. Kongsvold is the type locality for '*C. Baenitzii*', which was described in 1891 by Dörfler, and named after its discoverer, one Baenitz (1891), as a species, on the sole criterion of spineless spores. The leaf of Fig. 117*c* can hardly be other than *C. Baenitzii*, and it is not more different from *C. Dickieana* than is, for example, *C. alpina* from *C. fragilis*, though it lacks the congested pinnae of *C. Dickieana* from Scotland. The leaves of Fig. 117*b* are, however, of an extremely different type. They are from a plant brought back alive from Greenland in the autumn of 1948 by the Leeds University Expedition to that country. They represent an extreme arctic type, having been laid down in the bud in the original locality which was within five miles of the edge of the permanent ice-sheet which covers central Greenland. Immediately on arrival in Leeds the leaves expanded and within three weeks had given both a cytological demonstration of $n = 84$ and also proof of spineless spores. To what extent their appearance will alter in subsequent years after continuous growth under more temperate conditions cannot be predicted.

Preliminary search for spineless spores in herbaria has shown them to be distributed on a world scale, although in western Europe they are very infrequent. To the one locality in Scotland (that of var. *Dickieana* itself) can be added several records of

Fig. 119. Forms of '*Cystopteris fragilis*' with spiny spores and the low chromosome number ($n=84$), from living leaves grown in cultivation, natural size. *a*. From Swedish Lapland. *b*. From Rannoch Moor, Scotland. *c*. From Vancouver Island, Canada. *d*. From Preda, Switzerland.

C. Baenitzii Dörfl. in Norway, north Sweden and Finland and quotations of records in the flora of Russia (Komarov, 1934) from right across Siberia. From the south there are specimens in the Kew herbarium from Algeria, Asia Minor, Persia and the Himalayas. They also seem to be present in North America.

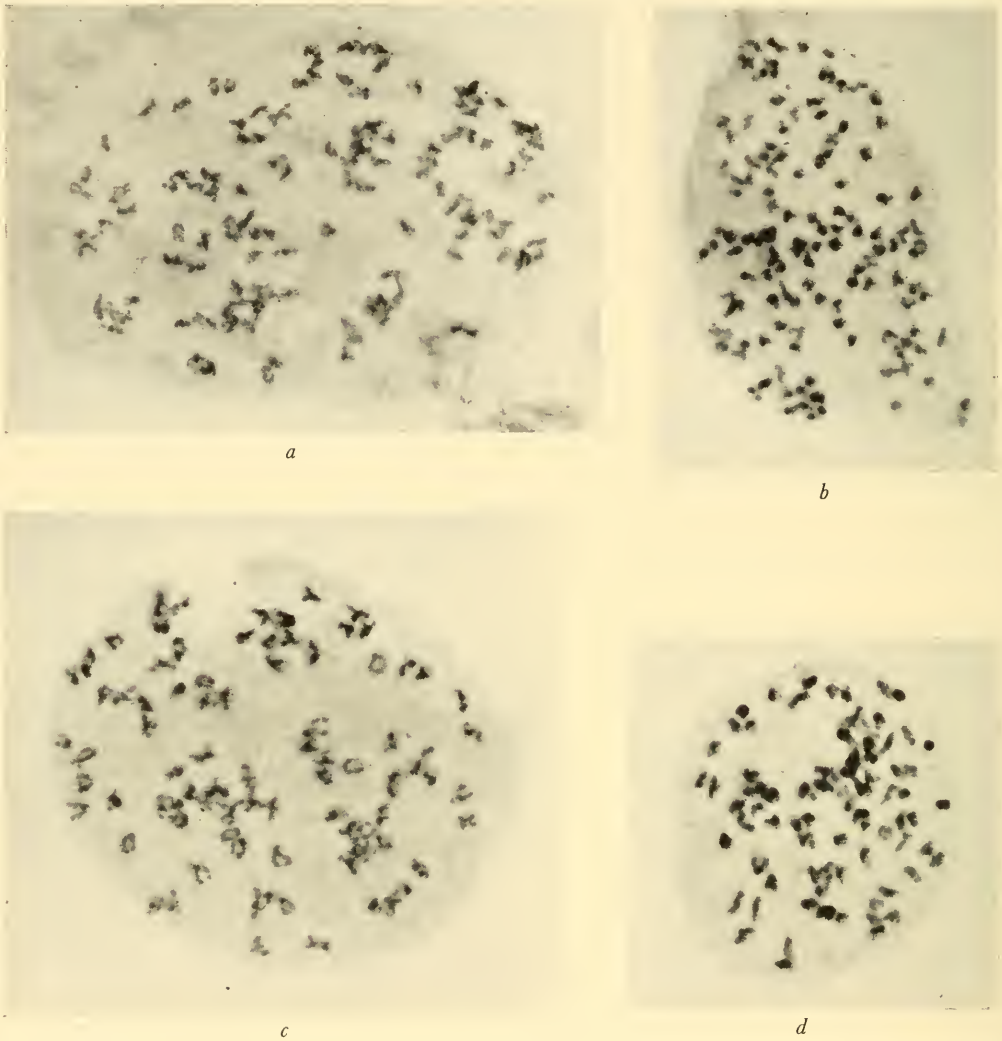
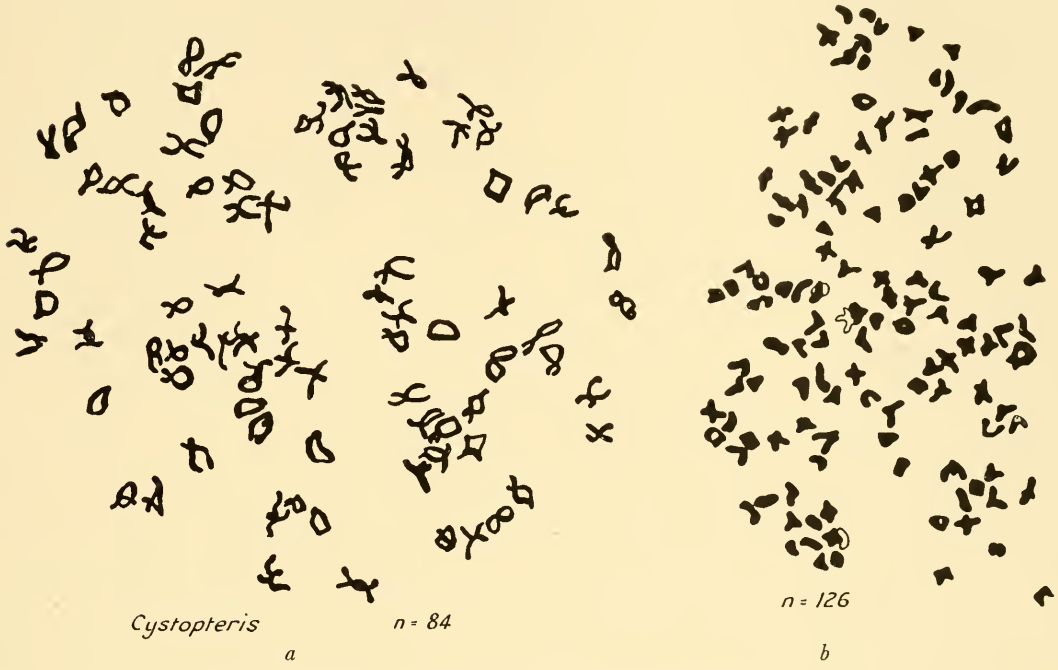


Fig. 120. Meiosis in various *Cystopteris* types, acetocarmine. × 1000. *a.* '*C. fragilis*' from Ingleborough, Yorkshire. $n=126$. *b.* '*C. alpina* forma *obtusa* Kestner' from Switzerland, from the leaf of Fig. 111*a*. $n=126$. For explanatory diagram see Fig. 121*b*. *c.* '*C. fragilis*' from Canada from the leaf of Fig. 119*c*. $n=84$. For explanatory diagram see Fig. 121*a*. *d. C. montana* (Lam.) Desv. $n=84$. For explanatory diagram see Fig. 122.

With such a vast range to explore a precipitate definition of species would be unwise. In terms of populations, however, we seem to be dealing with an ancient and perhaps relict stock of arctic affinities which is probably not co-specific with the spiny-spored *Cystopteris*, though whether it represents one species or several cannot yet be known.

It is also desirable to look for genetical evidence regarding the mode of inheritance of spore pattern, before final conclusions are drawn.

The status of the *C. Dickieana-Baenitzii* complex would be easier to determine if there were greater uniformity among the spiny-spored forms. These are, however, more diverse than the description so far given suggests. It is true that in Britain and Switzerland the principal populations have both spiny spores and the high chromosome number of 126, but even in these countries other types can be found, and elsewhere the relative proportions may prove to be quite different. Fig. 119 shows a small assemblage of



a *b*

Fig. 121. Explanatory diagrams to Fig. 120*c* and *b* to show the two chromosome numbers in '*Cystopteris fragilis*' with spiny spores. × 1500.

fronds from Britain, Switzerland, Scandinavia and Canada in which rather small spiny spores and the lower chromosome number of 84 have been found. Populations of this type have been met with so far in one place in Switzerland (Preda), in two places in Britain (Rannoch Moor in Scotland and the Lake District), in Iceland (Brekkufjall), in Finland (Piikkiö), and over large areas in Scandinavia, specific sites being Runmarö near Stockholm, Storlien in Jämtland, Swedish Lapland, and Trondheim and Hell in Norway. It is probable, indeed almost certain, that the high-numbered form also occurs in Scandinavia, though it is not so distinctly the dominant type there as it is with us. In Canada, on the other hand, the high-numbered form has not yet been encountered, although the low-numbered form has been obtained from Ontario, Ottawa and several places on Vancouver Island (Figs. 120*c*, 121*a*).

Taking the whole evidence assembled to date we have thus detected in a preliminary glance at *Cystopteris* in Europe, Iceland, Greenland and America three spore types (i.e. large and small spiny spores and verrucose spores), two chromosome numbers

($n = 126$ and 84) and a great variety of different forms of leaves. We may be certain of one thing only, which is that this degree of diversity cannot be all that will be found. For one thing the cytology as it stands is manifestly incomplete. The relationship between numbers as different as 126 and 84 may not at first leap to the eye, yet we can hardly doubt that we are dealing with the upper members of a polyploid series, the lower ones of which are still to seek. If a form with a gametic number of 42 could be found we should have a simple series of 42, 84 and 126 in which the plants bearing them would be diploid, tetraploid and hexaploid respectively. It may be that the diploid is extinct. In a world-wide range it is, however, scarcely necessary to postulate extinction as long as we are in ignorance of the nature of populations over vast regions. It is, therefore, desirable to continue the search for cytogenetic data on a scale far greater than has so far been obtained, in the light of which the speciation of this most troublesome group may perhaps become clearer.

Fig. 122. Explanatory diagram to Fig. 120*d*. × 1500.

In the meantime perhaps enough has been said to give some indication as to why the pure taxonomy of the *Cystopteris* populations of Europe is so intractably entangled when studied without cytology, and to hope that more extensive collections, especially from countries outside Britain, may ultimately bring order out of chaos.

The other British species, *C. montana* (Lam.) Desv., can be dealt with in one sentence. The chromosome number (Fig. 122) is 84, a fact which is of importance merely in showing that multiples of 42 are no innovation in the *C. fragilis* complex, but are probably fundamental to the genus. In this respect therefore the genus *Cystopteris*, though perhaps related to the Dryopteroids and to *Woodsia*, nevertheless stands somewhat alone.

Leaving the Dryopteroids now aside, the next major group to be placed near them by Bower (see diagram, p. 89) is that of the Blechnoids. The British representatives comprise two genera, *Blechnum* and *Scolopendrium** (*Phyllitis*), each with one species only in this country, namely *Blechnum spicant* (L.) With. and *Scolopendrium vulgare* Sm. (= *Phyllitis Scolopendrium* (L.) Newman). Both genera are commonly regarded as in some way related to *Asplenium*, though the interpretation of the nature of the relationship varies with different authors; Bower himself regards *Scolopendrium* as the end-result of a series of developments in the order *Asplenium—Blechnum—Scolopendrium*, but the alternate order, namely, *Asplenium—Scolopendrium—Blechnum*, is that more commonly adopted in Floras (cf. also Copeland, 1947).

The chromosome numbers of the British species are shown in the photographs (Fig. 123*e*, *f*). *Blechnum spicant* (Fig. 131*f*) has $n = 34$, the number having been established

*The retention of *Scolopendrium* instead of the technically more correct generic name *Phyllitis* for the purpose of this chapter is a matter of convenience in equating it with the bulk of the literature dealing with this species.

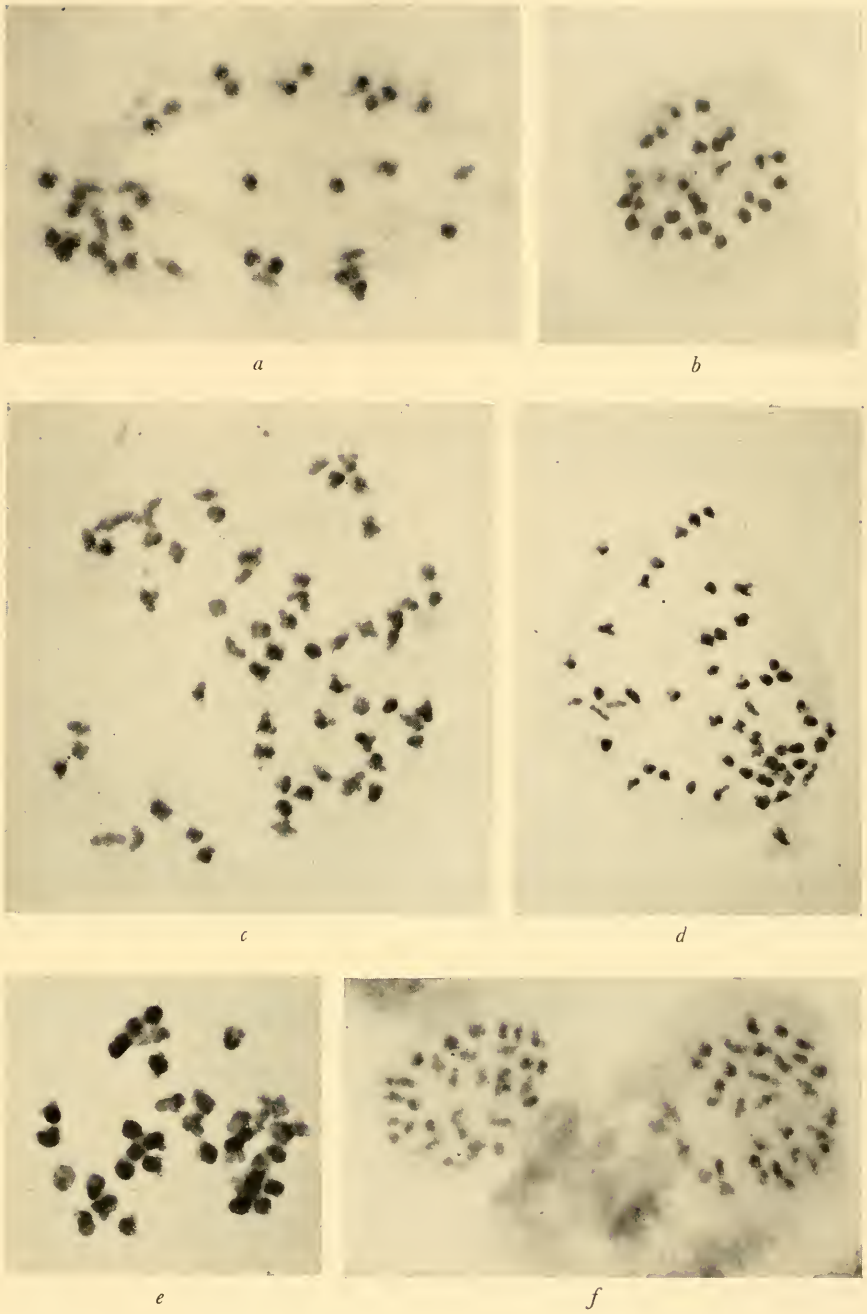


Fig. 123. Meiosis in various genera. Acetocarmine. × 1000. *a. Polypodium vulgare* var. *semilacerum* Hort., permanent preparation. $n=37$. *b. Adiantum capillus-veneris* L., permanent. $n=30$. For explanatory diagram see Fig. 125. *c. Cryptogramma crispa* (L.) R.Br., fresh preparation. $n=60$. *d. Pteridium aquilinum* (L.) Kuhn, permanent preparation. $n=52$. *e. Scolopendrium vulgare* (L.) Newm., fresh preparation. $n=36$. For explanatory diagram see Fig. 124. *f. Blechnum spicant* (L.) Roth, permanent preparation. $n=34$.

for specimens from both Scotland and England. *Scolopendrium vulgare*, on the other hand (Figs. 123*e*, 124), has $n = 36$. This result has been obtained for plants from both the south and the north of England and in some horticultural strains (cf. Chapter 11); there seems, therefore, to be no doubt as to its accuracy.

In so far as generic chromosome numbers based on single species mean anything, these numbers, while supporting the general resemblance of both genera to *Asplenium* (for which $n = 36$), also give some slight emphasis against Bower's interpretation of the form of the relationship and in favour of the older view that *Scolopendrium* is closer to *Asplenium* than is *Blechnum*. This view, it may be said in passing, is also that adopted by Holttum (1947).

Some additional information* about the genus *Scolopendrium* will be found in Chapters 8 and 11.

Fig. 124. Explanatory diagram to Fig. 123*e*. × 1500.

Fig. 125. Explanatory diagram to Fig. 123*b*. × 1500.

All the remaining genera of British ferns are, like the last two, either monotypic in this country or at least appear to be so at first sight. With one exception (see next chapter) they need not detain us long.

Of the Pteroid affinity (see diagram, p. 89) we have only the Common Bracken, *Pteridium aquilinum* (L.) Kuhn. This species is one of the most widespread plants known

*The establishment of $n = 36$ for *Scolopendrium vulgare* is also of interest in another connexion. This species is one of the relatively few ferns for which a considerable body of genetical information exists. It was the first member of the group for which simple Mendelian inheritance was demonstrated (Lang, 1923) and has since been the object of closer study by Andersson-Kottö. Of special interest in the latter work was the strain known as 'peculiar' for which the peculiar characteristic was the tendency of the edges of all the leaves to become prothalloid without special treatment to bring this about. As in other cases of apospory (cf. *Osmunda*) such prothalli could become free living if laid on soil and could produce new sporophytes from apparently normal sex organs. A sequence of such plants should give rise to a polyploid series, as in *Osmunda*. In *Scolopendrium*, however, an anomalous relation of chromosome numbers was described (Andersson-Kottö and Gairdner, 1938), but it is now evident that their analysis of 'peculiar' *Scolopendrium* is somewhat vitiated by the fact that their initial estimate of the chromosome number for the parent species was seriously in error. These authors had assumed that their material must have started with $n = 30$ and $2n = 60$. With the present demonstration that the correct numbers are $n = 36$ and $2n = 72$ it is clear that the cytology of 'peculiar' *Scolopendrium* needs to be reinvestigated before any interpretation is possible.

to science, for, without being a weed of cultivation, it is nevertheless to be found in every continent in the world. I am fortunate in having been able to compare a tropical and a temperate individual, fixed material of a specimen from Malay having been sent to me in 1938 by my friend Dr Chapman. The Malay material proved indistinguishable at meiosis from a British plant from Lancashire, both having $n = 52$ (Fig. 123*d*).

Of the Gymnogrammoid affinity we have the Parsley Fern *Cryptogramma crispa* (L.) R.Br. (= *Allosorus crispus* (L.) Bernh.), and the Maiden Hair, *Adiantum capillus-veneris* L. The little annual *Anogramme leptophylla* (L.) Link, though present on the island of Jersey, may perhaps be omitted from the present survey since it does not touch the mainland of Great Britain. Owing to the war it has in any case been impossible to obtain it.

Taking the Maiden Hair (*Adiantum*) first, this is a rare British plant though exceedingly common in many of the warmer parts of Europe. I have examined it cytologically in wild specimens from Italy and from Spain and in one British example brought back from the limestone pavement of Galway Bay in Ireland. All three specimens gave approximate root-tip counts of $2n = c.\ 60$, and they seemed indistinguishable. The Irish specimen unfortunately perished in an air raid before meiosis had been examined. Both the continental specimens, however, give $n = 30$ without any ambiguity (Figs. 123*b*, 125). This may therefore be accepted for the British plant also.

The Parsley Fern *Cryptogramma* (or *Allosorus*) is exceedingly abundant as a scree plant on siliceous rocks in many of our mountain regions, such as the Lake District and Wales. I have investigated it from the Lake District and, as shown in Fig. 123*c*, $n = 60$. The resemblance between this and the preceding is clearly of the sort which may be expected to be of use for taxonomic purposes when a greater number of species of the Gymnogrammoid affinity have been studied.

Reviewing this chapter we may note the rather wide range of different chromosome numbers to be met with in Britain as soon as we leave the relative uniformity of the Dryopteroid affinity behind. This may perhaps suggest that in the Pteridophyta as in the Cruciferae the aneuploid numerical changes, though rare, are actually concerned with the formation of larger evolutionary units than those which result with almost monotonous frequency from polyploidy. This conclusion is of some importance and should if possible be pursued outside the rather narrow confines of the European flora.

SUMMARY

Summarizing the facts of the chapter we may quote the following list:

Species	Source	*n*
Woodsia:		
W. ilvensis (L.) R.Br.	Wales	Probably 41 (41–2)
W. alpina (Bolton) S. F. Gray	Scotland	Probably 82 (82–4)
Cystopteris:		
C. montana (Lam.) Desv.	Hort. (Switzerland)	84
C. Dickieana Sim	Hort. (Scotland)	84
'*C. Baenitzii* Dörfl.'	Norway	84
Cystopteris, smooth spores	Greenland	84
C. fragilis (L.) Bernh., spiny spores	Norway, central and north Sweden, Runmarö (near Stockholm)	84
	Iceland, Finland	84
	Rannoch (Scotland), Lake District	84
	Switzerland (one locality)	84
	East and west Canada	84
C. fragilis	Britain, many localities	126
	Switzerland, many localities	126
	South Sweden	126
'*C. alpina*' Desv.	Switzerland	126
Blechnum:		
B. spicant (L.) With.	Britain	34
Scolopendrium:		
S. vulgare Sm.	Britain	36
Pteridium:		
P. aquilinum (L.) Kuhn	Britain and Malay	52
Adiantum:		
A. capillus-veneris L.	Britain, Italy, Spain	30
Cryptogramma:		
C. crispa (L.) R.Br.	Britain	60

CHAPTER 8

POLYPODIUM VULGARE

In the first draft of this manuscript one paragraph at the end of Chapter 7 was to have been devoted to the last of the higher British ferns, *Polypodium*, and the inclusion of a photograph of the chromosomes of '*Polypodium vulgare* var. *semilacerum*' on Fig. 123*a*, p. 123, is a reminder of that intention. As the work of writing has progressed, however,

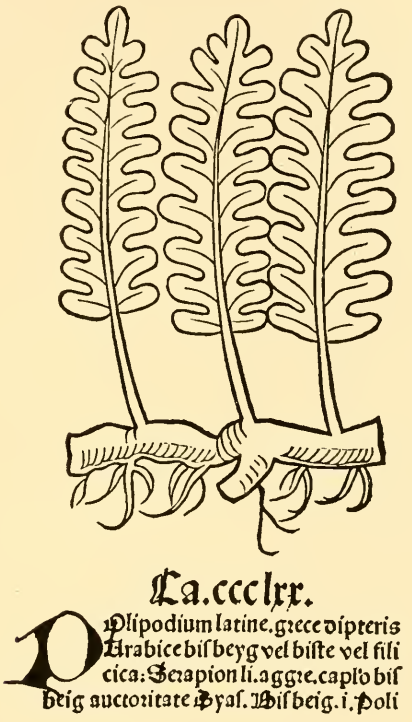


Fig. 126. Woodcut illustration of *Polypodium* in the *Ortus Sanitatis* of 1491. From a copy in the Rylands Library, Manchester. Slightly reduced. Portions of the Latin text are visible below the drawing.

the unexpected complexity of the *Polypodium* story has become increasingly revealed, until nothing less than a separate chapter will do justice to it even in a preliminary statement, which is all that this can claim to be. In making it, however, I am on this occasion drawing not merely on my own work but also on the collaborative effort of my colleague Miss Davies, who has contributed very greatly to the elucidation of the facts to be described below and without whom this chapter could not have been written.

The Common Polypody is a very familiar but at the same time a rather isolated fern. It was well known to our ancestors, being mentioned in a number of medieval herbals including the *Ortus Sanitatis* of 1491 (Fig. 126), in which the textual reference begins

with the words 'Polypodium latine, grece Dipteris . . .'. It is perhaps for this reason that the popular name of 'Polypody' is still based on the Latin rather than on a folk name in the vernacular as in other ferns, but it is probably only a coincidence that it is still bracketed with *Dipteris* in the phyletic view of Bower (cf. p. 89) as a somewhat doubtful member of the 'Dipteroid' affinity, since *Dipteris* at present denotes a very restricted genus confined to the Malay Peninsula and almost certainly unknown to the herbalists.

The European *Polypodium vulgare* L., although the type species of the type genus of the family Polypodiaceae to which all the ferns hitherto discussed except *Osmunda* have until recently been held to belong, has no well-authenticated near relatives. The genus is, in Bower's words, a comprehensive but phyletically confused one in which, owing to the loss of the morphological characters of the indusium, derivatives of widely different origin have been grouped together. Some of these have already been separated out and discussed in the context of their nearest indusiate relatives (*Phegopteris*, *Gymnocarpium*, Chapter 5; *Athyrium alpestre* and *flexile*, Chapter 6), but even without these we are left with a large and almost exclusively tropical genus in which it is by no means clear where exactly the one temperate species should be placed. Doubts about this have been repeatedly expressed, and it is only by invoking a resemblance to the tropical *Goniophlebium* that Bower has classified *Polypodium vulgare* at all. On the other hand, the idea that there could be doubts about the specific integrity* of *P. vulgare* is one which only a few specialists and no European writers of Floras appear ever to have entertained, and therefore the surprise with which the unequivocal fact was revealed by the cytology was very considerable.

'*P. vulgare*' is in fact a comprehensive name for a group of well-defined if closely related species possessing an aggregate range which extends right round the northern hemisphere together with South Africa, Kerguelen Island and perhaps Hawaii, to the first two of which it could, however, have been introduced from Europe. Each species within this range has a characteristic ecological or geographical area which only in certain cases overlaps that of others. Moreover, the cytological differences are such as to indicate quite clearly that populations of different ages are represented.

As was announced in a preliminary communication (Manton, 1947) there are three distinct cytological types in Britain and the nearer parts of the continent of Europe. The gametic chromosome numbers are 37, 74 and 111, which correspond therefore to sporophytes of diploid, tetraploid and hexaploid constitution in a polyploid series on 37. The monoploid number itself has already been illustrated for a horticultural variety of *Polypodium* known as *P. vulgare* var. *semilacerum* Hort. in Fig. 123*a*, p. 123, and it will be seen again in Fig. 143 on p. 141. The gametic number of the tetraploid ($n = 74$) may be seen in a specimen from Norway in Fig. 136, and that of the hexaploid ($n = 111$) in Figs. 127–129. The first of the hexaploid figures is that reproduced in the preliminary note in which an approximate count of *c.* 112 had been recorded earlier. That the gametic number is 111 without any equivocation is, however, clear, among other things, from Fig. 128, in which the whole array of chromosomes is dispersed with such spec-

* Compare, for example, the views of Christensen (1928), which summarized excellently the prevailing opinion with which this work began.

tacular clarity that the only difficulty in demonstrating the number with complete finality is that the area occupied by the flattened nucleus is so large that an unusually low magnification has had to be used in order to reduce the dimensions to that of the printed page.

At the time of publication of the preliminary note it was not certain whether some of these types might perhaps have been of horticultural origin. This matter is, however,

Fig. 127. Meiosis in hexaploid *Polypodium* from Windermere, fresh acetocarmine. × 1000. This was the first specimen obtained and was illustrated in the preliminary note (Manton, 1947) at a lower magnification. The chromosome number was at first thought to be '*c.* 112', but is now known to be 111. Compare with Figs. 128–129.

no longer in doubt. All three are characteristic and well-established components of the European flora over very large areas, and in the normal condition show none of the peculiarities which constitute the horticultural monstrosities to which varietal names have so often been given. Each has, however, a distinctive morphological character, and their separation in the field is a matter presenting no difficulty except where genuine admixture due to hybridization is occurring. There is, moreover, strong reason to suppose that not only are they distinguishable by their chromosomes and morphology but that they also have characteristically different ecological, or perhaps more correctly climatic, requirements which are reflected in differences of geographical distribution. Owing to obvious difficulties in sampling populations in central and eastern Europe at the present time, it has not yet been possible to investigate distributions fully. The compilation of a map will therefore be deferred, but such information as is available will be included in the description of each species in turn.

Fig. 128. Meiosis in hexaploid *Polypodium*, permanent acetocarmine. × 500. A very clear preparation showing $n = 111$ without equivocation but needing a low magnification for reproduction. For explanatory diagram see Fig. 129.

Fig. 129. Explanatory diagram to Fig. 128. × 750.

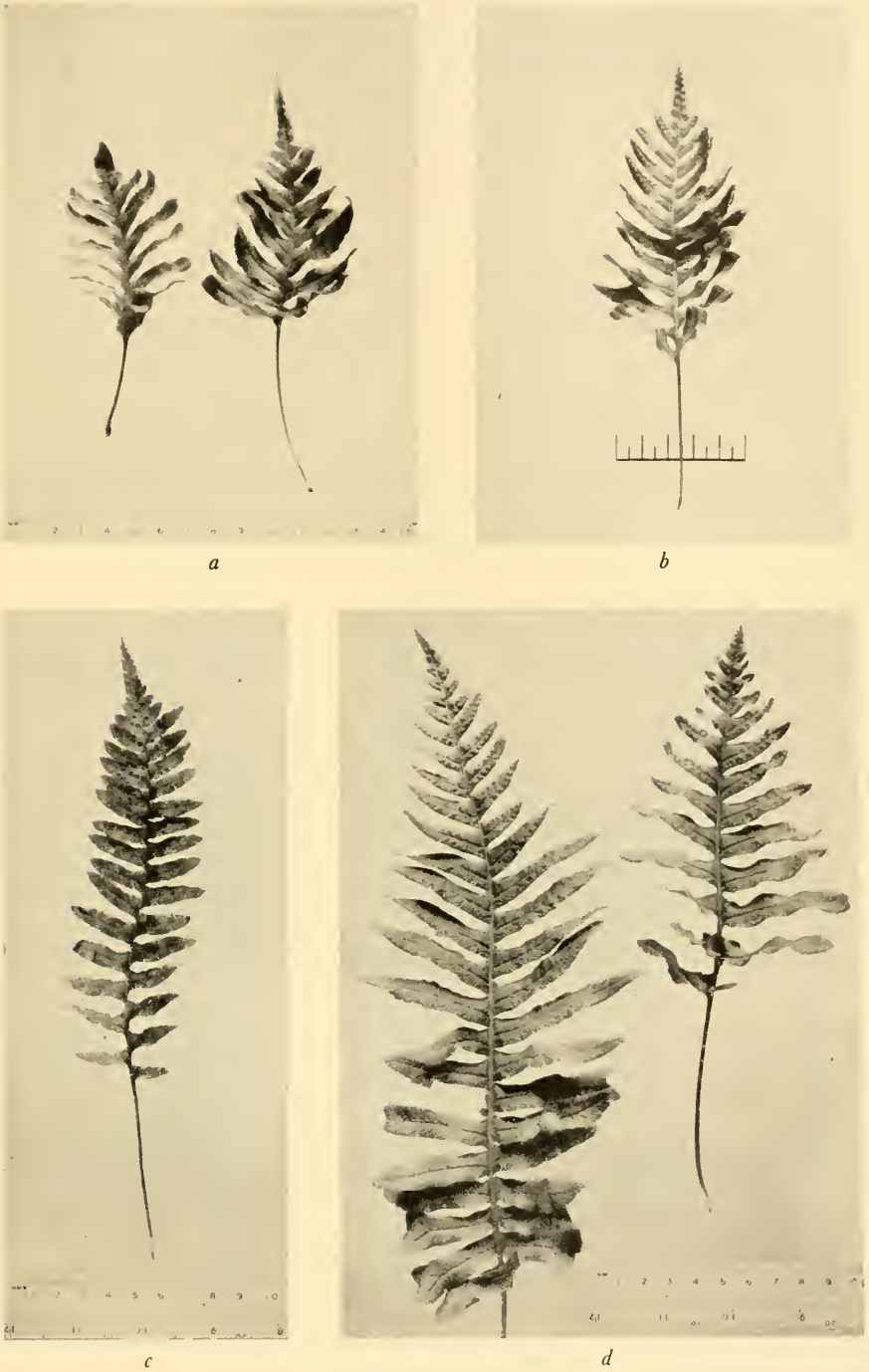
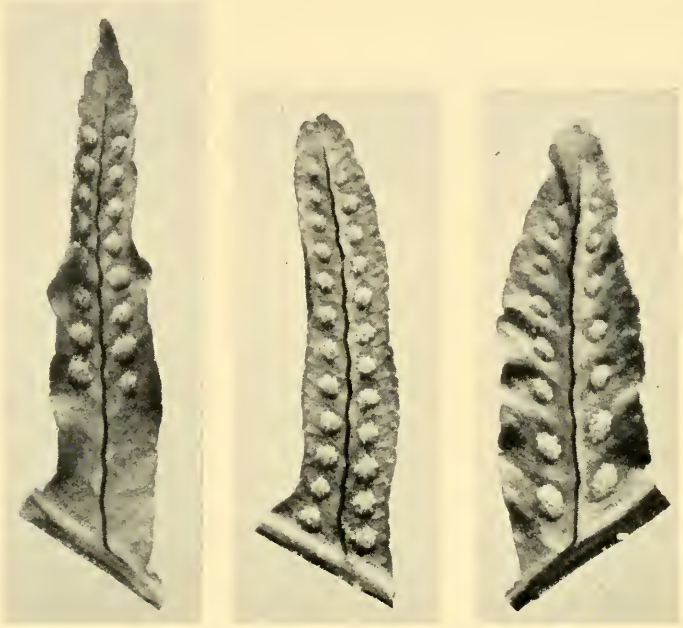


Fig. 130. Morphology of the three European species of *Polypodium* from living leaves grown under comparable conditions, half natural size. *a*. The diploid (var. *serratum*), the left-hand leaf from Cheddar (Somerset), the right-hand leaf from the south of France. *b*. A leaf from the same plant as the right-hand specimen of *a* to show forwardly projecting lower pinnae. *c*. The normal form of tetraploid *Polypodium* in Britain; note the narrow frond. *d*. A large and a small frond from the hexaploid in Britain.

The commonest form of *Polypodium* in most parts of western Europe from the north of Scandinavia to the Pyrenees is the *tetraploid.* This is shown photographically in Fig. 130*c* and in silhouette in Fig. 135*c*. It possesses characteristically the narrow outline for which the crude woodcut of the *Ortus Sanitatis* might be taken as a rough diagram. Many details of the shape of the pinnae are variable, but two fairly constant characters are the circular, as opposed to oval, sori and the number of indurated cells in the annulus. Attention was first drawn to the usefulness of this last character by Farquet in 1933 with special reference to *P. vulgare* var. *serratum* (Willd.) Milde to be



a *b* *c*

Fig. 131. *Polypodium* pinnae enlarged to twice natural size to show the shapes of the sori. *a*. The French diploid, sori oval. *b*. The tetraploid, sori round. *c*. The hexaploid, from a rather small frond, sori oval.

described below, and our experience strongly confirms his. In tetraploid *Polypodium* the range of numbers is from 11 to 13 cells with 12 as the commonest number. A sporangium showing this is reproduced in Fig. 132.

In contrast to the tetraploid the *diploid*, which corresponds in our experience with descriptions of *P. vulgare* var. *serratum* wherever we have found it, is of characteristically southern affinities. It appears to be the only type present at low altitudes in the Mediterranean basin. Living material has been cytologically examined by us from two places in the south of France (Pont-du-Gard and Perpignan) and from north Italy while herbarium records extend the range at least as far as North Africa and the Atlantic Islands (Madeira, Teneriffe, etc.). In northern Europe it is found in discontinuous patches, usually on limestone and often in districts containing other species of southern origin. Examples of such localities from which we have already obtained it are the

Rhone Valley in Switzerland at the eastern end of the Lake of Geneva at an elevation not exceeding 400 m., at Dartmouth in south Devon, abundantly in the Cheddar Gorge and some other limestone habitats in Somerset, on Ingleborough in Yorkshire and in the Burren in the west of Ireland. It may confidently be expected to occur in central

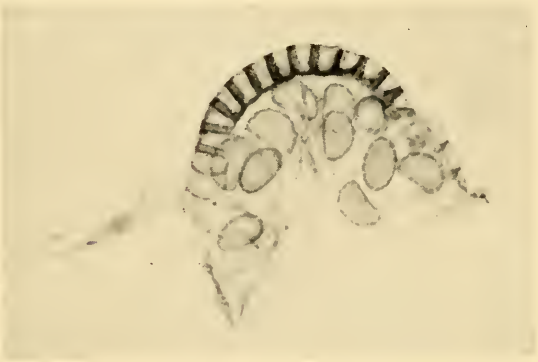


Fig. 132. Sporangium of tetraploid *Polypodium* from Cumberland showing twelve indurated cells in the annulus, from a glycerine jelly mount. × 100.

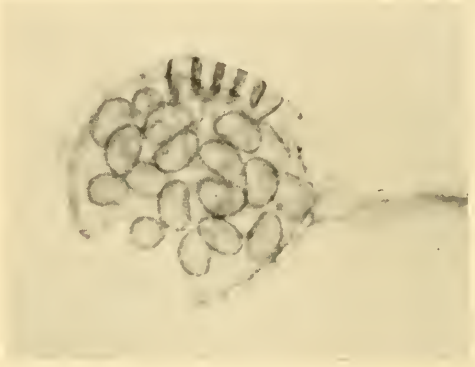


Fig. 133. Sporangium of diploid *Polypodium* from Cheddar showing five indurated cells in the annulus, from a glycerine jelly mount. × 100.

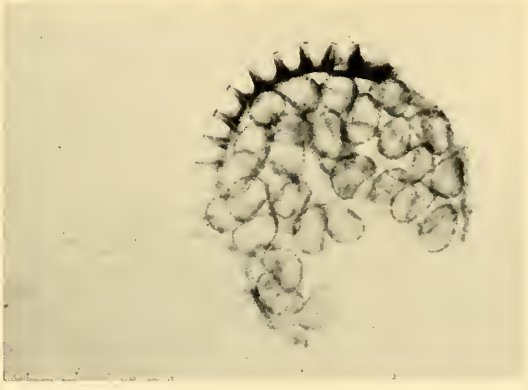


Fig. 134. Sporangium of hexaploid *Polypodium* from Anglesey showing approximately nine indurated cells in the annulus, from a glycerine jelly mount. × 100.

Europe, and profitable places to look would be the *Biscutella* districts in the river valleys of the Rhine, Elbe, Oder and Danube, but until these have been sampled the eastern limits of this 'variety' cannot be determined.

The morphological characteristics of the diploid can best be appreciated by a glance at Figs. 130*a*, *b*, 131*a* and 135*a*. The most striking character is the oval frond which even in small specimens widens out disproportionately much in the centre compared with the tetraploid. Another detail which not all leaves display but which can generally be found in some leaves on every plant is that the two lowest pinnae often project sharply forward in the manner so characteristic of the Beech Fern (*Phegopteris*). This character

is not always detectable when the leaf has been artificially flattened by herbarium treatment, though it can sometimes be inferred by the crushed condition of the lowest pinnae. Another detail which seems very constant is the shape of the sorus, which is characteristically oval and not round. This character is more clearly visible in a young leaf at or before the stage at which sporangia are ready for fixing than it is when the spores are ripe. A very important character, since it directly affects the ecological requirements, is the seasonal periodicity of the leaves. In the tetraploid, new fertile leaves are put up in early summer (May or June in Britain, early July in Scandinavia) and remain fresh unless battered to pieces at the end of the winter; the spores are ripe in July or August. In 'var. *serratum*', however, the principal dormant season is the summer during which, in times of drought, the leaves may die away. New fertile fronds only appear in autumn (August or September), and the spores are therefore ripe so late that it may be suspected that in bad years in the more northerly habitats they may perhaps fail to ripen at all. Lastly, the microscopic character of the number of cells in the annulus is found to be extremely helpful; in all the localities listed above, the average number of indurated cells is 5 with a total range from 4 to 6. A sporangium is photographed in Fig. 133.

It may be suspected that the claims of var. *serratum* to be regarded as a separate species would have been generally recognized long ago but for the existence of the *hexaploid* which is almost certainly in origin a hybrid between diploid and tetraploid and which therefore not unnaturally combines characters of both. Some leaves are photographed in Fig. 130*d*, and a silhouette appears in Fig. 135*e*. The hexaploid is always a coarse plant and often very large. The leaves are thicker and fleshier than either of the others, but their shape, size being discounted, is almost exactly intermediate between diploid and tetraploid. In some details, however, the characters of the diploid seem to show simple dominance. Thus the projecting lower pinnae otherwise characteristic of the diploid are often also present in the hexaploid. The sori likewise are oval, as in the diploid, but the number of indurated cells in the annulus is exactly intermediate, being on an average 9 (with a range of 8–10), although all the cells are distinctly larger than in either diploid or tetraploid. The shape of the sori and the nature of the annulus are illustrated in Figs. 131*c* and 134. With regard to seasonal periodicity the hexaploid differs from both the others in having an extended season from summer to autumn, and it was therefore probably no coincidence that when fixings were first attempted in September 1944 at Windermere in the Lake District, a region in which tetraploids abound, it was only on a hexaploid that a young fertile frond was found.

Ecologically and geographically the hexaploid seems to prefer a moister climate than do either of the others. It is the commonest type in Ireland, Wales, south-west England and the Channel Islands; indeed in Jersey, where it is abundant all over the island, no other form has so far been found. On the mainland of Europe its distribution is less well known, though it is certainly present in coastal districts from Portugal to Holland and inland it reaches the lower slopes of the Alps.

That the hexaploid has indeed originated from a hybrid between diploid and tetraploid which has attained stability by doubling its chromosomes is suggested not only by

Fig. 135. The various species and hybrids of *Polypodium* detected in Europe, silhouetted from living leaves. Half natural size. *a*. The diploid from Cheddar. *b*. A triploid wild hybrid between diploid and tetraploid from Roches, Switzerland. *c*. Tetraploid from north Wales. *d*. Pentaploid hybrid between tetraploid and hexaploid from Bolton Abbey, Yorkshire. *e*. Hexaploid from Ireland.

the morphology but also by the breeding behaviour of all three types when their native localities abut. Thus among the rather limited number of diploids collected at random on journeys as occasion offered, no less than three cases of triploids have been included. A leaf of one of these from the Rhone Valley in Switzerland is shown in Fig. 135*b*, and the chromosomes of another, from the lower slopes of the Pyrenees near Perpignan in Fig. 139. There is almost complete failure of pairing in the triploids, the shape of the univalents making a very striking contrast with that of the normal appearance of pairs in the putative parent species. These triploids, the third of which came from north Italy, can hardly be other than hybrids between diploid and tetraploid, and they could be the prototypes of the hexaploids before the chromosome number was doubled.

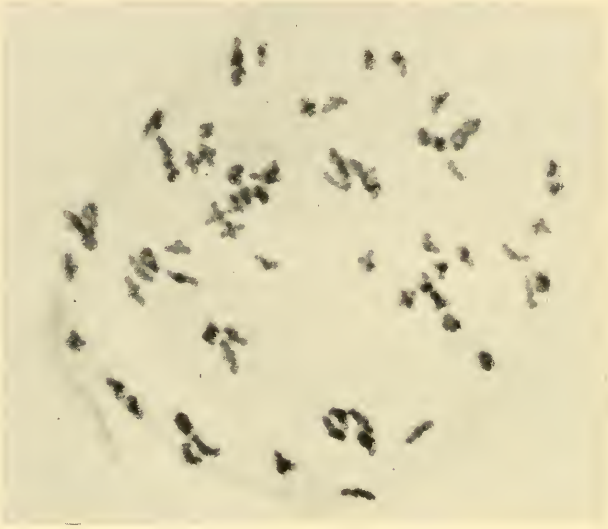


Fig. 136. Meiosis in tetraploid *Polypodium* from Norway to show the shapes of the 74 bivalents for comparison with the triploid of Fig. 139. × 1000.

Where tetraploids and hexaploids grow together, pentaploid hybrids are to be found, and some half-dozen of these have been included in our own collections made in different parts of north and south England and as far afield as Holland. One leaf is shown in Fig. 135*d* and the chromosomes in Fig. 138. As may be seen in the diagram (Fig. 137) there are exactly 74 pairs and 37 univalents.

This pairing is exactly what would be expected if the hexaploid is the allopolyploid between diploid and tetraploid as postulated. The triploid indicates that the diploid and tetraploid are sufficiently different from each other to have practically no chromosomes in common in spite of their readiness to breed together. On the other hand, the perfect pairing of 74 of the chromosomes of the hexaploid when backcrossed to the tetraploid indicates that the gametic complement of the tetraploid is present intact in the hexaploid, and that the unpaired chromosomes in the pentaploids are therefore almost certainly those of 'var. *serratum*', which have already been seen to be non-homologous with these by means of the triploid hybrid.

Fig. 137. Explanatory diagram to Fig. 138 with the 74 pairs in black and the 37 univalents in outline. × 2000.

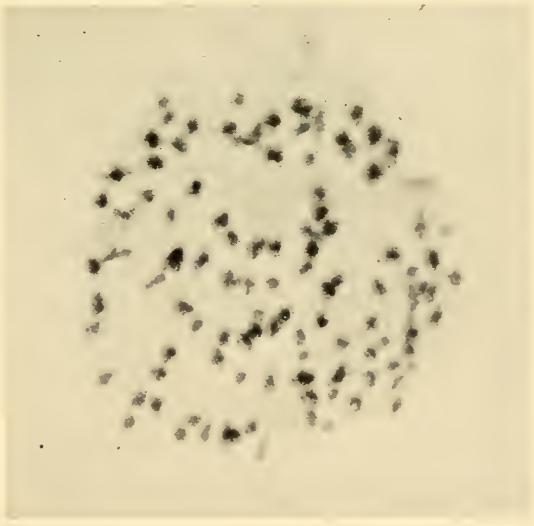


Fig. 138. Meiosis in pentaploid *Polypodium*, from a wild hybrid from Holland, permanent acetocarmine. × 1000. For explanatory diagram see Fig. 137.

The taxonomic conclusions for Europe would therefore seem to be that we are dealing with three distinct though closely related species of which the third, the hexaploid, is compounded of the other two and of very recent origin. The only point of uncertainty concerns their names. There is apparently some doubt as to the nature of Linnæus' type specimen since none is included in the Linnean herbarium, and it appears certain that the name *serratum* could not be accepted as a specific epithet since it has already been used for an entirely different species of *Polypodium*. This matter may therefore perhaps be laid before professional systematists and decision deferred as to nomenclature, since to act otherwise incurs grave risk of encumbering the literature with invalid epithets which might later have to be changed.

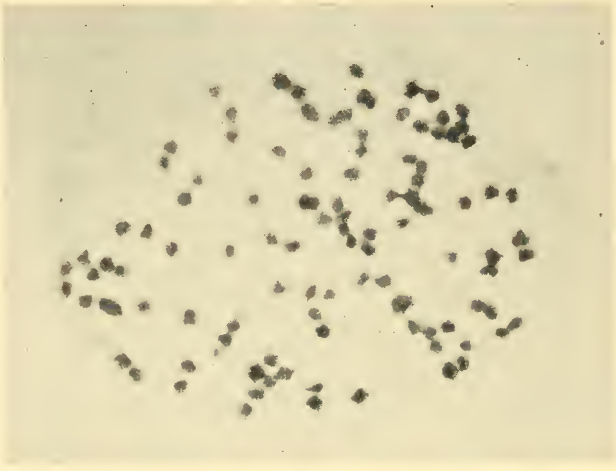


Fig. 139. Meiosis in triploid *Polypodium* from a wild hybrid believed to be between tetraploid and diploid. From the foot of the Pyrenees near Perpignan. Permanent acetocarmine. × 1000. Almost all the chromosomes unpaired ($3n = 111$).

There is, however, a little more to add to the cytological story. I have been fortunate in receiving from friends and correspondents some living wild material from both sides of North America. Fig. 141 shows a whole plant, natural size, of *P. virginianum* from Nova Scotia. Its general resemblance to the tetraploid of Europe is striking, although its identity as *P. virginianum* is attested by the possession of the very characteristic paraphyses (Fig. 142) only encountered in eastern America. Cytologically this plant is, however, diploid, and the same is true of specimens attributable to *P. vulgare* var. *occidentale* Hook. received from Vancouver Island and the Rocky Mountains, one leaf of which is visible in Fig. 140 and the chromosomes in Figs. 143 and 144. Since it is impossible to equate 'var. *occidentale*' with 'var. *serratum*' or *P. virginianum* with the European tetraploid, it seems probable that we shall have to accept at least two American species in addition to the three of Europe.

Lest, however, the reader should at this point lose patience thinking that only a troublesome tangle of nomenclature is involved, it is perhaps worth pointing out the extreme interest of the general situation which is beginning to become visible. In the

Fig. 140. '*Polypodium vulgare* var. *occidentale*' Hook. from Vancouver Island, from a living frond grown in cultivation. Natural size.

Fig. 141. *Polypodium virginianum* L. from Nova Scotia, from a dried frond of the plant used, before cultivation. Natural size.

summer of 1948 I was able to discuss the world distribution of *Polypodium* with Professor Hultén of Stockholm, who alone has adequate information regarding large tracts of central and eastern Asia, and he has kindly given permission to publish the gist of his verbal communication. This is that *P. vulgare* sens. lat. is to be found right round the northern hemisphere in a total area which is not now continuous though it may formerly have been so. The most conspicuous gaps are east of Lake Baikal in central Russia, the whole of central China, central North America and the whole of

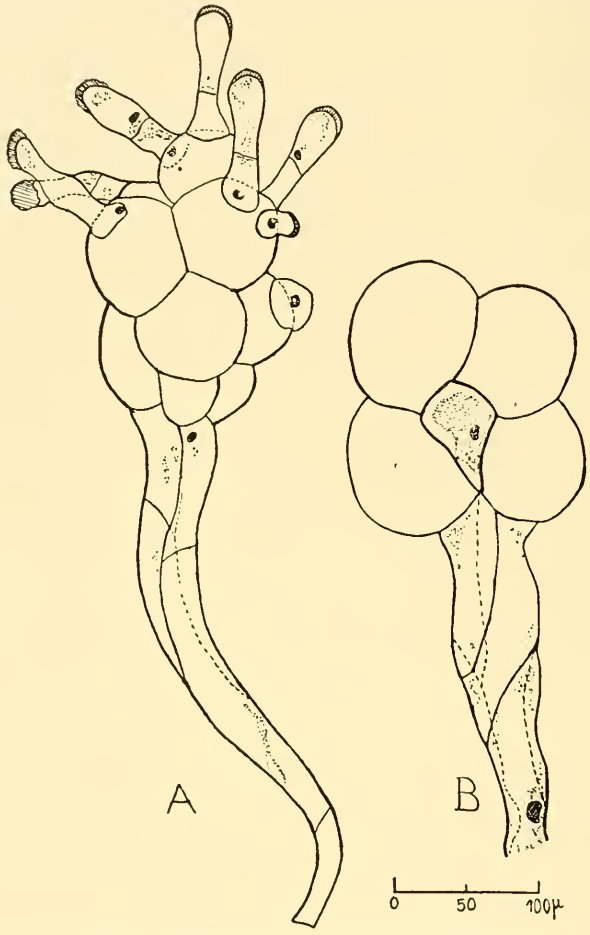


Fig. 142. Characteristic paraphyses diagnostic of *Polypodium virginianum*, after Martens (1943).

Greenland. The primary cause of disruption of this kind is believed to be glaciation, and the present distribution of diploid populations in Europe is in agreement with this.

If now the cytological facts so far obtained are superimposed on this general distribution we may interpret it as meaning that an ancient diploid stock must have spread round the world, breaking up as it went into ecospecies. During periods of extermination some of these ecospecies survived in major refuge areas of which we may discern at least four, namely, south Europe, eastern North America, western North America (including perhaps 'Beringia' as the unglaciated region of the Behring Straits has been

named by Hultén) and 'somewhere in Asia'. From these refuge areas under more equable conditions our present taxonomic species have spread again, though in most cases the areas occupied have not yet linked up with each other. An exception is, however, to be found for the tetraploid of Europe which may perhaps be a fairly recent species and which has certainly spread very vigorously over the heavily glaciated territories of our continent. Where this new tetraploid has encountered the remnant of the older population still persisting in the south it has hybridized with it, and the newest species, the hexaploid, is the result.

What are the nearest ancestors of the tetraploid or where in Europe or Asia it can have arisen can only be elucidated by further inquiry. We can likewise hope to investigate

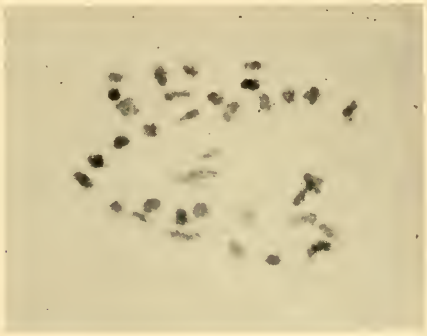


Fig. 143. Chromosomes of *Polypodium vulgare* var. *occidentale* from the Rocky Mountains. × 1000.

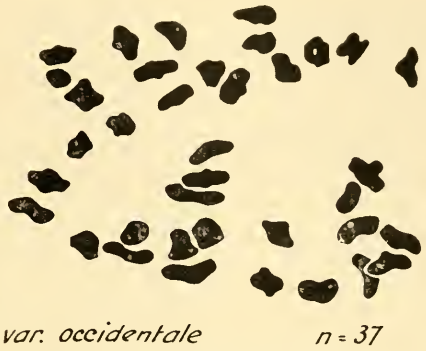


Fig. 144. Explanatory diagram to Fig. 143. × 1500.

the nature of the genetical differences which at present separate the various known diploid species from each other. The present state of knowledge is thus clearly not the end but the beginning of a problem which, if it can be unravelled, should illuminate the migrations of floras, their origins and developments, not only in Europe but over the whole of the northern hemisphere in the time lapse of a geological period. This hope is also inherent in the problem of *Cystopteris*, but the additional complexities of that genus may in the end impede progress irremediably. The relative simplicity of the situation in *Polypodium* is likely to be its greatest asset, and it is no exaggeration to say that even in the present state of knowledge *Polypodium* has turned out to be by far the most interesting member of the British fern flora which we have so far encountered.

SUMMARY

Summarizing this chapter, it is perhaps sufficient to say that morphological and cytological reasons have been given for recognizing three separate species of *Polypodium* in Europe and at least two from the *P. vulgare* complex in America. A preliminary discussion of geographical distribution is included. Some information regarding the mutual relationships of the European species has been supplied from wild hybrids.

CHAPTER 9

THREE SPECIAL CASES OF FERN HYBRIDS: *SCOLOPENDRIUM HYBRIDUM, WOODSIA* AND *POLYSTICHUM ILLYRICUM*

As a supplement to the foregoing account of the British fern flora attention may profitably be given to a few special cases of species hybrids of non-British origin which, nevertheless, add appreciably to our knowledge of species or genera already considered.

Scolopendrium hybridum Milde (= *Phyllitis hybrida* (Milde) Christensen) (Fig. 145) is

Fig. 145. *Scolopendrium hybridum* Milde. Natural size. *a*. From a dried leaf. *b*. From a living leaf of the next generation. Both grown in cultivation.

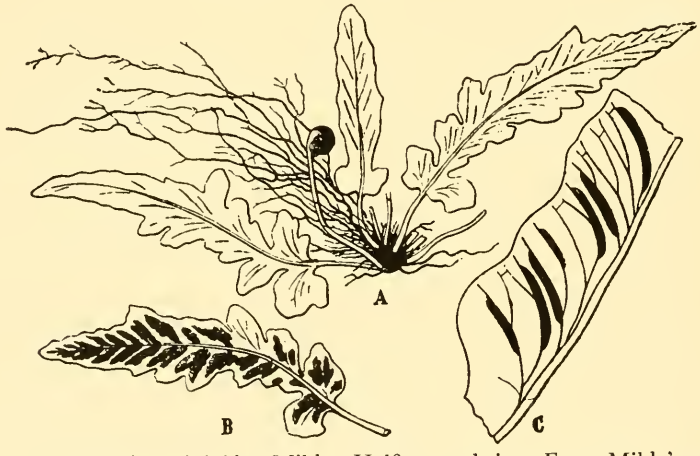


Fig. 146. *Scolopendrium hybridum* Milde. Half natural size. From Milde's original drawing after Luerssen in Rabenhorst's *Kryptogamenflora* (1889).

a somewhat problematical plant with a very restricted range. It is endemic to five small islands in the Adriatic Sea near the Dalmatian coast known as the Quarnero Group, and consisting of Lussino, Osiri, Arbe, Dolin and S. Gregorio. The first specimen known to science (cf. Fig. 146) was discovered on Lussino by Reichardt in 1862, who noticed it growing in a wall among a dense population of *Ceterach officinarum*. Reichardt's speci-

men was described in 1864 and named by Milde, who regarded it as a hybrid between *Ceterach* (Fig. 147*a*) and *Scolopendrium vulgare*, and since the latter species was not at that time recorded from the island, Milde predicted that it would subsequently be found. Another suggestion made later by Luerssen (1889) was that a more probable affinity might be with the south European *S. hemionitis* Lag., Garcia & Clem. (Fig. 147*b*). This species has a much more restricted range than *S. vulgare* and is the only other member of the genus *Scolopendrium* present in Europe; it occurs on many of the Mediter-

Fig. 147. Two of the imagined parents of *Scolopendrium hybridum*. Natural size. *a*. *Ceterach officinarum* DC. from the south of France with $n=72$. From a living leaf grown in cultivation. *b*. *Scolopendrium hemionitis* Lag., Garcia & Clem. from the south of France, from a pressed wild leaf of a small plant.

ranean islands and south European coasts. An affinity with *S. hemionitis* has been accepted as probable by most subsequent writers, and *S. hybridum* was indeed regarded as a subspecies of *S. hemionitis* by Ascherson and Graebner in 1896. The claim that it should be regarded as a totally distinct species had, however, already been made* by Heinz in 1892, and this may also have been suspected by Reichardt himself who comments (1863) on the fertility of the spores. Most subsequent discussion in the literature has accepted this view, while reiterating the probability that the species may have originated as a hybrid which had become fertile. Many lines of evidence have been quoted. In addition to the morphological comparisons introduced by Milde anatomical comparisons between *Scolopendrium* and *Ceterach* carried out by Hoffman in 1899 showed *Scolopendrium hybridum* to be intermediate. A similar result was obtained by comparison of the prothalli in 1922 by Howat, while at least two investigators have studied

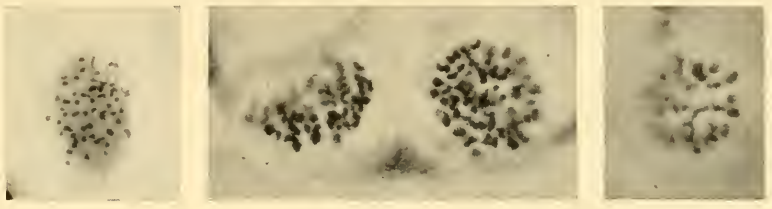
* A clear summary of the history of this controversy will be found in Morton (1914*a*, 1925).

the range of form to be found among natural populations of sporophytes. Thus Haračić in 1893, who appears to have been the first person to study natural populations of the plant, described three different morphological types and figured two of them under the names of forma *typica*, var. *Reichardtii* (Milde's original specimen) and var. *lobata*. Some additional evidence was added by Ivancich in 1923, who reinvestigated the ranges of form and spore fertility among natural populations. Haračić's var. *lobata* was not examined again, since it had been found only on the island of Osiri which Ivancich did not visit. Haračić's forma *typica* was, however, described as the most stable, and his var. *Reichardtii* as the most unstable. In addition, three other varieties were described, namely, var. *hemionitifolia*, which closely resembled *Scolopendrium hemionitis*, var. *erosa* which differed from forma *typica* in having large basal lobes and more irregular outline to the rest of the lamina, and lastly var. *ceterachifolia* which very closely resembled *Ceterach*. This last 'variety' was in effect only one individual plant, since it was sterile from spores and could only be propagated from the rhizome. By means of it, however, a series of fronds could be shown which almost entirely bridged the gap between one genus and the other.

This history has been given in some detail partly because *Scolopendrium hybridum* is somewhat unfamiliar to most botanists, but also because it differs from all the other suspected fern hybrids in combining characters not merely of two different species but of two different genera whose close relationship might not otherwise be suspected. It is thus clearly a plant of quite unusual interest, and I am extremely fortunate in having had access to living material of it. This has been of several kinds, all, however, ultimately referring to the one island of Lussino. The first material to reach me consisted of a pot of tiny sporeling plants raised by the late Dr F. W. Stansfield and presented to me after his death by his son. The origin of the pot was described by Stansfield in the *British Fern Gazette* (vol. VII, p. 91, 1936) as follows: 'A spore-bearing frond was sent to M. Kestner [of Lausanne] from the island of Lunin [accidental misreading of Lussin]. M. Kestner brushed off some spores and raised plants from them and, mainly from that fact, he formed the opinion that it was not a hybrid but a species *per se*. He afterwards sent on the frond to me and I was able to collect from it a few more spores which I sowed and have now about a dozen tiny plants as the result.' These plants grew to maturity and were the main source of cytological material. They were, however, supplemented, shortly before the war, by two consignments of adult plants very kindly supplied by Professor Lona of Trieste. The first consignment consisted of one plant of forma *typica* which had been in cultivation in Trieste Botanic Garden, and later, in 1938, some additional plants of various morphological kinds, though not including the extremes (var. *hemionitifolia* and var. *ceterachifolia*), were sent direct from the island of Lussino. Owing to the outbreak of hostilities the cytological study of this material was less exhaustive than it would otherwise have been; the fresh collections were, however, invaluable in confirming the validity of the results obtained with the Stansfield material. During the war itself almost the entire collection was lost, but spores saved from the last surviving individual have re-established the culture. A silhouette of the dry spore-bearing frond, together with that of a living leaf of the next generation obtained from it, have already been shown in Fig. 145*a* and *b*. They resemble somewhat the description

of Ivancich's var. *erosa*, though the general resemblance to Milde's type specimen (Fig. 146) is equally obvious. Comparable fronds of the putative parent species, *Ceterach* and *Scolopendrium hemionitis*, both from the neighbourhood of Marseilles, are illustrated in Fig. 147.

The cytological result which has been obtained from all this material is that *S. hybridum* on the island of Lussino is a tetraploid in comparison with the normal *S. vulgare*. Owing to the vicissitudes of war and the delicacy of the plants, the demonstration of chromosome number lacks the elegance which is attainable with the squash techniques, but the difference in size of the metaphase plates at meiosis can at once be seen by comparing Fig. 148*b* and *c*. A similar comparison of mitotic cells can be made from



a *b* *c*

Fig. 148. Chromosomes of *Scolopendrium hybridum* Milde with *S. vulgare* Sm. for comparison, from sections. × 1000. *a*. Root of *S. hybridum* forma *typica* from Trieste Botanic Garden. $2n = 144$. *b*. Meiosis in the plant of Fig. 145. $n = c.$ 70 (probably 72). *c*. Meiosis in *S. vulgare* for comparison. $n = 36$ (cf. Chapter 7).

Fig. 148*a*. Final accuracy is unfortunately not readily obtainable from sections, but the approximate results for *S. hybridum* are all of the order of 140 chromosomes for roots and *c*. 70 at meiosis. Since the exact chromosome number of *S. vulgare* is known to be $n = 36$ (Chapter 7), the exact gametic number for *S. hybridum* is almost certainly $n = 72$.

The detection of tetraploidy makes a hybrid origin for *S. hybridum* more rather than less probable, though the parentage is by no means self-evident. *Ceterach officinarum*, the only European species of that genus, is also a tetraploid (cf. Chapter 6) with $n = 72$, both in Britain and in France, and any direct hybrid with *Scolopendrium vulgare* would be triploid at first and hexaploid after chromosome doubling. With regard to *S. hemionitis*, however, there has hitherto been no cytological information.

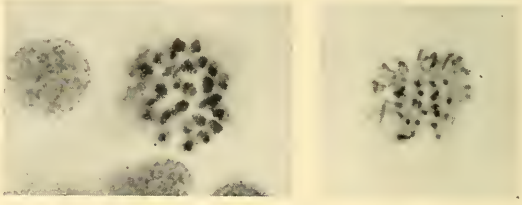
After many years of fruitless efforts to obtain living material of *S. hemionitis* I was fortunate in 1946 to be able to visit the Mediterranean coast near Marseilles and to collect some spores and adult plants of this species. A leaf of a small plant at the time of collection is reproduced in Fig. 147*b*, with a larger leaf produced from the same plant two years later in a warm house at Kew in Fig. 149. The apparent fimbriations of the margin in the latter specimen are due to undulations of the leaf lamina produced, no doubt, by the somewhat abnormal growing conditions. The very characteristic shape of the auricles is, however, fully displayed in both specimens, and both have also the fleshy texture characteristic of this rather uncommon species.

The chromosomes of *S. hemionitis* are illustrated in Figs. 150 and 151. Fig. 150*b* shows mitosis in a root with *c*. 70 (actually no doubt 72) chromosomes. Figs. 150*a* and

151 represent meiosis in which the number is quite certainly $n = 36$. *S. hemionitis*, like *S. vulgare*, is therefore a diploid species, and both contrast equally strongly with *S. hybridum*.

Fig. 149. *Scolopendrium hemionitis* Lag., Garcia & Clem. Living leaf of the same plant as Fig. 147*b*, grown in cultivation showing characteristic auricles. Natural size. For description see text.

At this point it is greatly to be regretted that the programme of experimental work planned for this species but interrupted by the war has not yet been resumed. The



a *b*

Fig. 150. Chromosomes of *Scolopendrium hemionitis* Lag., Garcia & Clem., from sections. × 1000. *a*. Meiosis. $n=36$. For explanatory diagram see Fig. 151. *b*. Mitosis in a root. $2n=72$. For comparison with *S. hybridum* see Fig. 148 *a*.

S hemionitis $n=36$

Fig. 151. Explanatory diagram to Fig. 150 *a*. × 1500.

origin of *S. hybridum* is therefore still uncertain, and, with the experience of *Dryopteris remota* and *Asplenium germanicum* in our minds it would be unwise to predict a conclusion before the evidence is fully assembled. We have three suggested parents to consider. The two species of *Scolopendrium* are both suitable in chromosome number, but whether either is actually related can only be determined by crossing *S. hybridum* with each and examining chromosome pairing in the triploids so obtained. There remains, however,

Ceterach. This cannot lightly be dismissed, even though an intergeneric hybrid seems at first sight improbable and the chromosome number hitherto found ($n = 72$) is too high. We cannot, however, assume that a form of *Ceterach* with a lower chromosome number may not exist, and the fact that if the known number were halved it would be identical with that of diploid *Scolopendrium* may indicate a closer relationship than one might otherwise have expected to find. Further investigation of this problem is therefore very much to be desired.

The second case to be discussed, that of *Woodsia*, came to my notice almost accidentally during the summer of 1948, and a full investigation has been impossible to carry

Fig. 152. Hybrid *Woodsia*. Series of leaves from the island of Runmarö near Stockholm, each from a different plant, grown in cultivation. Natural size. For description see text.

out before going to press. Even in a preliminary form, however, the results are so surprising to a British botanist that reference to them seems profitable.

The extreme rarity and alpine associations of *W. ilvensis* and *W. alpina* in Britain have already been commented upon in Chapter 7, and it is therefore at first an unfamiliar experience to find both species growing, often in great profusion, at sea-level in Scandinavia. Once this experience has been gained it is perhaps not so surprising to learn that both in Sweden, Norway and also in Alaska (Hultén, 1941) both species can sometimes be found together and that in some of these localities hybrids are formed. One of the best known of such places is the island of Runmarö in the archipelago near Stockholm which was described in detail by Qvarfort in the *Svensk botanisk Tidskrift* for 1931 (vol. xxv, p. 36). I was not able myself to visit the island, since in 1948 it was still included in

a military defence area forbidden to foreigners. At my request, however, Professor Halle of Stockholms Riksmuseet very kindly undertook to send out two members of the museum staff (G. Haglund and R. Rydberg), who visited the island on my behalf and brought back a very large and varied collection of living plants. A selection of these was dispatched by

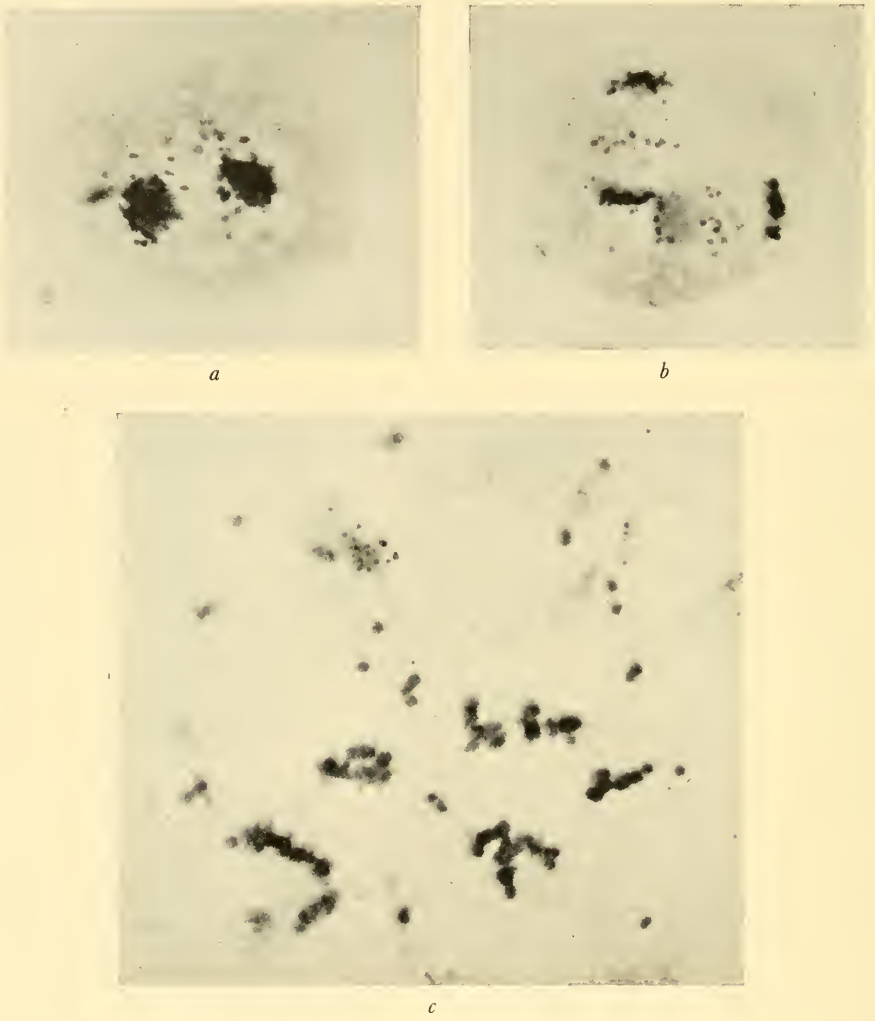


Fig. 153. Meiosis in hybrid *Woodsia*, permanent acetocarmine preparations. *a*. Anaphase of first meiotic division from the leaf of Fig. 152*b*, showing lagging univalents. × 1000. *b*. Anaphase of second meiotic division showing lagging halves of univalents, from the leaf of Fig. 152*c*. × 1000. *c*. First meiotic metaphase from the leaf of Fig. 152*d*. × 1500. For explanatory diagram see Fig. 154.

air to Kew where they throve amazingly, and one month after arrival there, namely, at the end of August 1948, a new crop of fronds had been put up on every one, from which fixations and the following information were obtained.

Fig. 152 shows the range of leaves represented in this collection. If comparison is made with Fig. 107, p. 110, it will be clear that the leaf of Fig. 152*a* is most like that of

W. ilvensis though somewhat coarse and flaccid, as greenhouse fronds are apt to be. Fig. 152*e* is the most *alpina*-like leaf, and Figs. 152*b–d* are of the intermediate type which suggests hybridity.

Fixations from all these leaves and from some others were made, and though none was sufficiently perfect to establish the chromosome numbers in full detail had the genus been unknown, the information presented in Chapter 7 is sufficient to make them interpretable. The plant of Fig. 152*a* had a reduced chromosome number of approximately 40 and few if any unpaired chromosomes; it therefore resembles *W. ilvensis* in

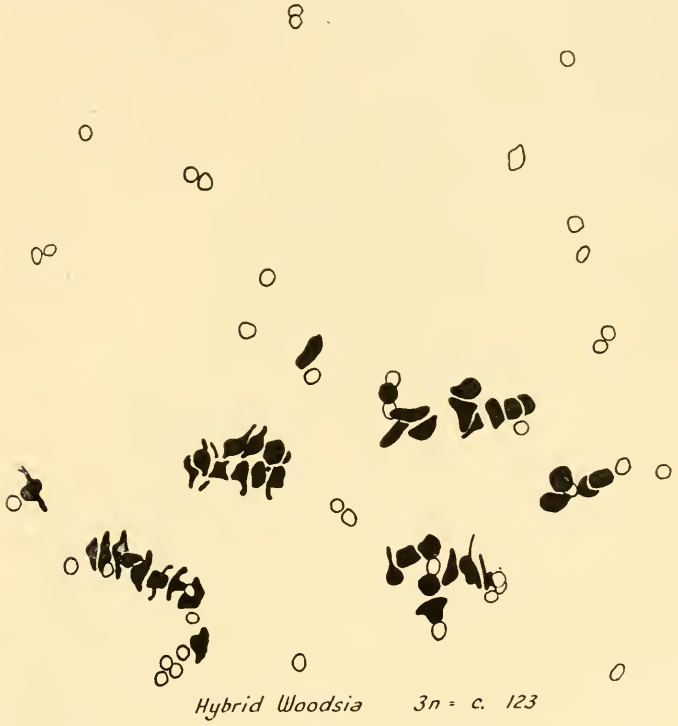


Fig. 154. Explanatory diagram to Fig. 153*c*, probable univalents in outline, probable pairs in black. ×2000.

Britain. The plant of Fig. 152*e* had no unpaired chromosomes and approximately 80 bivalents at diakinesis; it therefore approximates to and is probably identical with the normal state of *W. alpina* in Britain for which $n = c.$ 82. In each of the plants of Figs. 152 *b–d* on the other hand numerous unpaired chromosomes were present. Some characteristic anaphase figures showing lagging univalents at both meiotic divisions are reproduced in Fig. 153*a* and *b*, while Fig. 153*c* gives a polar view of a spread first meiotic metaphase, in which an approximate though not an exact count can be made. An explanatory diagram is given in Fig. 154, which may perhaps help the reader to distinguish between pairs and univalents. In some parts of the figure the groups are too closely crowded to be fully analysed, but there is no doubt that pairs and univalents are present in almost equal numbers and that the approximate number for each is of the order of 40. Since we know from Chapter 7 that, in *Woodsia*, $n = c.$ 41 this

Fig. 155. *Polystichum illyricum* Hahne, living fertile leaf from Switzerland grown in cultivation, somewhat depauperate. Natural size.

is sufficient demonstration that some at least of the putative hybrids are triploids, as they should be if the interpretation of their nature has been correct.

The relevance of this to our general field of inquiry is close. In spite of the imperfection of the cytology at this preliminary stage we are undoubtedly seeing a type of behaviour which has much in common with the Male Fern story of Chapter 4. As in the case of the artificial hybrid between *Dryopteris abbreviata* and *D. Filix-mas*, we have a triploid in which two gametic sets of chromosomes have paired with each other and one has remained unpaired. In the case of *Woodsia* the gametic sets which have paired seem necessarily to be those of *W. ilvensis*, and we therefore reach the somewhat surprising conclusion that the diploid species *W. ilvensis* must be part-parental to the tetraploid species *W. alpina* in the same sense that *Dryopteris abbreviata* has shown itself to be part-parental to the Male Fern.

Woodsia alpina seems therefore to be another member of the British flora for which a hybrid origin must be assumed, though, as in the case of the Male Fern, we know one parent but not the other. That *W. glabella*, the third European species, may perhaps be the other parent of *W. alpina* is quite possible though the fact can only be determined by experiment. This problem also must therefore be left undecided until some future occasion.

The last case to be discussed in this chapter is that of *Polystichum illyricum* Hahne and related forms. This is the name given to the putative hybrid between *P. aculeatum* and *P. Lonchitis*. As already pointed out in Chapter 6 these two species in Britain occupy rather different habitats, the Holly Fern, *P. Lonchitis*, being almost always an alpine while *P. aculeatum* is a lowland or at most a montane rock plant. Under most normal conditions they do not therefore encounter one another; occasionally, however, this occurs. In Britain the only locality known to me where this happens is in the limestone pavement of the Craven area in the northern Pennines, and comparable admixture has been described from time to time from various parts of the Continent. Only in such places is *P. illyricum* to be found. It is represented by single individuals (as opposed to homogeneous populations), which betray their hybrid nature both by the possession of morphological characters (cf. Fig. 155) intermediate between those of the putative parents and also by marked spore sterility.

The material of *P. illyricum* available to me was obtained on each of two visits made in 1937 and 1947 respectively to one of the classic localities for this particular mixture of species, namely, that adjacent to the alpine garden at Pont-de-Nant above Bex in the Rhone Valley in Switzerland. I am greatly indebted to the authorities of the University of Lausanne for facilitating both visits.

The locality in question is a *Picea* wood on the south-facing slopes of a tributary valley at an altitude of 4100 ft. The soil is calcareous and the floor of the wood is composed of gigantic boulders, partly moss covered, in the cracks under which *Polystichum Lonchitis*, *P. aculeatum* (*P. lobatum*), together with putative hybrids, grow in great profusion and in very close proximity to each other. Some characteristic old plants of all three types had been transferred to the alpine garden some years before my visit, and fixings of sporangia were made on all of these. Other plants of all three types were collected on both occasions and posted alive to England for further study.

Investigation of the purest forms of the parent species in the alpine garden showed that the cytological nature of these was exactly as in England (cf. p. 92), *P. Lonchitis* being a diploid species and *P. aculeatum* tetraploid. This may perhaps be sufficiently demonstrated by Fig. 157*a* and *b*, which are from the original fixings made on the journey in 1937. These fixings are very inferior in quality to those otherwise obtainable, but as an addition to what has previously been given in Chapter 6, they will perhaps serve as a rough demonstration that the two species on the Continent, as in Britain, differ in chromosome number. This circumstance is a very fortunate one, since a simple root-tip count should confirm or refute the correctness of the diagnosis

Fig. 156. Series of pinnae from dried wild fronds of different adult plants from one locality (Les Plans sur Bex, Switzerland) to show range of form of hybrid *Polystichum*. *a*. *P. Lonchitis* (L.) Roth. *b*, *c*. *P. illyricum* Hahne. *d*. *P. aculeatum* (L.) Roth, (=*P. lobatum* (Huds.) Woynar).

of the putative hybrid, for *P. illyricum*, if it really is the interspecific cross between *P. Lonchitis* and *P. aculeatum*, ought to be a triploid with 123 chromosomes in its roots.

Among the plants suspected to be *P. illyricum* on morphological grounds three were shown to be triploid by root-tip counts. It had, however, been at once apparent from scrutiny of the living populations before collection that a considerable range of morphological types grading between the pure forms of the putative parents could be found, and since a number of these had also been sent to England it was not surprising to find that not all were actually triploid. In the 1937 visit one suspected hybrid, rather closer than the average to *P. aculeatum*, turned out to be a tetraploid, while on the 1947 visit two plants rather closer to *P. Lonchitis* than usual, though with more deeply cut-up pinnae, proved on examination to be diploid or nearly so. Some examples of the range of pinnae found on the 1947 visit are shown in silhouette in Fig. 156, in each case the pinnae chosen being from large fertile fronds. The parental types, together with diploid and triploid putative hybrids, are represented, and they obviously make an almost continuous morphological series.

The interpretation of this series is fairly straightforward. The presence of the triploids is in itself sufficient evidence for the correctness of the original diagnosis of the cross. The presence of the others seems therefore necessarily to mean that this hybrid when first formed is not completely sterile. There is no direct evidence of the nature of the descendants which would be produced, but the behaviour of autotriploid *Osmunda* is a helpful indication. If the expected proportion of balanced spores with the even polyploid numbers can be formed, they would certainly be viable and the reversion to the parental chromosome numbers would therefore not be surprising. That some signs of hybridity still persist in such progeny would merely seem to imply that some extra chromosomes belonging to one or other species are still present or that a measure of gene exchange can occur between the chromosomes of the two species.

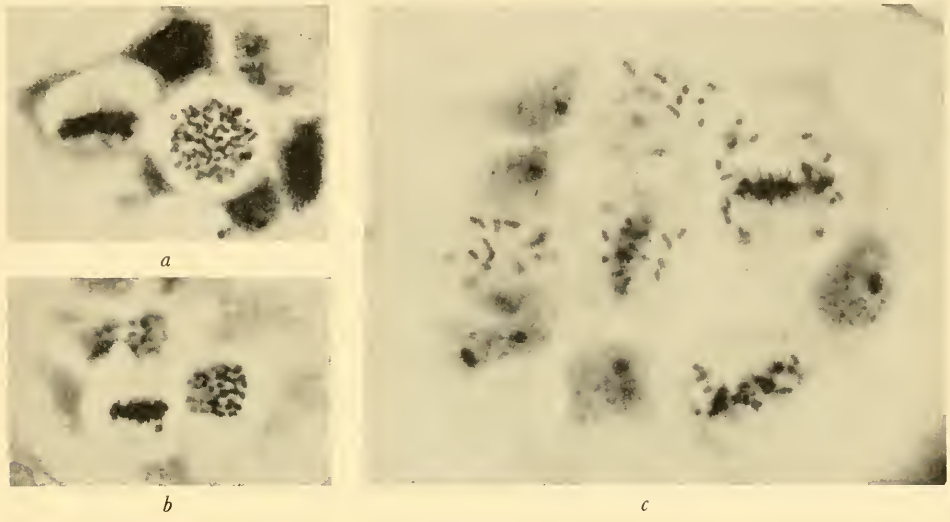
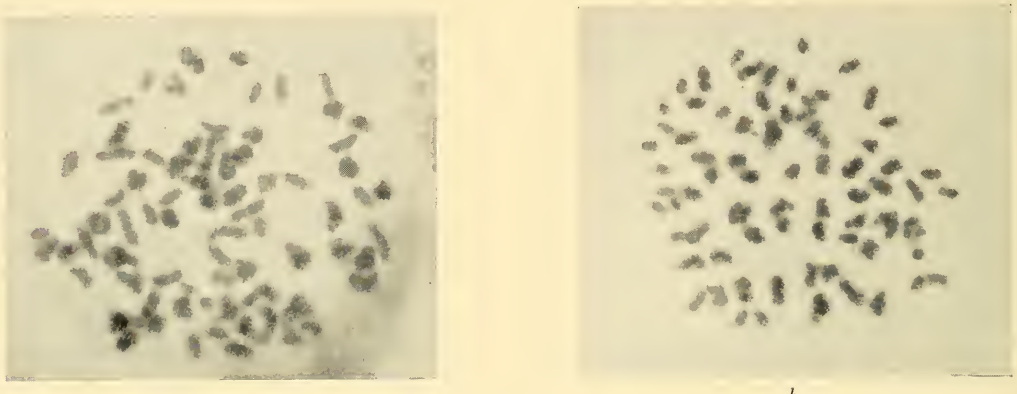


Fig. 157. Meiosis in *Polystichum illyricum* Hahne and its parents, from sections. *a*. *P. aculeatum* (L.) Roth, wild fixation. × 750. *b*. *P. Lonchitis* (L.) Roth, wild fixation. × 750. *c*. *P. illyricum* Hahne, grown in cultivation, good fixation. × 1500.

Be that as it may, an important taxonomic deduction follows from the study of meiosis in the triploid plants. The evidence on this is presented in Figs. 157*c*, 158 and 159. Fig. 157*c* shows meiosis in a triploid plant from the 1937 collection which had been sent to England and grown on; exactly comparable results were also obtained in that year from a hybrid plant fixed in the garden at Pont-de-Nant. Paired and unpaired chromosomes are present in both in almost equal abundance, and the formation of trivalents is so inconspicuous as to be negligible. More completely analysable evidence is presented in Figs. 158 and 159 from acetocarmine preparations from one of the 1947 triploids which had been sent to England and fixed the following year. This does not resemble autotriploid *Osmunda*, but again agrees very closely with the triploid hybrid between *Dryopteris Filix-mas* and *D. abbreviata*. The conclusion which appears necessarily to follow from this is that *Polystichum aculeatum* and *P. Lonchitis* are related in a way comparable to *Dryopteris Filix-mas* and *D. abbreviata*, namely, that *Polystichum aculeatum* is an allotetraploid and *P. Lonchitis* is one of its parents.

This was somewhat unexpected, and the problem of the other parent is immediately raised. As far as the British flora is concerned there is here little choice, since the only other species is *P. angulare* (Kitaib.) Presl (*P. setiferum* (Forsk.) Woynar*), which, as already explained (cf. p. 90), was for many years regarded as co-specific with *P. aculeatum* and which certainly resembles it more closely in the adult state than does *P. Lonchitis*.



a *b*

Fig. 158. Meiosis in *Polystichum illyricum* Hahne, permanent acetocarmine. × 1000. *a*. Diakinesis with approximately 41 pairs and 41 univalents. For explanatory diagram see Fig. 159. *b*. First meiotic metaphase showing total number of approximately 82 bodies.

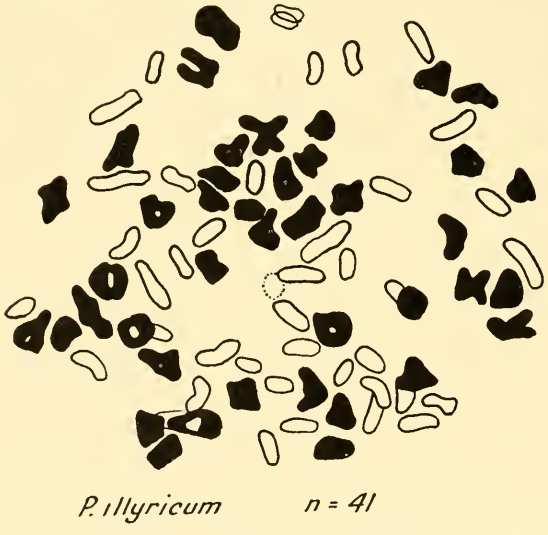


Fig. 159. Explanatory diagram to Fig. 158*a*. × 1500. Pairs in black, univalents in outline.

To test this idea the attempt was made to hybridize *P. aculeatum* with *P. angulare*. As parental stocks *P. aculeatum* from north Italy was used as a source of archegonia and *P. angulare* from Dartmouth as a source of sperm. The hybrid (Fig. 160) proved easy to

* According to the International Rules the valid name for this species is *P. setiferum*. The retention of the older name of *P. angulare* for the purposes of this chapter is merely a temporary expedient to maintain consistency with the scheme of nomenclature indicated in the Preface.

Fig. 160. Leaf of hybrid between *Polystichum aculeatum* (L.) Roth and *P. angulare* (Kitaib.) Presl for comparison with *P. illyricum* Hahne (Fig. 155) and the parent species (Figs. 76 and 77, Chapter 6).

make and a number of examples were attested as such by a triploid chromosome count. In appearance they resemble pure *P. aculeatum* so closely that their hybrid nature might escape detection in a herbarium specimen unless the spores were examined and seen to be abortive. Meiosis is, however, characteristic. The first sporangia were produced in 1947, and the plants were fully fertile in 1948. Figs. 161 and 162 show a sample cell. As expected, pairing closely resembles that of the other triploid. In the figured cell there appear to be 41 pairs and 40 identifiable univalents. Since $n = 41$ this is sufficiently close to expectation to confirm the suggested close relationship of *P. angulare* with *P. aculeatum*.

If this conclusion is correct it ought therefore to be possible to resynthesize *P. aculeatum* by crossing *P. angulare* with *P. Lonchitis* followed by colchicine treatment. It is hoped

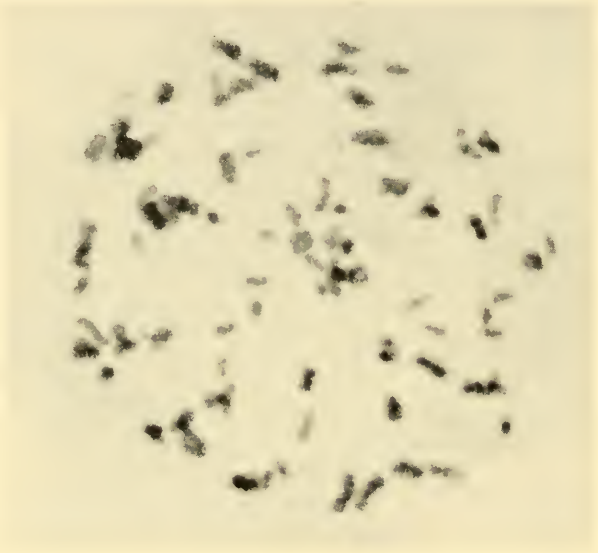


Fig. 161. Meiosis in the triploid hybrid between *Polystichum aculeatum* (L.) Roth and *P. angulare* (Kitaib.) Presl (Fig. 160), permanent acetocarmine. × 1500. For explanatory diagram see Fig. 162.

that this will indeed be done, and that the resulting plant will in fact resemble *P. aculeatum*. If it does not it would be necessary to look at other diploid species of the genus sufficiently close to the two in question to be likely to have chromosomes homologous with these. To pursue this matter further at the moment, however, is impossible, since even the synthesis from known parents would take at least 5 years to carry out and test. The importance of *P. illyricum* in a general inquiry such as this is, however, obvious. It has provided a clue to the genesis of yet one more polyploid hybrid species, and by doing so has completed the analysis of a common British plant in terms not of suspected relationship or certain affinity on one side only, as in the case of the Male Fern and *Woodsia*, but with very strong probability indeed with respect to both the parents of the hybrid species. This degree of completeness is a very welcome addition to the analyses already discussed in this and previous chapters.

Fig. 162. Explanatory diagram to Fig. 161. ×2000. Pairs in black, univalents in outline. One univalent has not been identified.

SUMMARY

Three non-British wild hybrid ferns have been described.

(1) *Scolopendrium hybridum* Milde, endemic to some Adriatic islands, is shown to be a tetraploid. One possible parent, the south European *S. hemionitis* Lag., Garcia & Clem., is shown to be diploid as expected, but nothing is yet directly known about the relationship.

(2) *Woodsia.* Some Swedish hybrids between *W. ilvensis* (L.) R.Br. and *W. alpina* (Bolton) S. F. Gray have been shown to be triploid and to have chromosome pairing of a type which suggests that the diploid species, *W. ilvensis*, is part-parental to the tetraploid *W. alpina.*

(3) Two collections of *Polystichum illyricum* Hahne from Switzerland have been shown to contain triploids which indicate by their chromosome pairing that *P. Lonchitis* (L.) Roth is part-parental to *P. aculeatum* (L.) Roth. The other possible parent of *P. aculeatum* is thought to be *P. angulare* (Kitaib.) Presl (*P. setiferum* (Forsk.) Woynar), and this is confirmed by chromosome behaviour in triploid hybrids between these two species. The synthesis of the tetraploid *P. aculeatum* from a hybrid between *P. Lonchitis* and *P. angulare* is therefore awaited.

CHAPTER 10

APOGAMOUS FERNS. THE GENERAL PHENOMENON

It may now perhaps be appropriate to consider a little more closely some of the remarkable cytological peculiarities accompanying obligate apogamy in the Polypodiaceous ferns. Some examples of this type of life history have already been introduced incidentally when describing *Dryopteris Borreri*, *Phegopteris* and others, but the peculiarity is not confined to members of the Dryopteroid affinity. It has been met with from time to time in widely scattered genera, in each of which it must have arisen *de novo*, yet the main characteristics wherever they occur are so similar that all the known cases may profitably be dealt with together.

The characteristics of this particular type of life history are that in spite of the existence of normal-looking and fully functional spores, the prothalli which develop from them are devoid of archegonia though antheridia may be, and usually are, present in abundance. A sexual fusion takes no part in the initiation of the new sporophyte which is formed directly from the central tissue of the prothallus at a stage of its development which, in a sexual gametophyte, would just precede the thickening of the central cushion. The first leaf of the new sporophyte is in most cases of a more adult type than is the first leaf of a sexually produced young plant, but otherwise the only additional external difference that can easily be detected is that the sporangia from which the functional spores are derived contain 32 spores instead of the customary 64.

A list of species in which this type of life history is known to me is as follows:

Pteris cretica L.
Cyrtomium falcatum Presl
C. Fortunei J.Sm. = '*Aspidium falcatum*'
C. caryotideum (Wall.) Presl
Dryopteris Borreri Newm. = '*Nephrodium pseudomas*' and also many varieties referred to '*D. Filix-mas*'
D. remota (A.Br.) Hayek
D. atrata (Wall.) Ching = '*Nephrodium hirtipes*'
Phegopteris polypodioides Fée
Pellaea atropurpurea (L.) Link
Asplenium monanthes L.

All of these species have been available to me for study, and though the list will certainly be found to be incomplete with regard to ferns as a whole, most of which have never been examined from this point of view, the series is sufficiently wide to be a fair sample of the general phenomenon. That many species should be reviewed by one person is perhaps of importance in that a number of incomplete accounts of single species have been produced at various times since the discovery of apogamy by Farlow

and de Bary at the end of the last century, and the available literature on the subject is in a rather unsatisfactory state. From the cytological point of view there is, in my experience, only one existing account which can be accepted as adequate, namely, that by Döpp (1932) on *Dryopteris remota*. Other well-known and much-quoted papers, e.g, Farmer and Digby (1907) on '*Nephrodium pseudomas* vars. *polydactyla*' Wills and Dadds, Allen (1914) on '*Aspidium falcatum*', Steil (1919) on '*Nephrodium hirtipes*', while containing some correct observations are so seriously incomplete as to be actively misleading.

The central cytological problem posed by all these plants is that of reconciling the absence of a sexual nuclear fusion with the presence of an apparently normal meiotic process in the development of the spores. It is obvious that some compensating process must exist at some point in the life cycle to stabilize chromosome numbers and to prevent the progressive diminution which a repeated succession of meioses would otherwise very rapidly bring about.

Some authors have looked for this compensating process in the gametophyte, and Farmer and Digby (1907) in particular believed that they had found it when they detected nuclear migrations in the vegetative cells of some of the prothalli of '*Nephrodium pseudomas* (= *Dryopteris Borreri*) var. *polydactyla*', and interpreted this as 'pseudo-fertilization'. The observational evidence for the existence, under certain circumstances, of nuclear migrations may be accepted as valid, but no demonstration was offered of either nuclear fusion or the origin of sporophytic tissue from cells with a higher chromosome number than the rest of the prothallus. At that date, indeed, it is doubtful whether cytological technique was sufficiently advanced to permit of a numerical demonstration of this kind, and certainly these authors, having examined meiosis, were unable to detect any numerical or other peculiarity about the process. Since in fact the vars. '*polydactyla*' differ in no way from the other cases, in all of which, as will shortly be seen, the compensating process looked for is in the sporangium, it is to be hoped that 'pseudo-fertilization' after passing as a fact for a quarter of a century will shortly cease to be quoted.

All subsequent authors have correctly concluded that the process which compensates for the apparently normal meiosis is to be looked for in the sporangium. That the process itself was not at once discovered may perhaps be explained in part by the unexpected complexity of the sporangial development, and also probably in part by the fact that all the earlier workers appear to have been consciously looking for a pseudosexual process and were therefore predisposed to find it, in spite of incompleteness, now obvious, in the evidence before them.

The first serious investigation of sporangial development in an apogamous fern was by Allen in 1914 on '*Aspidium falcatum*', now more correctly known as *Cyrtomium falcatum*. She observed, correctly, that sporangia could be found with eight spore mother cells and with sixteen spore mother cells, and from this she concluded prematurely that one type turned into the other by fusion of the mother cells in pairs, a view which was apparently supported by the detection of occasional intermediate stages. Allen's evidence on the chromosome numbers was very seriously defective, as will be shown below, but she deduced, correctly, that the meiotic process, though normal in

appearance, nevertheless produced spores with the same chromosome number as the parent plant and that sporophyte and gametophyte were identical in nuclear content.

The correct interpretation of Allen's intermediate fusion stages was first given by Steil in 1919 working on '*Nephrodium hirtipes*' now known as *Dryopteris atrata*. Steil's work was favoured by the fact that in *Dryopteris atrata* these 'intermediates' are unusually abundant, being, in my experience, more numerous in this species than any other type of sporangium. At first, in a preliminary note, Steil accepted Miss Allen's view that these were signs of nuclear fusion, but in his fuller account (1919) he realized that they were better interpreted as signs of incomplete division, and that the true nature of the compensation process was an incomplete nuclear division immediately preceding meiosis, by means of which the nuclear content of the spore mother cells is momentarily doubled.

Steil's account is incomplete in that he did not fully realize the extent of the variation in possible sporangial developments within one sorus, which is indeed less obvious in *D. atrata* than in other species, and this is where Döpp's account of *D. remota* (1932) is greatly to be preferred. While authenticating the reality of an incomplete nuclear division immediately preceding meiosis as the basic abnormality without which the continued reproduction of the species would be impossible, Döpp showed clearly that the abnormality is only effectively accomplished in a proportion of sporangia, the remainder being affected by differences of detail, most of which result in abortive or non-viable spores. In Döpp's account three distinct types of development were recognized, and though in most species the number can be extended to four, for most purposes his account of *D. remota* is adequate. In his last paper to appear before the war Döpp (1939) extended his observations to '*D. Filix-mas* var. *cristata* Hort.' and to the two *polydactylas*, and showed that they agreed exactly with *D. remota* in essentials.

The account which will be given here is in no sense based upon Döpp's work, but is the result of an independent investigation begun (cf. Manton, 1932) in the early 1930's and continued in the first instance in ignorance of the parallel observations being carried out in Germany. This should mean that something like finality may now be claimed for the straightforward descriptive facts, as far as these go, for Döpp's observations and those to be described below confirm and supplement each other where they relate to similar material, while the present work also extends the description to a number of additional species. Since the actual number of species to be discussed is rather high, it is proposed in the first instance to give a generalized description of the main features in which they all agree, illustrating this fully with reference to a limited number of sample types. This description will occupy the rest of this chapter, after which the separate peculiarities of individual species, together with the evolutionary analyses of all of them, will be added in the chapter which follows.

The choice of *Cyrtomium falcatum* (Fig. 163) as the main illustrative sample type has been dictated partly by historical reasons and partly for convenience. It was a fern grown extensively for the market as an ornamental plant in the neighbourhood of Manchester before the war, and unlimited supplies of material were made available to me by the kindness of Messrs Clibran of Altrincham, who, without charge, gave me free access to their nurseries. Although, as will be seen in the next chapter, I have since

supplemented this by several important samples of material of wild and botanic garden origin, the normal strain of commerce was in the first instance my principal source of information. Other species, notably *Pteris cretica*, at a later stage showed themselves to be more amenable to cytological treatment and gave better preparations more easily; yet others, e.g. *Dryopteris Borreri* and *Phegopteris*, were detected as relatively abundant sources of material in the British flora. All these advantages have been utilized in the evolutionary analyses, but the chief historical interest still lies with *Cyrtomium*. Besides

Fig. 163. Part of a frond of *Cyrtomium falcatum* (L.f.) Presl from a pressed greenhouse plant of commercial origin. Natural size.

being one of the first three apogamous ferns to be discovered (de Bary, 1878), the other two being *Pteris cretica* and *Dryopteris Filix-mas* (or *D. Borreri*) var. *cristata* Hort., the existence of Miss Allen's rather imperfect description of the cytology quoted above makes it seem of greater scientific value to emend this rather than to utilize some other species, such as *Pteris cretica*, about which no confusion is likely to arise. Before starting the description, however, it may be well to anticipate what will be said in the next chapter to the extent of explaining the change of nomenclature (Christensen, 1930) which has been introduced since Miss Allen's time. The old species '*Aspidium falcatum*', besides being relegated to a separate small genus *Cyrtomium* composed of about a dozen species, has itself been split into three, each with a different geographical area in eastern Asia and Africa. Miss Allen's material was from Wisconsin Botanic Garden and was probably *C. falcatum* proper, since this is the most commonly

grown. The other two species are *C. Fortunei* and *C. caryotideum*, both previously treated as varieties. Their separation as species does not, as it turns out, seriously affect the issue. I have had wild material of both *C. Fortunei* and *C. caryotideum* and botanic garden material of *C. falcatum* from several sources including Wisconsin. All are cytologically indistinguishable, although no doubt slightly different genetically, and therefore for the present purpose they can be used interchangeably. Illustrations of the various types of fronds will be found in the next chapter.

The early stages of sporangial development in all Polypodiaceous ferns, whether apogamous or sexual, are identical, and consist of a limited but very precise set of

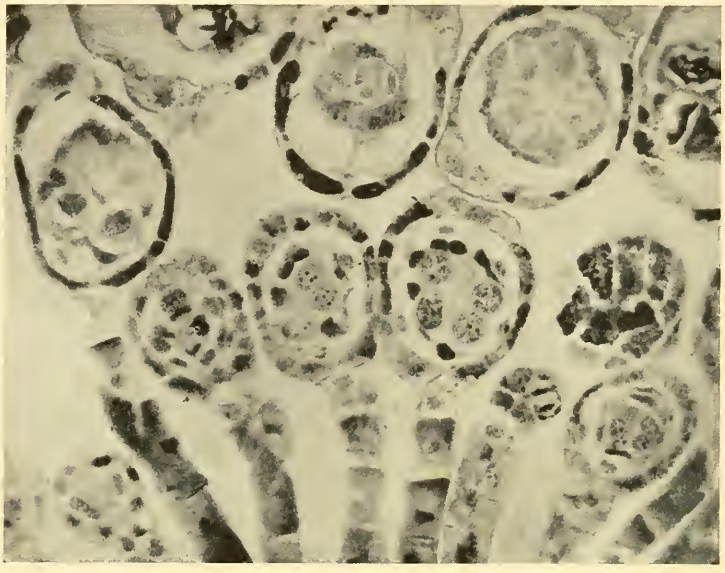
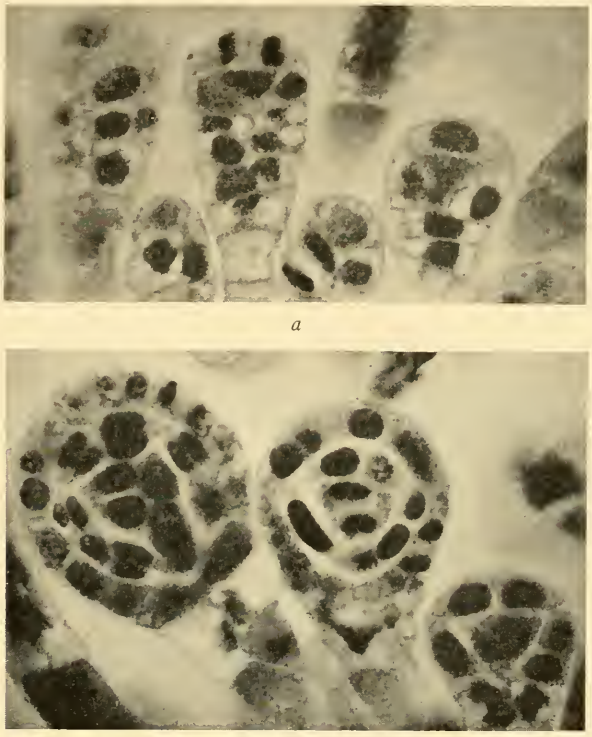


Fig. 164. Section of part of a sorus of *Cyrtomium falcatum* (L.f.) Presl to show various stages of young sporangia. × 250.

cleavages in what was originally a single superficial cell. After cutting off the stalk, which soon becomes a short filament, the terminal cell undergoes four oblique cleavages which separate the sporangium wall from a central tetrahedral cell (Fig. 165*a*). From this central cell (which appears triangular in all types of section) a further set of cleavages parallel to the four sides separates the tapetum from the archesporium (Fig. 165*b*, centre). The tapetum gives rise to two layers of cells which are nutritive in function (Fig. 165*b*, left), and the archesporium undergoes a sequence of four synchronized mitoses (cf. Fig. 166) to give a central mass of cells which, in the sexual species, are ultimately sixteen in number. These sixteen cells then enlarge considerably and begin to round up to become mother cells, each of which will give rise to four spores after undergoing meiosis. During the rounding up which accompanies the early meiotic prophases the mother cells tend to separate somewhat from each other, and the interstices between them become filled with protoplasm from the inner tapetal layer which becomes plasmodial. This is visible in many of the photographs of meiosis in section included in previous chapters. The walls of the inner tapetal cells finally disappear,

and protoplasm and nuclei from this layer closely invest the mass of mother cells until the spores are ripe. At the same time the sporangium enlarges considerably owing to increase of its layer of wall cells, so that during the act of meiosis the mass of tapetum and mother cells appears as if suspended in a cavity far too large for it and which will not be filled until the spores themselves reach their final size. Some of these stages can be seen in Fig. 164 and elsewhere in this chapter and the next.



a

b

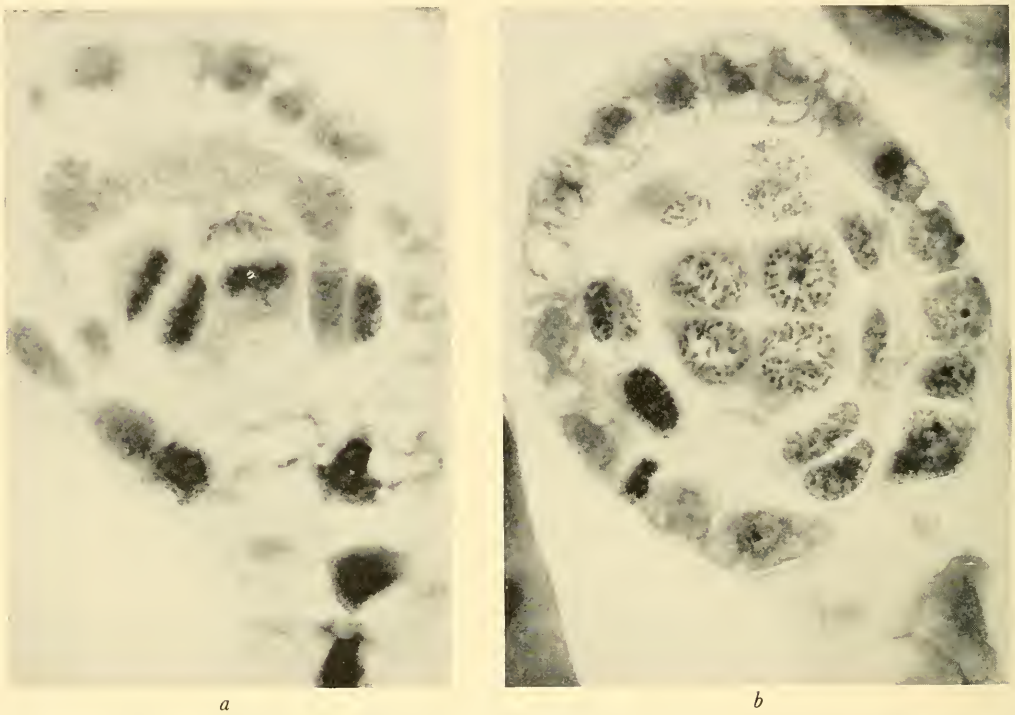
Fig. 165. Sections to show very young stages of sporangial development in *Cyrtomium falcatum* (L.f.) Presl. × 500. For description see text.

In the apogamous ferns extreme uniformity prevails over the early stages up to the first few synchronized mitoses of the archesporium (Figs. 166, 167*a*) after which one of four different things may happen.

(1) In some sporangia all four successive archesporial cleavages may be completed and sixteen spore mother cells result (Fig. 170*a*). The proportion of such sporangia varies somewhat from species to species; in some they are abundant and in others extremely rare though they never seem to be entirely absent. Sporangia of this type are of extreme interest from an evolutionary point of view for they display the true pairing homologies of the chromosomes. In most cases, however, they are of no importance for the reproduction of the species and their spores abort.

(2) The second type of sporangium is the one which is responsible for reproduction. In this the first three archesporial cleavages are normal, but the last division which

should change the eight-celled archesporium into the sixteen-celled is imperfect. Metaphase starts in each of the eight archesporial cells in the usual way, the split chromosomes taking their places on the spindle (Fig. 167*a*); but there the division ends. There is no anaphase separation of half-chromosomes and cleavage of the cytoplasm is also omitted. The mass of split chromosomes remains in the centre of the cell losing something of its regular outline (Fig. 167*b*) and then reverts to the resting state (Fig. 167*c*). As may be seen by comparison of Fig. 166*b* with Fig. 167*c* before and after this abnormal division, the number of cells present remains unaltered, four of the eight being generally contained in any one median section no matter what the plane of cutting. The nuclei



a *b*

Fig. 166. Young sporangia of *Cyrtomium falcatum* (L.f.) Presl from sections. × 1000. Showing two stages in the formation of the eight-celled archesporium, (*a*) the younger, (*b*) the older.

of the later stage are, however, distinctly larger and their shape at first less regular. In due course they become mother cells (Fig. 167*d*). Meiosis is then exceedingly regular (Fig. 168), every chromosome pairing with its sister half, and thirty-two large well-filled spores result. An accurate chromosome count made during meiosis will, however, at once show what has occurred. The number of pairs which present themselves at metaphase of the first meiotic division or at diakinesis is not half that of the single chromosomes in a root or other somatic cell of the parent plant but identical with it. A demonstration of this has already been given for *Phegopteris* (Figs. 69 and 70, Chapter 5), and it is one of the ways in which apogamy can be detected before the spores are sown. The explanation is that the abnormal premeiotic mitosis has momentarily doubled the chromosomes present and therefore meiosis merely restores the condition to

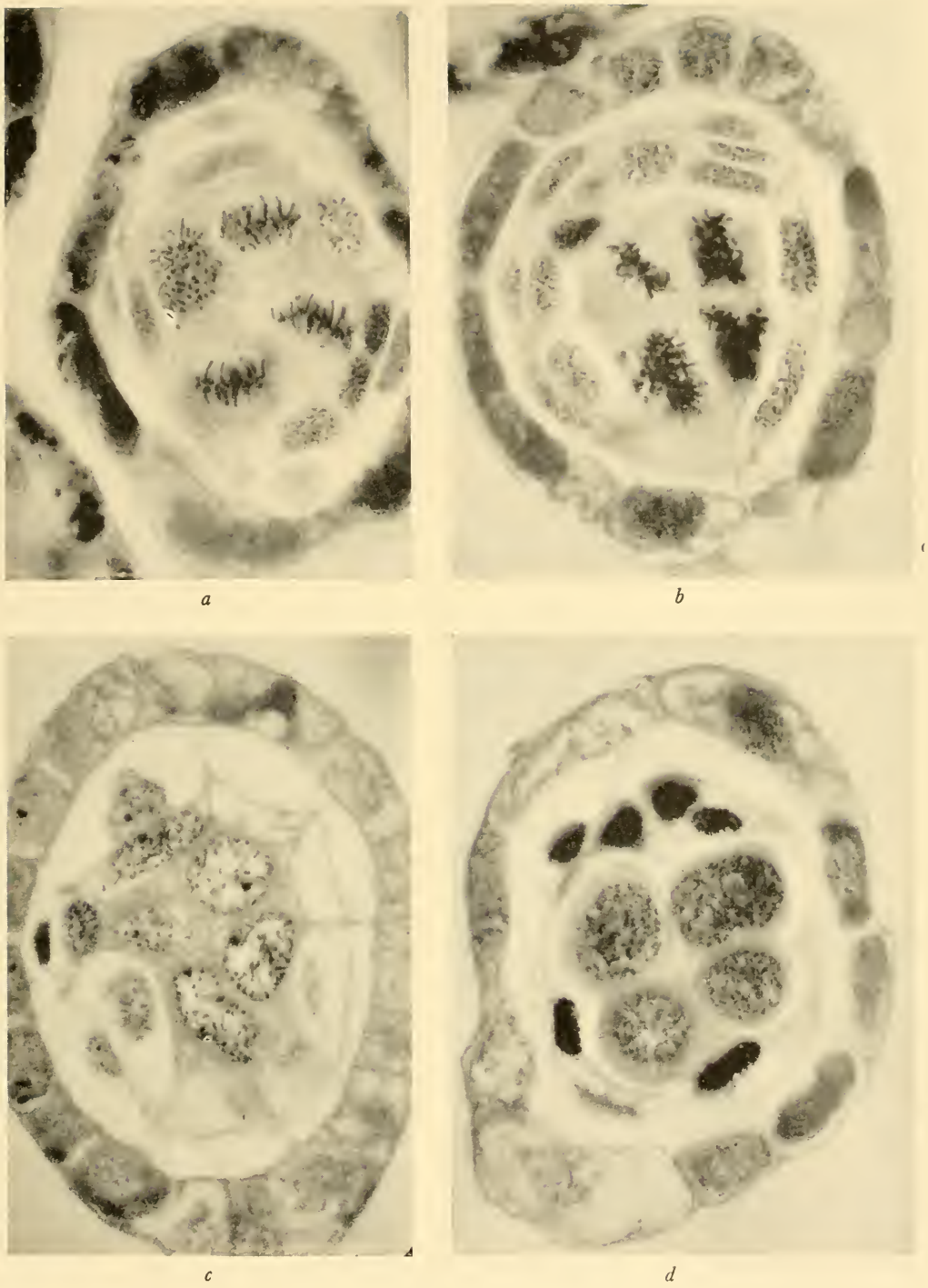
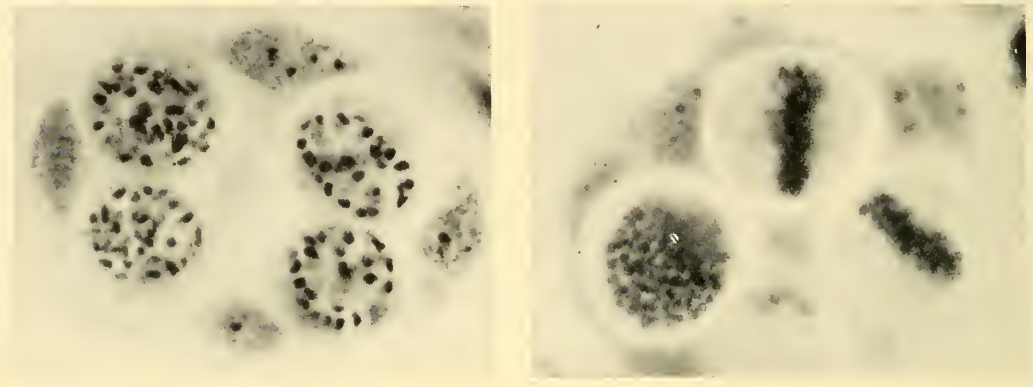


Fig. 167. *Cyrtomium falcatum* (L.f.) Presl, the abnormal mitosis in the eight-celled archesporium. In each case only four cells are visible. From sections stained with haematoxylin and Bismark brown. × 1000. *a*. The premeiotic metaphase still normal. *b*. The premeiotic 'anaphase' with the chromosome still on the equatorial plate. *c*. The premeiotic telophase. Restitution nuclei have been formed in each cell without cytoplasmic division. *d*. The beginning of meiosis. Eight (four visible) giant mother cells each with twice the previous number of chromosomes. Note signs of cytoplasmic cleavage in one of them.

that characteristic of the rest of the sporophyte, but the haploid state is not thereby attained.

(3) The third type of sporangium is a variant on that just described and is in a sense an imperfect version of it. The cytoplasmic activities which normally result in cell cleavage may not be entirely suppressed but may be present in unco-ordinated forms which affect in a very striking way the shapes and behaviour of the resulting mother cells. Sporangia of this kind are those which were interpreted as stages of nuclear migration and fusion when first seen. A superficial resemblance to such a process they undoubtedly possess. The nuclei, after loss of the spindle, may become irregularly lobed (Fig. 169*a*, *b* and *d*), cell walls partially crossing the cell may be laid down, often in relation to such lobes (cf. Fig. 169*a*, *b*), and sometimes complete cleavage into two unequal portions containing different-sized pieces of the restitution nucleus may result



a *b*

Fig. 168. Meiosis in *Cyrtomium falcatum* (L.f.) Presl from sections. × 1000.
a. Diakinesis. *b*. The first metaphase.

(Fig. 169*c*, *f*, *g*). That the cleavage, in the last event, involves a passive amitotic constriction of a restitution nucleus and not a mere inequality of anaphase distribution of normally separating half-chromosomes is proved by the complete regularity of chromosome pairing no matter how large or small a piece of nucleus may have been constricted off; this could not be attained by any process of random distribution of non-homologous half-chromosomes but must denote the random separation of groups of split chromosomes with their halves still in close contact. As may be seen from several of the figures quoted, which refer to a number of different species, not all the mother cells in a sporangium may be affected in this way, but only one or a few. Since the distribution of chromosomes to the constricted portions is certainly at random the nuclei so formed can hardly fail to be genetically unbalanced, and abortion of the resulting spores is therefore virtually certain.

(4) The fourth type of sporangium was not observed by Döpp, but I have come across it from time to time in almost every species. Occasionally the abnormality affecting the premeiotic division in the eight-celled sporangia may occur twice running and affect the four-celled stage also. In such cases only four giant mother cells are to be found at

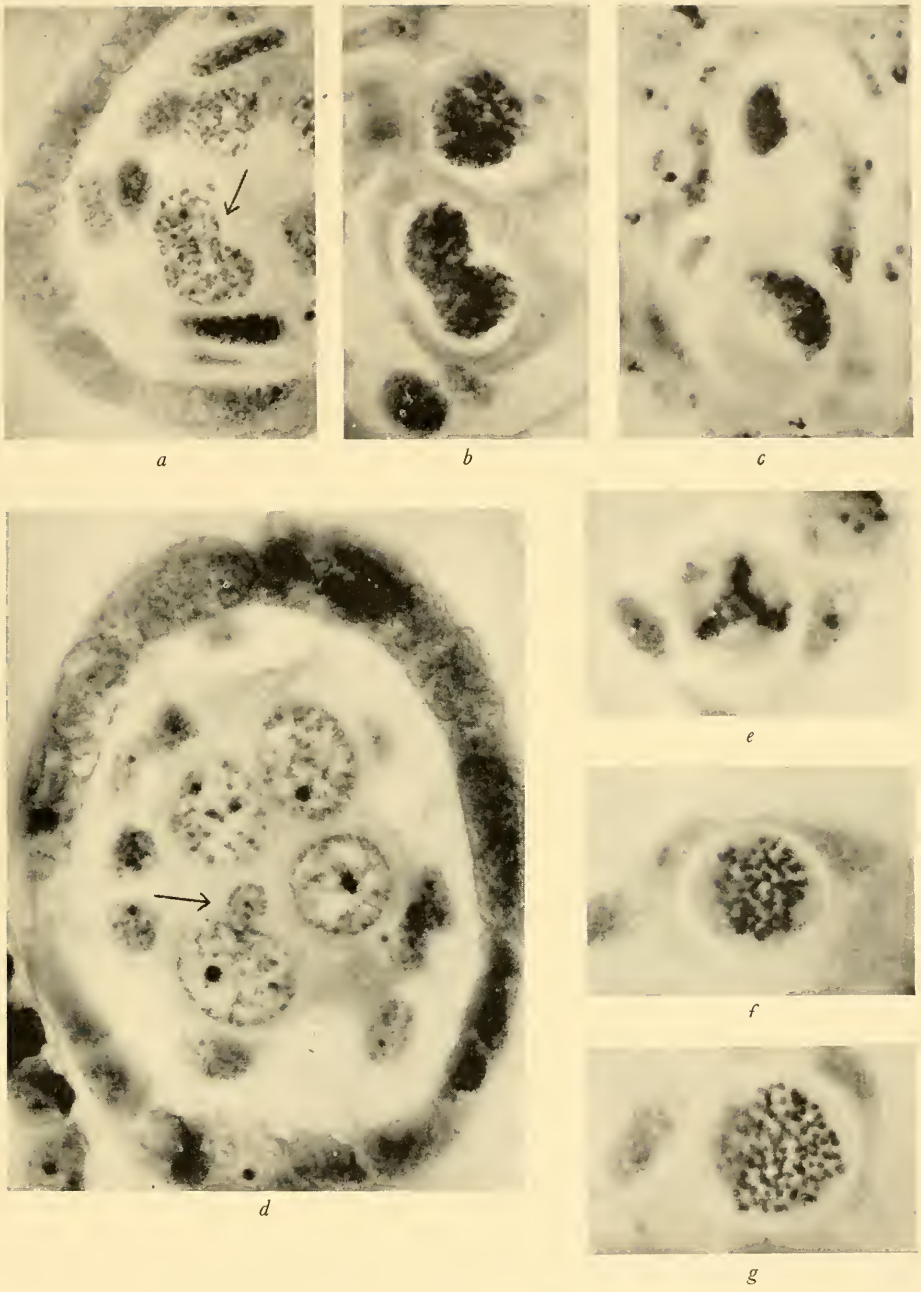


Fig. 169. Various examples of imperfect cleavage resulting from the abnormal premeiotic division (= developmental type 3, p. 166) from sections. × 1000. *a*. *Dryopteris atrata* (Wall.) Ching (*Nephrodium hirtipes* (Bl.) Hook.) showing partial cleavage of one cell. *b*. The same in *Dryopteris remota* A.Br. at early meiotic prophase. *c*. The same in *Pteris cretica* L. var. *albolineata*. A small cell has been cut off from a large one, but both are now in an early state of meiosis. *d*. *Dryopteris Borreri* var. *polydactyla* Wills. *e*. Tripolar meiotic spindle in *Pteris cretica* var. *albolineata*. *f, g*. *Dryopteris Borreri* var. *polydactyla* Dadds. Two very unequal-sized meiotic metaphase plates in the same sporangium.

meiosis, each with four times instead of twice the somatic chromosome number (cf. Fig. 69*b*, Chapter 5). In spite of their high chromosome number such mother cells are quite normal in their subsequent behaviour. It might reasonably be expected that quadrivalents would be formed, since each chromosome is certainly present in quadruplicate and all four homologues lie close together since they are quarters of the same

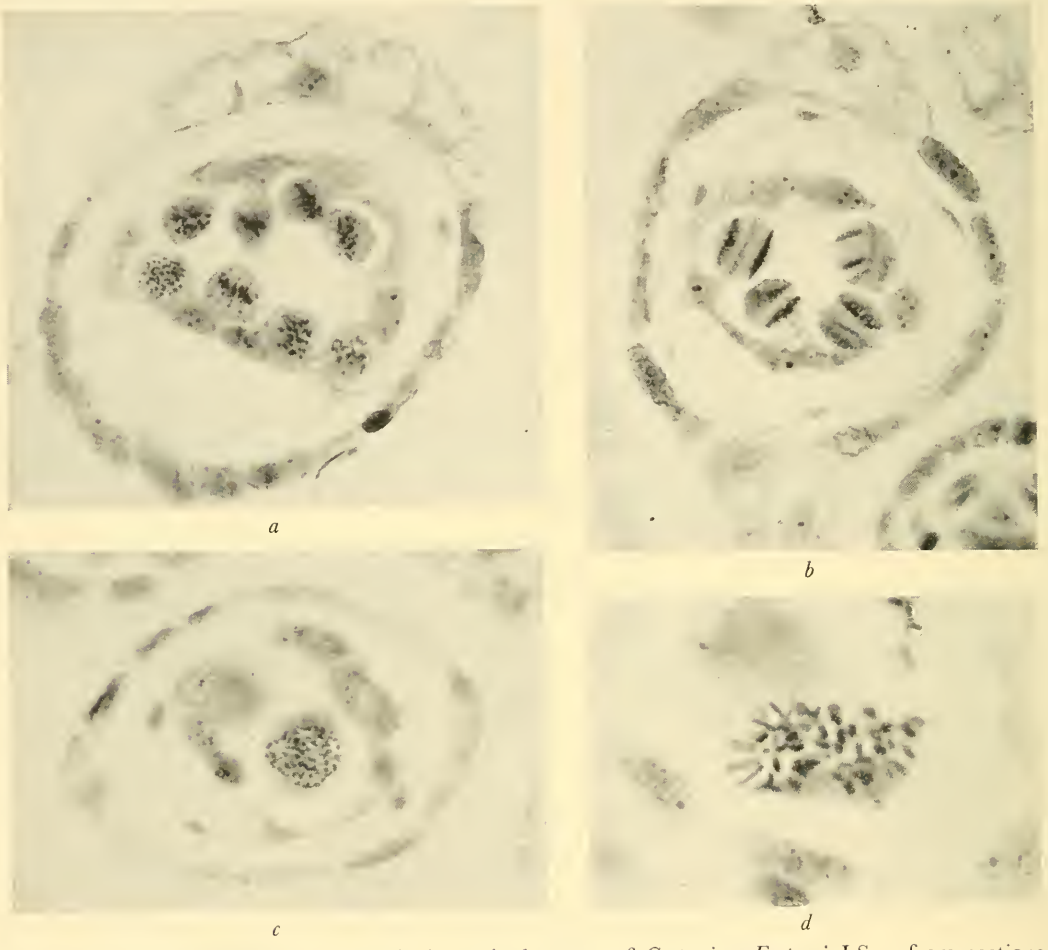
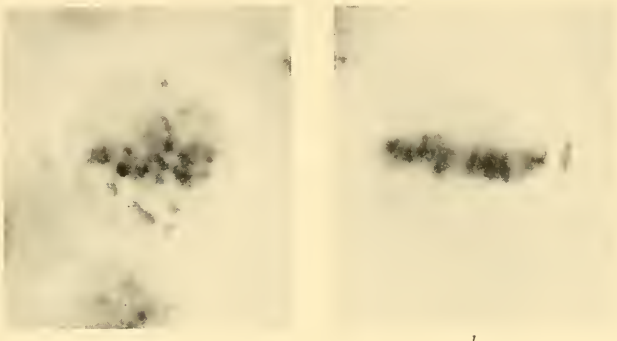


Fig. 170. Different types of sporangia in a single sorus of *Cyrtomium Fortunei* J.Sm. from sections. *a*. Sixteen-celled sporangium. × 500. *b*. Eight-celled sporangium. × 500. *c*. Four-celled sporangium. × 500. *d*. The second abnormal mitosis in a potential four-celled sporangium by means of which the chromosome number is quadrupled. × 1000.

original chromosome (Fig. 170*d*). This, however, does not occur. Pairing seems to be confined to sister chromosomes only, and the result is a meiosis which is as undistorted in all details as is that of the eight-celled sporangia. Such sporangia when ripe contain only sixteen giant spores, each with twice the nuclear content of the plant which bears them. Their subsequent fate is unknown, but it is highly probable that they may be responsible for some of the cases of polyploidy by simple chromosome doubling which have been met with in apogamous ferns, notably in *Pteris cretica* (see next chapter).

Figs. 170 and 171 summarize some of these facts for one particular sorus of *Cyrtomium Fortunei* in which sixteen-celled (Fig. 170*a*), eight-celled (Fig. 170*b*) and four-celled sporangia (Fig. 170*c*) were found in full meiosis simultaneously. This is somewhat unusual, although four-celled sporangia are sufficiently common to be listed as a separate type. Their frequency almost certainly varies with the genetical condition of the plant, but only once have they been seen in such relative abundance as to dominate the reproductive picture. This was in a monstrous form of *Dryopteris Borreri*, almost certainly a descendant of a hybrid between that species and one of its sexual relatives; but unfortunately nothing further is known about this plant except a dried frond and some cytological preparations.

It is clear that diversity of this degree between the developmental histories of the different sporangia in a sorus is a matter of great cytological, genetical and physiological



a *b*

Fig. 171. Details of chromosome pairing in two of the sporangia of Fig. 170, from sections. × 1500. *a* A sixteen-celled sporangium showing irregular pairing. *b*. An eight-celled sporangium showing very regular pairing, since the chromosome number in this has been doubled by the abnormal premeiotic division.

interest. Much further work is likely to be necessary before the causal mechanism will be understood, but a few further facts of a genetical kind will be forthcoming at the end of the next chapter, while others of the many purely cytological points of interest must be left aside for consideration elsewhere, since they are irrelevant to the evolutionary study which is the main purpose of this book.

Even in this limited context, however, the situation revealed is of unusual interest. In the life history of the apogamous ferns a very remarkable compensating process is present which is curiously difficult to equate with anything of a comparable nature in other groups of plants or animals. It is, moreover, a complicated process in which purely cytological aberrations in the sporangia must occur in the same organism as the morphological aberrations in the prothalli which result in apogamy, or the continued existence of the species would be impossible. That the somatic organization of the leptosporangiate ferns lends itself rather easily to these aberrations is shown by the relative frequency with which identical behaviour is found in isolated examples from quite unrelated genera. That the whole abnormality must have arisen in each case suddenly seems almost inescapable, since it is difficult to visualize any mechanism

which would at the same time be reproductively effective for its development by stages. For similar reasons a simple qualitative mutational mechanism seems unlikely. We seem rather to be dealing with the effects of a generalized disturbance of a quantitative kind involving several processes. We are ignorant of the chemical nature of these processes, though some further information on the mode of origin of a generalized disturbance will emerge from the next chapter.

Another point of interest to comment upon in passing is the surprising variety of sporangial life histories which all these ferns possess and to note its evolutionary consequences. If any of the spores produced by the types of sporangia described as (1), (3) and (4) on pp. 163, 166 above should prove viable, a considerable saltation in morphology and genetical constitution might result. That under certain circumstances changes of this kind do, in fact, occur, will also be shown in the next chapter.

SUMMARY

A general description of the sporangial development found in all known cases of apogamous ferns is given, with photographic illustrations principally selected from *Cyrtomium* (= *Aspidium*) *falcatum* sens. lat. There are four main types of sporangia, all of which may be found together in one sorus. These may be designated according to the number of spore mother cells undergoing meiosis as sixteen-celled, eight-celled, eight-celled with partial cleavage and four-celled. Only the eight-celled sporangia are normally effective in reproducing the species and they give rise to spores with the unreduced chromosome number.

CHAPTER 11

APOGAMOUS FERNS (*cont.*)

EVOLUTION OF THE SEPARATE SPECIES

The nature and possible mode of origin of the various apogamous species listed on p. 158 may next claim our attention. And here the reader should perhaps be warned to expect a rather extreme measure of compression in the description of each case as it arises, for only so can the results of an extended inquiry be brought within the compass of a single chapter. Each species must be analysed on its own evidence without a preconceived interpretation. But in the concise presentation of evidence it will scarcely be possible to convey anything of the charm of the plants themselves or of the many details of interest in their structure, behaviour or distribution which reward the investigator for the labour involved in piecing the evidence together. In the colourless description of species after species the impression of repetition and sameness may indeed predominate. This, it should be remarked, is, in a sense, what we are after. The differences between species give personality to them, but only the resemblances are likely to show us anything of the general principles which they all share, and the principles which we may hope to discern in this context are those which underlie the development of apogamy in ferns as a whole.

Sources of information about the evolution and origin of an apogamous species are of three, or perhaps four, kinds. The chromosome number alone may be highly informative if enough is known about the cytology of related sexual species; in the absence of such knowledge chromosome number by itself is, of course, uninformative. Of greater value is observation of the mode of pairing of the chromosomes in the sixteen-celled sporangia (type (1), p. 163 above). This can give a very great deal of information regarding the cytogenetical make-up of the plant involved, even in the complete absence of related species for comparison. Thirdly, significant differences can sometimes be detected in the relative frequency with which the various types of sporangia appear. Observations of this kind are sometimes of value (notably in *Dryopteris Borreri*) for confirming cases of suspected hybrids between an apogamous and a sexual species. Lastly, as was shown by Döpp (1939) it is sometimes possible to synthesize hybrids between apogamous and sexual species, and in such cases observation of chromosome pairing should be as informative as in other cases of hybrids of known parentage. Owing to the labour involved, very little work has yet been done using this last method, but the first two, and to a less extent the third, have given information of considerable value about almost every species.

It will be convenient to start this chapter with *Pteris cretica*, which will serve both to round off de Bary's account previously quoted and to supply us with a species more amenable than most to cytological treatment and of interest as representing a genus not of the Dryopteroid affinity.

Fig. 172. Living sterile frond of *Pteris cretica* L. var. *major* Hort. Natural size.

Pteris cretica L. is widespread and abundant in the tropics of the Old World, although, somewhat surprisingly, it has a number of outlying stations in Europe and, owing to its ease of cultivation and tolerance of the dry air of dwelling houses, it is an even greater favourite as an ornamental plant of commerce (cf. Fig. 172) than is *Cyrtomium*. Most of the considerable range of horticultural varieties have arisen in cultivation, and it is doubtful whether the genuine wild species is present at all in greenhouses at the present

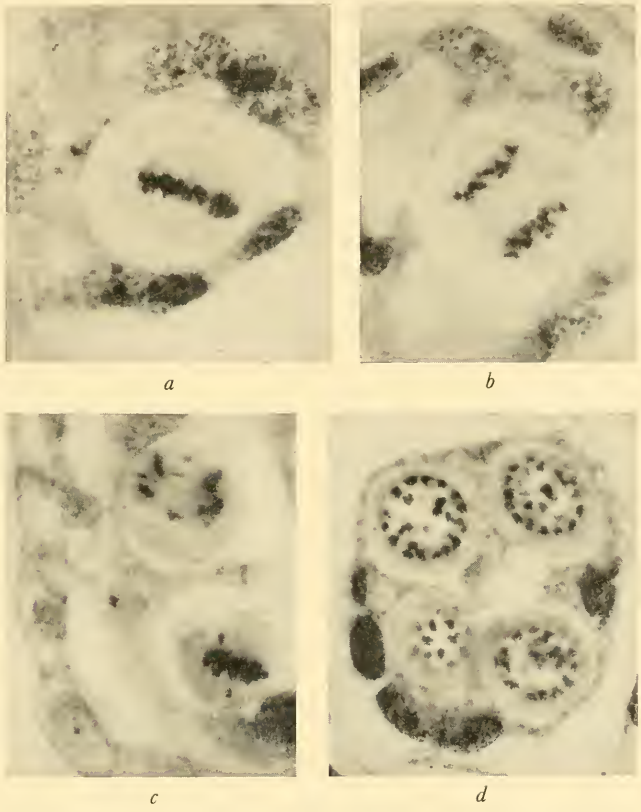


Fig. 173. Meiosis in *Pteris cretica* L. from sections stained in haematoxylin and counter-stained with Bismark brown to show unusual excellence of fixation. *a*. Diploid *P. cretica* first meiotic metaphase in an eight-celled sporangium. × 1000. *b*. The same, anaphase. *c*. The same in a sixteen-celled sporangium. Note smaller cells and lagging chromosomes at both metaphase and anaphase. *d*. Triploid *Pteris cretica* var. *albolineata*. Diakinesis in an eight-celled sporangium. × 750.

time. An exception is, perhaps, var. *albolineata*, for this was described by Hooker as a wild variety with variegated fronds native to the Far East, and comparison which I have been able to make with a reputedly wild specimen from Ceylon supplied by Peradenya Botanic Gardens showed no detectable difference of any kind between it and examples of the variety already in cultivation in England. It has, however, long been known (cf. de Litardière, 1920) that the native European species has fewer chromosomes than the morphologically normal 'var. *major*' of commerce. The numbers given by de Litardière were $2n = 60$ and 120 respectively, the low number being

obtained from Italy and from Corsica. In my experience these numbers are very nearly correct (see Fig. 174), and the wild European species may thus be regarded as diploid in contrast to 'var. *major*' which is tetraploid. All other commercial strains that I have investigated, notably var. *Wimsetti* and var. *Childsii*, are also tetraploids which is the reason for thinking that the wild type had fallen out of use.

The principal material of *Pteris cretica* used by me has been the following:

(1) The horticultural strains 'var. *major*' and 'var. *Wimsetti*' grown for the market, together with *Cyrtomium falcatum*, by Messrs Clibran of Altrincham, to whom I am extremely grateful for unrestricted access to their greenhouses.

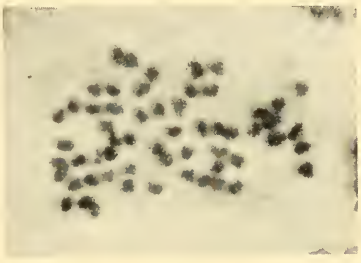


Fig. 174. Meiosis in diploid *Pteris cretica* L. permanent acetocarmine, from an eight-celled sporangium to show detailed chromosome count ('n'=58). × 1000.

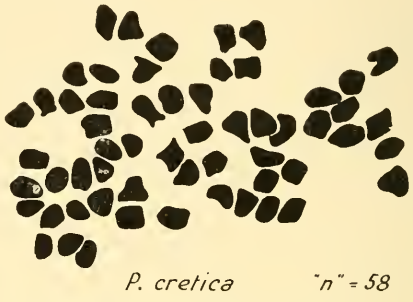


Fig. 175. Explanatory diagram to Fig. 174. × 1500.

(2) var. *albolineata* from Kew and Ceylon together with var. *albolineata-cristata*, a crested form of *albolineata* otherwise closely resembling it, from Kew.

(3) Wild European material collected by myself from a locality near the western shore of Lake Maggiore in 1937 and since maintained in cultivation.

(4) Wild tropical material brought alive to Kew from Uganda in 1938 but unfortunately lost as a result of the war.

Although the horticultural varieties first mentioned (1) were the earliest to be investigated and from them the general outline of the behaviour obtained, in the account which follows attention will be almost confined to the three wild samples listed under (2), (3) and (4), owing both to the obvious advantages of wild over horticultural material but also to the spread of chromosome numbers displayed, which recalls in some ways what has already been noted in *Dryopteris Borreri*.

Chromosome counts of all these strains showed that whereas the Italian material, as expected, was of a low chromosome number with $2n = c.$ 60 (actually 58, see Figs. 174, 175), which may be interpreted as diploid, the Uganda material was tetraploid with $2n = c.$ 120 (cf. Fig. 176*e*). It is of some interest to have traced a tetraploid to a known geographical locality. On the other hand, 'var. *albolineata*' is triploid with $3n = c.$ 90 (Fig. 176*c*), and so is 'var. *albolineata cristata*'.

All forms of *Pteris cretica* give an exquisite quality of fixation readily, as may perhaps already have been seen in Fig. 173 and others. It is also fortunate that sixteen-celled

sporangia are more abundant than in *Cyrtomium falcatum*, and details of chromosome pairing in such sporangia are therefore available for all the polyploid types. The greatest interest naturally attaches to such pairing in the diploid which in this case is likely to be the oldest type, a view with which its apparently relict and disjunct occurrence in Europe is in full accord.

The first impression given by chromosome pairing in the sixteen-celled sporangia of *Pteris cretica* is its irregularity. Pairs are numerous and absorb the majority of the

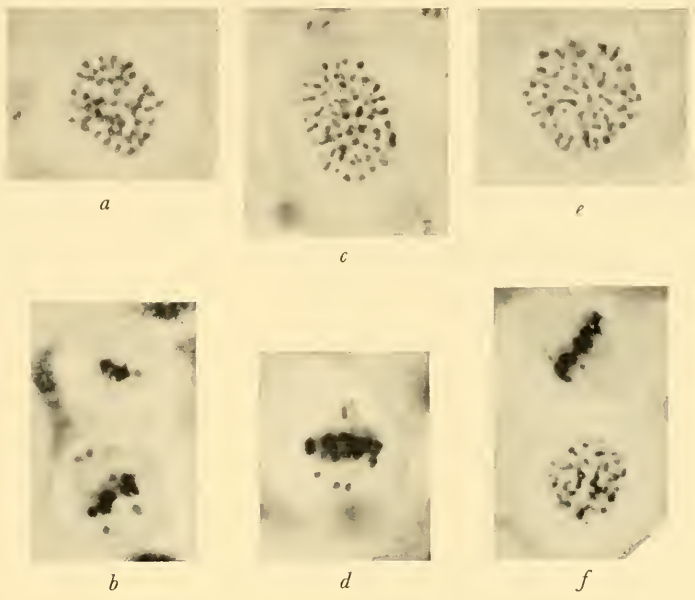


Fig. 176. Meiosis in the polyploid series of *Pteris cretica* L. from sections. × 1000. *a*. First meiotic metaphase in an eight-celled sporangium of the diploid ($2n=58$). *b*. The same in a sixteen-celled sporangium. *c*. Triploid var. *albolineata* ($3n=c.$ 90) an eight-celled sporangium. *d*. The same in a sixteen-celled sporangium. *e*. The wild tetraploid from Uganda ($4n=c.$ 120) an eight-celled sporangium. *f*. The same in a sixteen-celled sporangium.

chromosomes, but there are also some trivalents together with a few probable quadrivalents and a residue of unpaired chromosomes remains. Various views are shown in Fig. 177*b* and *c*.

In contrast to this, the sixteen-celled sporangia of triploid and tetraploid show fewer unpaired chromosomes and more multivalents involving larger numbers of chromosomes. Some of these, as found in the Uganda tetraploid, are shown in Fig. 178, in which a very large multivalent group is seen at diakinesis. Another expression of the same thing is the reduced number of lagging univalents visible in side views of metaphase; this is perhaps detectable by comparing the bottom row of Fig. 176 in which this stage in diploid, triploid and tetraploid are placed side by side.

With regard to the interpretation of these observations, the presence of multivalents in tetraploid *P. cretica* need not surprise us, since some are also to be found in the diploid.

Since a mechanism for obtaining tetraploids from diploids is known to exist in the four-celled sporangia, there is no reason to regard the Uganda plant as anything other than a derivative by simple chromosome doubling from the simpler state still retained in

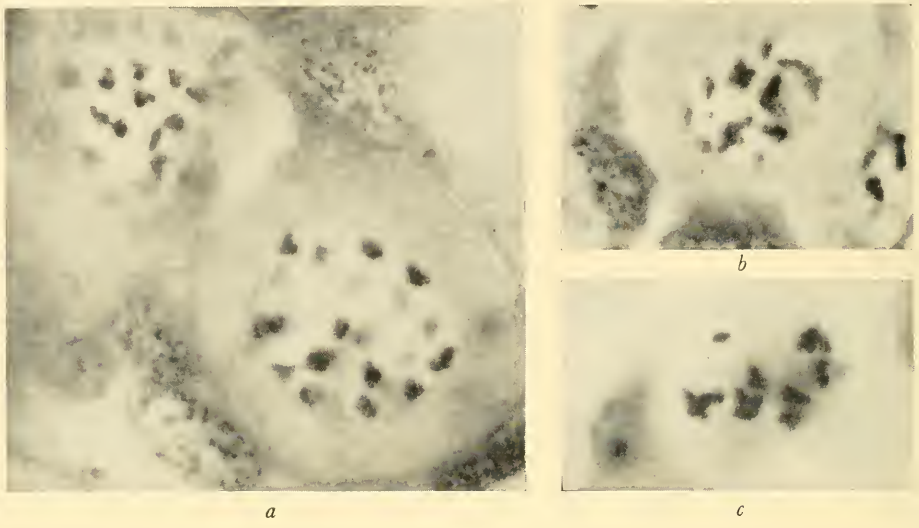


Fig. 177. Details of chromosome pairing in diploid *Pteris cretica* L. from sections. × 2000. *a*. Diakinesis in an eight-celled sporangium with very regular pairing. *b*. Diakinesis in a sixteen-celled sporangium showing univalents and multivalents. *c*. The same at first metaphase showing a univalent a trivalent and other groups.

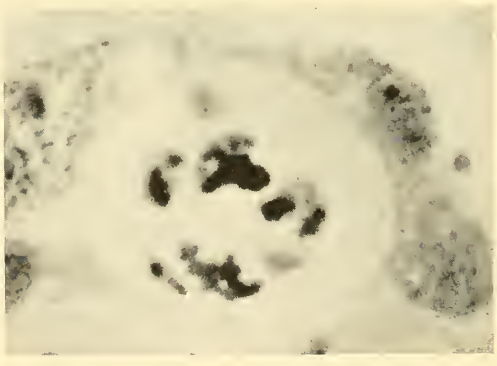


Fig. 178. Pairing at diakinesis in a sixteen-celled sporangium of tetraploid *Pteris cretica* L. from Uganda showing complex multivalents.

the European form of the species. With regard to the triploid we are in the same dilemma as in triploid *Dryopteris Borreri*, already to some extent discussed in a previous chapter. A hybrid between apogamous *Pteris cretica* and some related sexual species seems the most probable explanation.

With regard to diploid *P. cretica* there are perhaps two alternatives. The first and most probable is that it is a hybrid between two related species with much though not complete homology between the chromosomes of the two gametic sets. Another

possibility must, however, be recognized. The wide geographical range and apparently relict status of *P. cretica* in Europe must denote a high antiquity for that species during which time chromosome changes (e.g. translocations and the like) may have taken place, and in the absence of natural selection their effects may have accumulated. It is conceivable that all the irregularity of pairing observed both in production of multivalents and of univalents may actually have arisen in this way *after* the apogamous habit was established. This would mean, if true, that for this one species the possibility exists that apogamy itself could perhaps have arisen in a formerly sexual species, not by an act of hybridization, but by a process of internal differentiation of a genetical kind. This explanation is not the most likely one, for it would be expected that traces of a sexual form of similar morphology would be found if mere mutation had produced the apogamy, and there is so far no indication that such a sexual form exists. It is, nevertheless, perhaps of importance to raise this alternative explicitly at this point, for, as will shortly be seen, it is an explanation which on cytological grounds is virtually excluded for every other apogamous species analysed.

It may now be suitable to complete the account of *Cyrtomium*. As already explained (Chapter 10) there are now three species instead of one to consider owing to the splitting of the old aggregate '*Aspidium falcatum*' into three microspecies now known as *Cyrtomium falcatum* Presl, *C. Fortunei* J.Sm. and *C. caryotideum* Presl. The first has already been illustrated in Fig. 163, the second is represented in Fig. 180 and the third in Fig. 179. All three have been

Fig. 179. *Cyrtomium caryotideum* (Wall.) Presl from Uganda. A young, live frond in cultivation. Natural size.

in cultivation for many years in Europe and America, both in botanic gardens and as ornamental plants of commerce.

Owing to the rather wide discrepancy between my results and those of Miss Allen (1914) with regard to the chromosome numbers, it was necessary to examine a wide sample of the various species with rather pedantic attention, since a superficial comparison with *Pteris cretica* might easily have led to erroneous conclusions. The material available to me was as follows:

(1) Horticultural plants of *Cyrtomium falcatum*, grown for the market in the nurseries of Messrs Clibran of Altrincham, Cheshire.

(2) *C. falcatum* var. *Rockfordii* Hort. in the Royal Botanic Gardens, Kew.

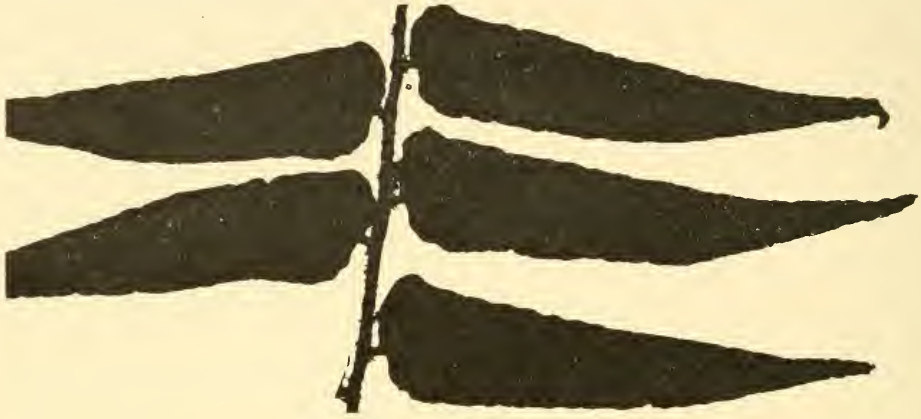


Fig. 180. *Cyrtomium Fortunei* J.Sm., part of a dried cultivated frond. Natural size.

(3) *C. falcatum* var. *Rockfordii* Hort., raised from spores obtained from the Botanic Garden, Wisconsin, U.S.A., and thought to be identical with Miss Allen's material.

(4) *C. Fortunei* of unknown origin, long established at Manchester University Experimental Ground.

(5) *C. Fortunei*, wild from the neighbourhood of Pekin, raised from spores sent by Dr C. Ching.

(6) *C. caryotideum*, wild from Uganda, conveyed alive in 1938 to Kew, together with the *Pteris cretica* previously referred to, but, like that species, subsequently lost owing to enemy action during the war.

From this rather extensive range of specimens the greatest scientific interest naturally attaches to the wild specimens (numbers (5) and (6)) and to the Wisconsin material (number (3)). None of these was, however, obtained until the work was far advanced, and in the general account already given in Chapter 10, numbers (1) and (4) were principally quoted. Enough has been seen of the others, however, to make it quite certain that they are not different in any way which can be detected cytologically.

Evidence on the chromosome numbers of the three species of *Cyrtomium* is contained in Figs. 181–184. In sections (Figs. 181, 182), only approximate counts can be made which, in each of the three cells figured, place the number as 'not less than 119 nor more than 123'. The cell of Fig. 181*a* and 182*a*, it should perhaps be pointed out, is from Wisconsin. Only with squashes can greater precision be reached, and in each of

the cells shown in Fig. 183*a* and *b*, which relate to *C. falcatum* and the wild *C. Fortunei* from China respectively, the number is 'not less than 122 nor more than 123'. In the cell of Fig. 183*c* of wild *C. caryotideum* from Uganda the number is exactly 123.

The significance of this chromosome number is considerable. It is about twice that recorded by Allen, but it is not a tetraploid and there seems little doubt, especially in view of the results obtained by reinvestigating Wisconsin material (Figs. 181*a*, 182*a*),

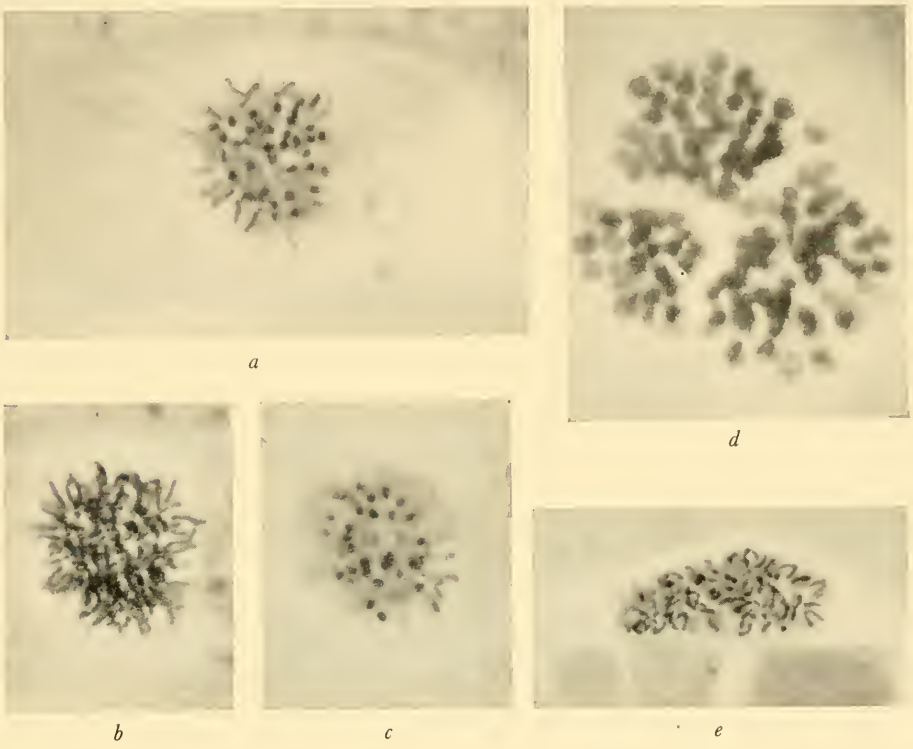


Fig. 181. *Cyrtomium* chromosome counts from section. *a*, *b*, *c*. Three different focal levels of a plate of chromosomes in a root of the Wisconsin plant (see text). × 1500. For explanatory diagram see Fig. 182*a*. *d*. Meiosis in *C. falcatum* (L.f.) Presl, plate of chromosomes slightly dismembered by pressure. × 2000. For explanatory diagram see Fig. 182*b*. *e*. Mitosis in a root of *C. falcatum* var. *Rockfordii* from Kew. × 1000. For explanatory diagram see Fig. 182*c*.

that Miss Allen's estimate must have been due to technical error and not to genetical difference of her specimen.

The obvious interpretation of the actual number found is that it is that of a triploid, since the monoploid number 41 is known to characterize a number of very closely related genera, notably *Dryopteris* and *Polystichum*. This interpretation is to some extent confirmed by the evidence from chromosome pairing in the sixteen-celled sporangia. Trivalents are certainly present (Fig. 185), together with pairs and a large number of univalents. Trivalents are not in themselves diagnostic of triploidy if present in small numbers, and if the subject is not an autotriploid, nevertheless, they are some confirmation of the status of the parent plant if this had been diagnosed from other evidence.

In addition, the general appearance of the cells in question, especially the relatively large number of unpaired chromosomes, is very strong confirmation of a necessary concomitant of triploidy (whether this be auto- or allo-), namely, hybridity. For the attainment of the triploid state wherever found would seem to necessitate an immediate or antecedent cross between two dissimilar plants, at least one of which must have been sexual.

Since all three of the wild species and the one horticultural variety, var. *Rockfordii*, have the same chromosome number, it must be further assumed that the morphological

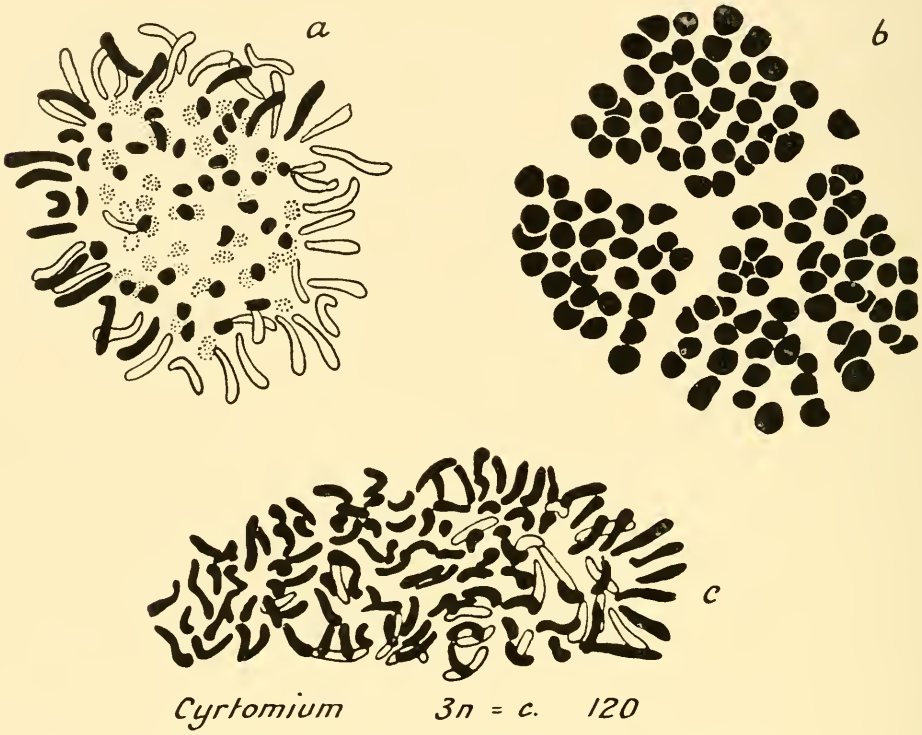


Fig. 182. Explanatory diagrams to Fig. 181. All ×3000. *a*. The Wisconsin plant. Chromosomes in focus in Fig. 181*a* shown in black, those in focus in Fig. 181*c* in dots, and the remainder in outline. Approximate count is 119–123. *b*. The cell of Fig. 181*d*. Approximate count *c*. 121. *c*. The cell of Fig. 181*e*.

differences which distinguish them are secondary to their apogamous habit and have been developed later than this by a process presumably of genic mutation. It is obvious that in any apogamous species non-injurious mutations would tend to be transmitted indefinitely without disturbance or segregation.

The next species to claim our attention can be *Dryopteris atrata* (Wallich) Ching (Fig. 186), formerly known as *Nephrodium hirtipes* and partially investigated by Steil (1915, 1919) in work which has been already quoted (p. 160 above). The species in the wild state occurs from China to the Himalayas and is available only in botanic gardens, since it is not a fern of commerce. My material was obtained from Kew and since

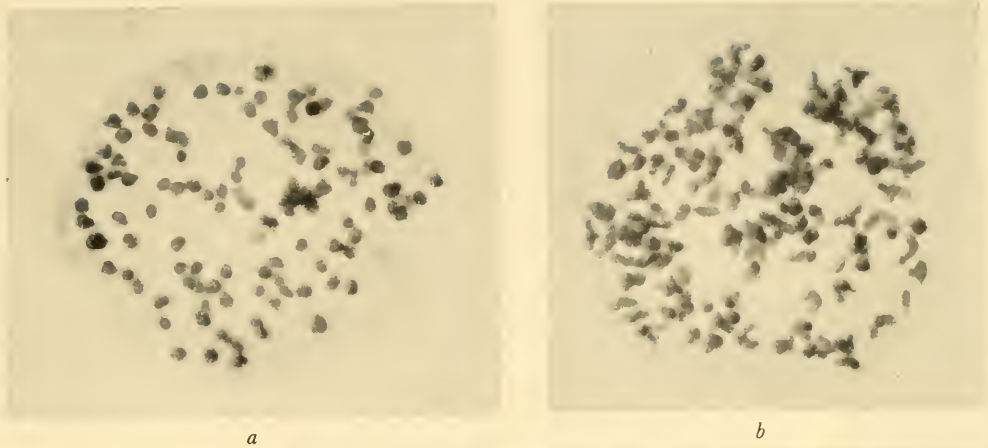
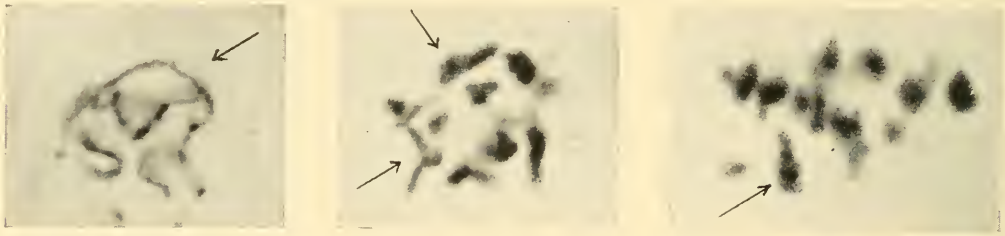


Fig. 183. Squash preparations of meiosis in *Cyrtomium*. × 1000. *a*. *C. falcatum* (L.f.) Presl in balsam after acetocarmine. *b*. *C. Fortunei* J.Sm., the wild plant from China, in liquid acetocarmine. *c*. *C. caryotideum* (Wall.) Presl in balsam after acetocarmine. For explanatory diagram see Fig. 184.

Fig. 184. Explanatory diagram to Fig. 183*c*. × 1500. '*n*' = 123.

Steil's was also of botanic garden origin it is possible that the two are identical. This supposition is not affected by the change of name, which is quite recent* (Ching, 1933).



a *b* *c*

Fig. 185. Details of chromosome pairing in sixteen-celled sporangia of *Cyrtomium Fortunei* J.Sm., from a section. × 3000. *a*. Pachytene showing a trivalent. *b*. Diakinesis showing trivalents. *c*. Metaphase showing a trivalent, a univalent and other groups.

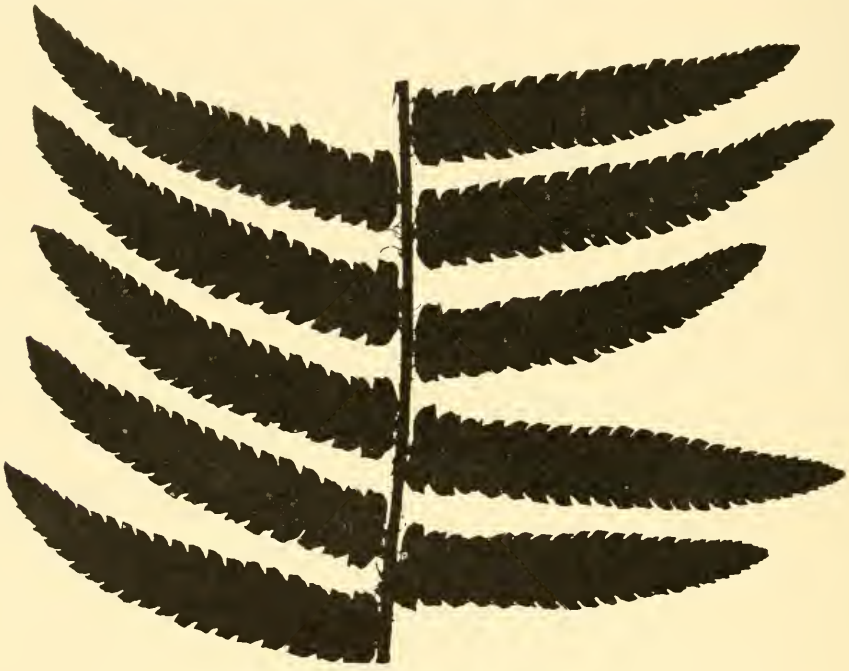


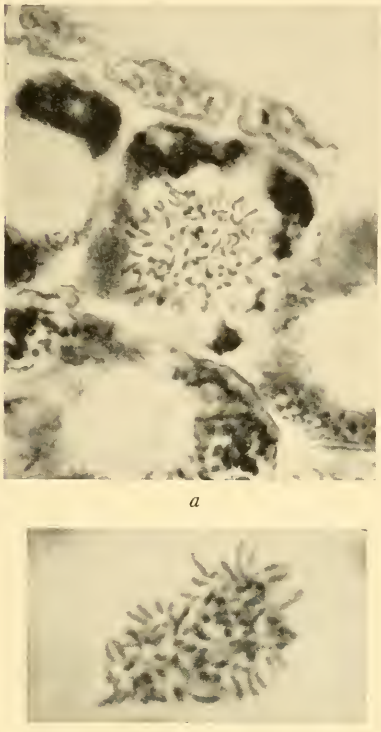
Fig. 186. *Dryopteris atrata* (Wallich) Ching. Part of a dried frond of the plant used. Natural size.

Some of the sporangial peculiarities of *Dryopteris atrata* have already been mentioned in a previous chapter, and it only remains to add such details as may be of evolutionary importance. The most significant detail is again the chromosome number. As in the case of *Cyrtomium* I differ considerably from previous investigators, in this case Steil (1919) and Andersson and Gairdner (1930), for reasons which I can only interpret as

* The change of name results from a splitting of the former collective species *Nephrodium hirtipes* into two, the Chinese portion now constituting *Dryopteris atrata* and the Indian portion retaining the epithet *hirtipes*. On comparison with Ching's diagnoses (1933), the botanic garden form, the origin of which is unknown, was found to conform to the Chinese variant. The labels were emended in consequence, though the plant is the same as that formerly disseminated under the older name.

technical deficiency in the earlier workers. As determined by me (Figs. 187–190) the chromosome number of *Dryopteris atrata* is indistinguishable from that of *Cyrtomium* and is of the order of 120 on the imperfect evidence of sections and exactly 123 when squash methods are available.

Chromosome pairing in the sixteen-celled sporangia of *Dryopteris atrata* is shown in Fig. 189*a*. Unlike the case of *Cyrtomium*, pairs are few and multivalents apparently absent; the resemblance is therefore with *Dryopteris remota* described by Döpp. Since from the chromosome number and systematic position of the species it may safely be assumed to be another triploid, the absence of homology among the chromosomes



a

b

Fig. 187.

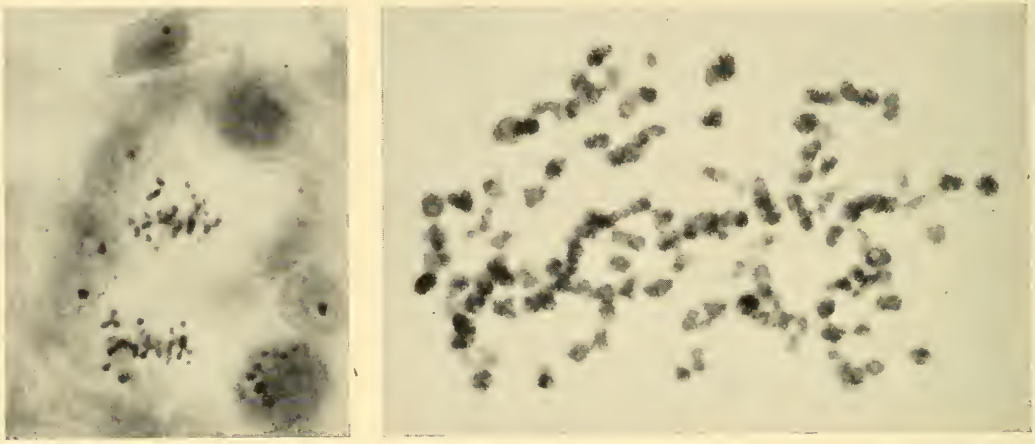
Fig. 188. Explatory diagram to Fig. 187*a*. × 3000.

Fig. 187. *Dryopteris atrata* (Wallich) Ching. Mitotic chromosome counts from sections. *a*. A root, showing a good metaphase plate surrounded by heavily staining cytoplasmic inclusions. × 1000. For explanatory diagram see Fig. 188. *b*. A dividing cell in a young sporangium. × 1500.

present would seem in this case, for the first time in this chapter, to rule out the possibility of really close affinity between the two parents. Autopolyploidy followed by minor evolutionary changes cannot be wholly excluded from either *Pteris cretica* or *Cyrtomium*, but in *Dryopteris atrata* there is no reason of any kind to bring it to mind. The species here seems to be a triploid hybrid, either formed directly or by descent from some other polyploid in which there is virtually no affinity between the chromosomes of the hybridising parents. In this case it is therefore necessary to accept an interspecific and not an intervarietal cross in its immediate ancestry. [For another similar case see footnote on *Asplenium monanthes* L. added in proof on p. 195.]

Phegopteris, the Beech Fern, has already been dealt with at some length in Chapter 5, and there is nothing to add to that description. The sixteen-celled sporangia in this

species are unfortunately so rare that I have not yet seen this type of meiosis, in spite of annual fixations made for the purpose over many years. The possible origin of the species is therefore at present unknown, since the chromosome number (90) is unlike



a *b*

Fig. 189. Meiosis in *Dryopteris atrata* (Wallich) Ching. *a*. Two mother cells surrounded by tapetum from a sixteen-celled sporangium in a section showing irregular meiotic figures composed largely of unpaired chromosomes. × 1000. *b*. Spread metaphase from an eight-celled sporangium in balsam after acetocarmine. × 1500. For explanatory diagram see Fig. 190. '*n*'=*c*. 122= probably 123.

Fig. 190. Explanatory diagram to Fig. 189*b*. × 2000.

that of any of the other genera with which *Phegopteris* has from time to time been classed and there is therefore as yet no clue to its nearest affinities.

Somewhat similar uncertainty hangs over the genus *Pellaea*, of which one species, *P. atropurpurea* (L.) Link, has been available to me from spores collected wild in Cali-

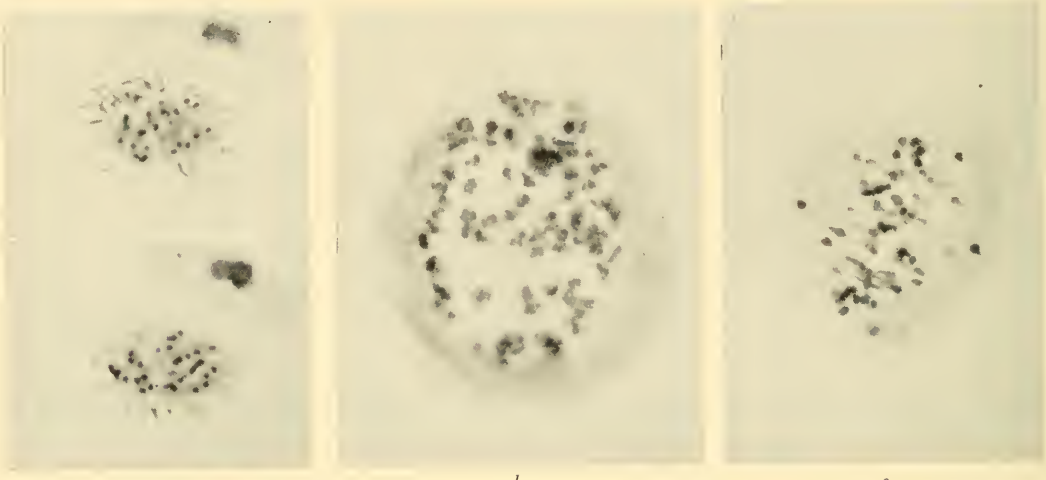
fornia and communicated to me by the kindness of Mr Alston of the British Museum. In this case the internal facts are easily ascertained, but their interpretation is hampered by the absence of any other information regarding related species. The form of the leaf of the specimen used is shown in Fig. 191, and the chromosomes in a root, an eight-celled sporangium and a sixteen-celled sporangium, are shown in Figs. 192 and 193. The chromosome number is approximately (and perhaps exactly) 87, and pairing in the sixteen-celled sporangia falls into approximately equal numbers of pairs and univalents. Both this pairing and the chromosome number itself are strongly suggestive of another triploid, although acceptance of this interpretation requires some independent evidence that a monoploid complement of 29 exists in this group. If it does the pairing could be regarded as that of a backcross between an allotetraploid and one of its diploid parents, one at least of which must have reproduced sexually. The search for sexual species of *Pellaea* or related genera* with the required chromosome numbers may therefore be recommended to local botanists to whom these plants may be accessible in the wild.

Fig. 191. *Pellaea atropurpurea* (L.) Link, live frond of a young plant grown in cultivation. Natural size.

Returning now to *Dryopteris* we have *D. remota* A.Br. and *D. Borreri* Newm. still to consider. With regard to *D. remota* much has already been said in Chapter 5, and the chief thing to recall is the hope that it will shortly be synthesized. Whether or not the parentage deduced for it on p. 79 is correct, however, the evidence from the sixteen-celled sporangia shows clearly that, as in *D. atrata*, there is almost complete lack of homology between the chromosomes of the two component species. As Fig. 194*b* displays, the almost complete failure of pairing of all the chromosomes in the sixteen-celled sporangia of this species, in marked contrast to the regularity of pairing in the eight-celled sporangia, is as characteristic a feature of the Irish specimen, as it appears, from Döpp's description (1932), to be of continental material.

* The existence of $n = 29$ in the related genus *Pteris* has, of course, actually been demonstrated in this chapter by finding that diploid *Pteris cretica* has $2n = 58$ (p. 174 above).

The last of the apogamous species, *D. Borreri*, remains, and here we have richer material than in any other recorded case, since the species is not only widespread and abundant in our own flora but, unlike the Beech Fern which is also abundant but more



a *b* *c*

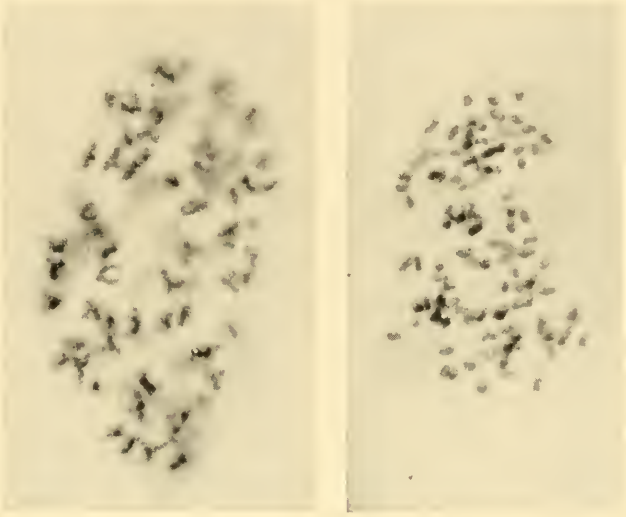
Fig. 192. Cytology of *Pellaea atropurpurea* (L.) Link. *a*. Two focal levels of mitosis in a root from a section stained in gentian violet. × 1500. *b*. Meiosis in an eight-celled sporangium in balsam after acetocarmine. × 1000. *c*. Meiosis in a sixteen-celled sporangium showing pairs and univalents in balsam after acetocarmine. × 1000. For explanatory diagrams see Fig. 193.

Fig. 193. Explanatory diagrams to Fig. 192*a* and *b*. × 1500.

uniform, we have a considerable variety of different forms to examine from which some genetical evidence can be obtained even in the present state of the inquiry.

Two types of material should, in the first instance, be called to mind. On the one hand, there are the host of monstrosities beloved of fern collectors of which the var. *polydactyla* Wills and the var. *polydactyla* Dadds may be taken as samples, and on the

other there is the unmodified natural wild species to which reference has already been made in Chapter 5, with its range of polyploid chromosome numbers. Taking the monstrosities first, the *polydactylas* need not detain us long. Evidence has already been presented, both by Döpp and in Figs. 45–7 of Chapter 4, that these two forms follow the normal story common to apogamous ferns in general, and it was already known to



a *b*

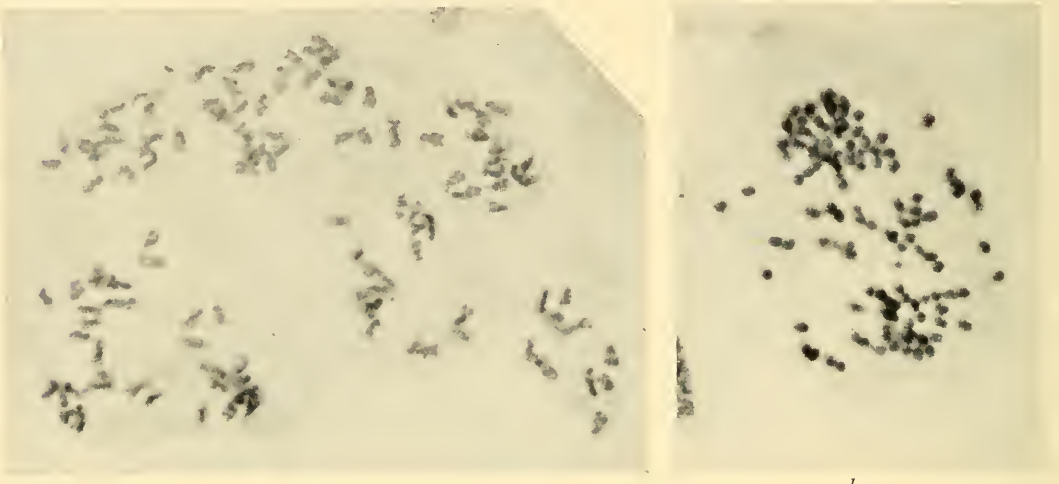
Fig. 194. Meiosis in *Dryopteris remota* A.Br. in balsam after acetocarmine. × 1000. *a*. From an eight-celled sporangium with regular pairing. *b*. From a sixteen-celled sporangium with virtually no pairing.

Fig. 195. One pinna of *Dryopteris Borreri* var. *polydactyla* Dadds. Natural size. Compare with var. Wills on p. 58.

Farmer and Digby (1907) that two different chromosome numbers are represented. It is not surprising that Farmer and Digby's actual counts are incorrect, considering the early date of their investigation, and it is perhaps sufficient to emend their statement by the demonstration already given (Fig. 45) that var. *polydactyla* Wills is a diploid with 82 chromosomes in both generations, whilst var. *polydactyla* Dadds (Fig. 196) is approximately triploid. Further, as Fig. 196*b* illustrates, there is complete failure of pairing in the sixteen-celled sporangia, at least in the latter variety.

Of far greater importance than the monstrosities which, at most, have a historic interest, is the study of the naturally occurring wild species. Here we have evidence from Britain, Switzerland, Germany, and more recently Norway. Fig. 197 indicates

the approximate distribution of the species in Europe as compiled in a recent study by Nordhagen (1947).



a *b*

Fig. 196. Meiosis in *Dryopteris Borreri* var. *polydactyla* Dadds in balsam after acetocarmine. × 1000. *a*. From an eight-celled sporangium with regular pairing. *b*. From a sixteen-celled sporangium with virtually no pairing.

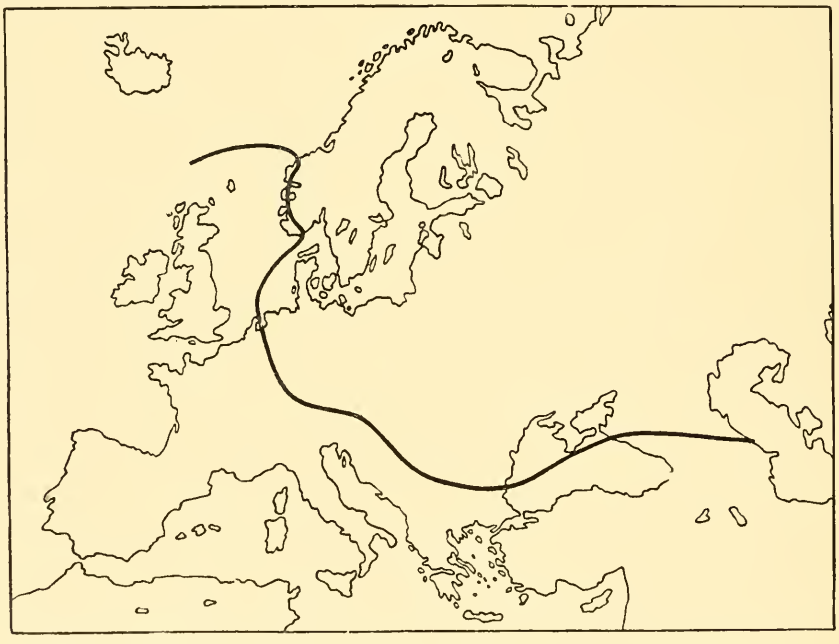


Fig. 197. Map of the northern limits of *Dryopteris Borreri* Newm. in Europe. After Nordhagen (1947).

With regard to chromosome numbers in *D. Borreri* it has already been shown in Chapter 4 that a polyploid series is present in grades ranging from diploid to pentaploid, though not all of the same constitution. We are still imperfectly informed about the

geographical details of members of the series, although some preliminary facts are available from the four countries mentioned above. In an unpublished communication from Mrs G. Knaben of Oslo, which I am permitted to quote, the first chromosome

Fig. 198. Comparable pinnae of the polyploid series in *Dryopteris Borreri* Newm. Natural size. *a*, diploid; *b*, triploid; *c*, tetraploid; *d*, pentaploid.

count for Norway is 'approximately 125'. In Germany we have Döpp's record of approximately 130, and in Britain and Switzerland there are my own records of '*n*' = 123.. There seems little doubt therefore that the triploid is the commonest form

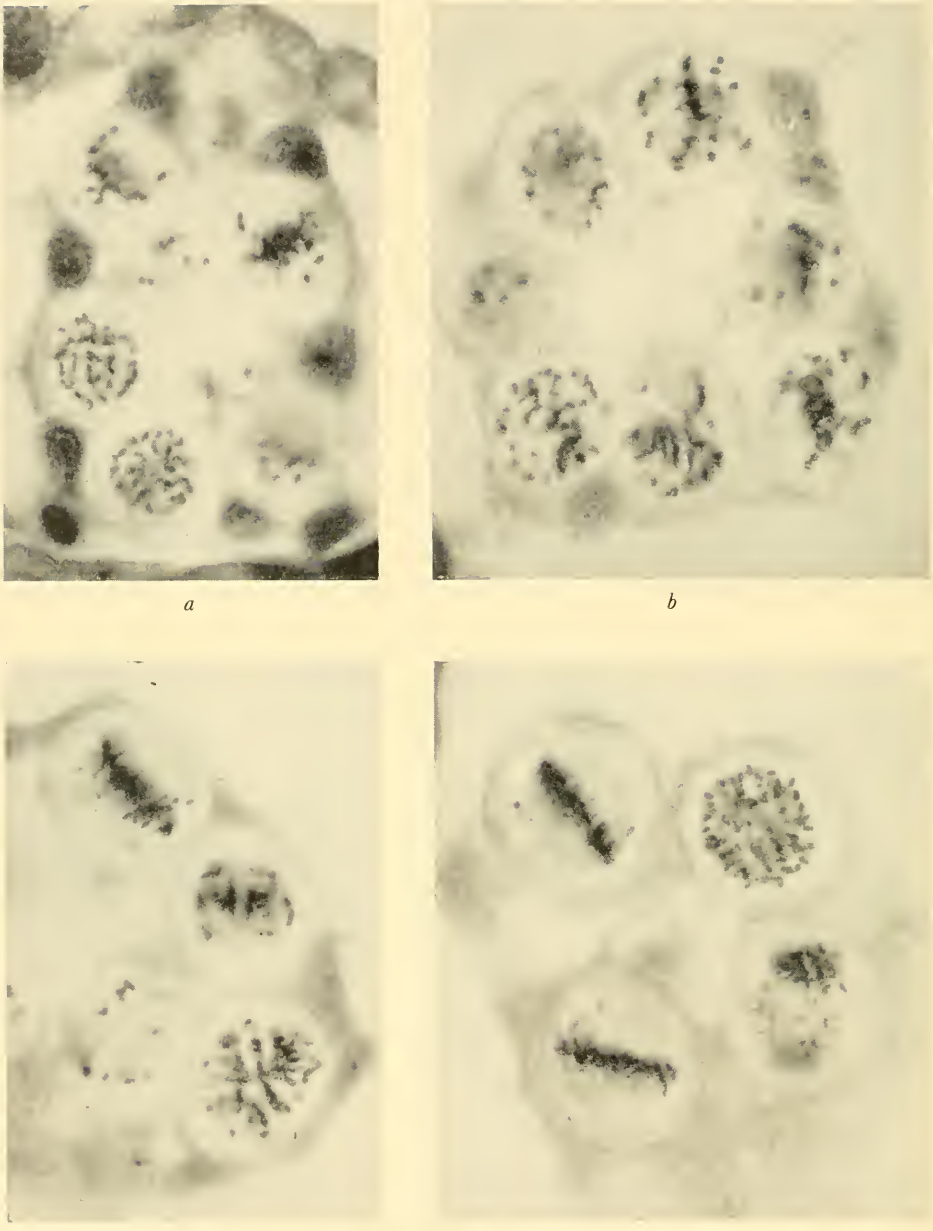
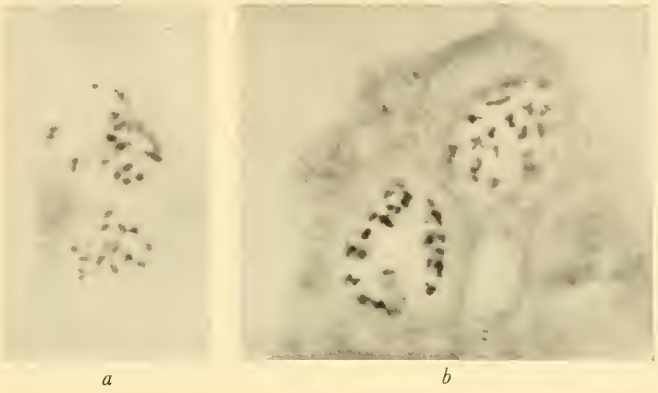


Fig. 199. Meiosis in sixteen-celled sporangia of *Dryopteris Borreri* Newm. from sections. × 1000. *a*, diploid; *b*, triploid; *c*, tetraploid; *d*, pentaploid.

of the species over most of its range. Local populations of diploids* have, however, been met with both in Britain and in Switzerland, the only two countries in which a range of material has been studied. Tetraploids and pentaploids have so far only been encountered in England and Ireland, though they will probably be detected elsewhere if search is made for them. They differ from the diploids and triploids in being single individuals and not local populations, and all those so far met with have suggested by their appearance that they are hybrids between *D. Borreri* and *D. Filix-mas*. Such an origin is in agreement with their observed chromosome numbers, since a cross between *D. Filix-mas* and diploid *D. Borreri* would be tetraploid and between *D. Filix-mas* and triploid *D. Borreri* would be pentaploid. That hybrids of this constitution can be synthesized readily has already been shown by Döpp (1939), and the experiment has been repeated by myself more recently though the resulting plants are still too young to provide full evidence.



a *b*

Fig. 200. Enlarged detail of chromosome pairing in sixteen-celled sporangia of *Dryopteris Borreri* Newm. × 1000. *a*. Diploid with only univalents. *b*. Pentaploid with numerous pairs.

Samples of comparable pinnae from diploid, triploid, tetraploid and pentaploid specimens of the various types referred to above are contained in Fig. 198. The close resemblance of diploid and triploid will perhaps be recognized. It is perhaps remarkable that no tetraploid or hexaploid populations with the pure *D. Borreri* morphology have yet been encountered, although a mechanism for their production (i.e. from four-celled sporangia, cf. Chapter 10) undoubtedly exists. The reason for this is uncertain, and further search may still perhaps reveal them.

Chromosome pairing in the sixteen-celled sporangia of one example of each of the four grades of polyploidy is shown in Fig. 199 and in greater detail in Fig. 200. In all cases pairing is irregular though it is not equally so throughout. Pairs are numerous in tetraploid and pentaploid, though not so complete even in the tetraploid as to suggest

*For anyone interested in acquiring material of such local diploid populations four, which are well known to me, may be listed as: (1) The Kentmere Valley near Kendal, Westmorland, both wood and scree populations. (2) A wood near Bangor, north Wales. (3) A wood near Dartmouth, Devon. (4) The neighbourhood of Dublin, a very golden yellow type. (Note that the tawny appearance of the Dublin diploids is not diagnostic of the diploid state in other places.)

an origin by simple chromosome doubling. Pairing in the diploid, on the other hand, is virtually absent (see Fig. 200*a*). In certain cases (e.g. var. *polydactyla* Dadds) a triploid may also show complete failure of pairing, but in the more normal forms of the species some pairs are present in triploids, suggesting that not all the triploids found wild are of the same genetical nature; some may perhaps be primitive triploids (i.e. the ancestral type of the species whatever that may have been), while others (e.g. *polydactyla* Dadds) may be triploids of secondary origin such as could be formed by a cross between an apogamous diploid and a related sexual diploid. Until more is known about the nature

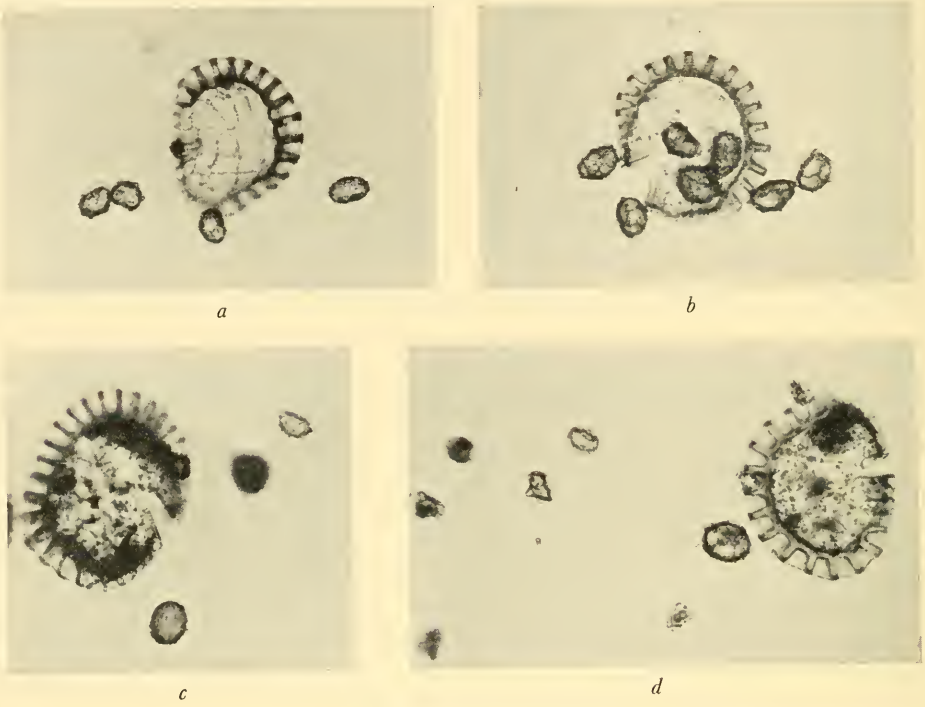


Fig. 201. Spores of the polyploid series in *Dryopteris Borreri* Newm. from glycerine jelly mounts. × 1000. *a*, diploid; *b*, triploid; *c*, tetraploid; *d*, pentaploid.

of related sexual species this problem cannot be solved, and it is equally impossible to state with assurance which of the two first members of the series (diploid or triploid) is in fact the older, for while a derivation of the latter from the former can have occurred along the lines just indicated, a reverse derivation is also conceivable. Should any of the spores produced by the sixteen-celled sporangia be viable, we know enough about the breeding behaviour of triploids (cf. *Osmunda*, Chapter 3) to be certain that the viable types could not fail to include those in which the regular diploid chromosome number had by chance been reassembled.*

That the production of an occasional viable spore from a sixteen-celled sporangium

* Since the above was written some positive evidence in favour of the primitive form of *D. Borreri* having been *diploid* has come to hand by finding that the population of this species on the unglaciated island of Madeira is exclusively diploid.

is not a negligible possibility is made clear by the behaviour of the two upper members of the series (the tetraploids and pentaploids), which also provide such genetical evidence as we possess. That they are hybrids between the lower numbered forms of *D. Borreri* and the Male Fern is suggested not merely by their isolated occurrence, their chromosome number and their mixed morphology, but also by a curious circumstance affecting the total spore output. This is always low, as may perhaps be seen in Fig. 201, in which the relatively good spores of diploids and triploids contrast quite strongly with the high proportion of shrunken spores found in tetraploids and pentaploids. So marked is this character that inspection of the spore output of an unknown plant in which hybridity of this kind is suspected can provide almost as reliable a guide to its chromosome number as in other cases of hybrids in which apogamy is not involved. In these plants there are, however, always a few very large good spores produced which, on sowing, give a sparse crop of apogamous prothalli which reproduce the parental type exactly. The low spore output is therefore presumably the reason why homogeneous local populations are not formed.

The reason for the low spore output is at once to be seen on sectioning a sorus. Whereas in diploid and triploid (with the exception of var. *polydactyla* Dadds) the eight-celled type of sporangium is so conspicuous as to dominate the general field, in tetraploids and pentaploids it is the sixteen-celled type which predominates to the extent that in one tetraploid for which accurate counts of the two types were made, sixteen-celled outnumbered eight-celled by 17:1. It is, therefore, a rather exceptional sporangium which is able to carry through the normal apogamous development, and viable spores are therefore correspondingly scarce.

This is a very curious type of genetical expression of a character. Apogamy, though inherited, is not behaving at all like a simple Mendelian dominant, nor is it even a partial dominant in the usual sense. Combination of an apogamous with a sexual species might perhaps be expected to produce the intermediate type of sporangium described as type 3 on p. 166 if dominance were imperfect, yet we find merely a reduced percentage of eight-celled sporangia in which, however, the apogamous qualities are fully developed.

This type of inheritance, it may be said in passing, provides strong confirmation of the hybrid origin of the tetraploids and pentaploids under discussion (and perhaps also of some triploids such as var. *polydactyla* Dadds), since it agrees exactly with what has been found in a hybrid of known origin described and synthesized by Döpp. Döpp's hybrid was between a diploid crested form of *D. Borreri* and normal *D. Filix-mas*, and it must therefore have been tetraploid. The crested character proved to be recessive, though the hybrid resembled *D. Borreri* in the shape of the pinnae and indusium. It also resembled *D. Borreri* by reproducing apogamously, though the spore output was low owing to a preponderance of sixteen-celled sporangia.

These facts are not only interesting in themselves but they provide an opportunity for testing the possibility that occasional viable spores may be formed in the sixteen-celled sporangia. An experiment of this kind was carried out on a pentaploid from Ireland. This was lifted one autumn and transferred in a large pot from the mixed population of the experimental garden to an isolated greenhouse about a mile away on

the roof of the University in which no other sporing ferns were present. Next spring, when new leaves had expanded, uncontaminated spores were collected and sown on to soil after sterilization of soil and pot in an autoclave. During culture the pots were kept covered with a glass lid and were watered only from below by standing them in a zinc tray; a chance admixture of stray spores from outside seemed therefore excluded. On germination, the usual sparse crop of apogamous prothalli was produced which were removed to another pan as soon as they appeared. The residue was then closely scrutinized. A few abortive apogamies were found, as described by de Bary, but, in addition to these, five specimens were found with definite and fairly abundant archegonia in the usual position. All these five looked more or less abnormal, recalling indeed the type of aberrant and depauperate prothalli which result from a sowing of spores from triploid *Osmunda*. Attempts to induce the formation of sexually produced young plants on them were unsuccessful and all died without offspring, although one remained alive for two years making very slow growth before it succumbed. None showed the slightest attempt at apogamous developments of the usual kind. With regard to their nature it seems difficult to avoid the conclusion that they had originated from sixteen-celled sporangia in which a minute proportion of viable but genetically unbalanced spores had been formed. With a larger sowing the still smaller proportion of viable and balanced types might have been detected.

The importance of this experiment is that it shows at least one way in which saltations can occur in spite of apogamy, although to what extent, if any, these have actually occurred in nature is unknown. It also demonstrates the importance of the nucleus in the determination of this type of apogamy, since here we have what is tantamount to segregation of sexual versus apogamous characters in the offspring of one individual, and the peculiar genetical behaviour of apogamy can therefore not be explained by any type of maternal cytoplasmic inheritance.

The genetical inference would appear to be this. Apogamy cannot be determined by a simple Mendelian mechanism involving one mutant factor which is either dominant or recessive. It seems rather to be the expression of a generalized unbalance of a quantitative kind involving several (i.e. at least two and possibly many) different processes the interaction of which is required for development of sporangia and prothalli, and some or all of which are liable to fluctuate from environmental causes internal or external to the plant. The exact fate of any individual sporangium is therefore to some extent indeterminate, though the statistical frequency of the different types of development may be constant and characteristic for a given plant in a given environment.

Such a condition could perhaps be produced in a pure line by the simultaneous occurrence of two or more genetical mutations of appropriate type; such an occurrence is, however, in a high degree improbable. For this reason it need cause no surprise that no case has yet been recorded of apogamy of this kind occurring as a local variant within a pure species. Multiple unbalance of many genetical factors can, however, readily occur at one step by the mating of gametes of different constitution, and it can be no accident that interspecific hybridization has been suspected or proved for every example about which sufficient information is available. That such hybrids often involve the mating of gametes of different grades of polyploidy is perhaps only incidental,

since polyploidy as such is by no means confined to apogamous ferns; but that triploidy may perhaps in some way predispose to the type of unbalance required is nevertheless perhaps indicated by the remarkable preponderance of triploids among the species analysed. No reason for this can at present be advanced, but the fact gives emphasis to the general conclusion that, at least as regards the ferns, hybridization is (as was first suggested by Ernst in another connexion) a cause and not merely the occasion for the manifestation of apogamy, whatever the physiological mechanism may be.

One last conclusion is perhaps worthy of comment. The whole phenomenon of apogamy of the type under discussion is a complicated departure from the normal which is nevertheless repeated with almost monotonous identity of detail in every case which has arisen. The somatic organization and normal development of sexual ferns is presumably such as to lend itself rather easily to this particular innovation, but whatever may be the facts regarding this, a more striking example of parallel evolution would be hard to find. This is of some importance, since we have already had occasion in Chapter 6 to comment on the evidence for parallel evolution in the developmental changes affecting soral structure and the form of the indusium. We seem forced to conclude that wherever (for reasons known or unknown) certain types of change can occur more easily than others, they will make their appearance repeatedly as long as the structural conditions facilitating them prevail. This is perhaps the reason for the taxonomists' perennial difficulties in tracing phylogeny and why, in the well-known phrase, a phyletic 'tree' so often resembles less a trunk with branches than a bundle of sticks.

SUMMARY

The following list summarizes the basic facts for each species mentioned in the chapter, together with the country of origin of the actual material used:

Grade of polyploidy	Species	Chromosome no.	Country of origin	Derivation
Diploid	*Pteris cretica* L.	58	Italy	Probably hybrid
	Dryopteris Borreri Newm.	82	Britain and Switzerland	Certainly hybrid
Triploid	*Pteris cretica* L. var. *albolineata* Hook.	*c.* 90 (probably 87)	Ceylon and Hort.	,, ,,
	Dryopteris Borreri Newm.	123	Britain, Switzerland, Germany, Norway	,, ,,
	D. remota (A.Br.) Hayek	123	Britain, central Europe	,, ,,
	D. atrata (Wall.) Ching	123	Hort. (probably China)	,, ,,
	Cyrtomium falcatum (L.f.) Presl	123	Hort.	,, ,,
	C. Fortunei J.Sm.	123	Hort. and China	,, ,,
	C. caryotideum (Wall.) Presl	123	Uganda	,, ,,
	Asplenium monanthes L.*	108	Madeira	,, ,,
	(?)*Pellaea atropurpurea* (L.) Link	87	California	Probably triploid
Tetraploid	*Pteris cretica* L.	*c.* 120 (probably 116)	Hort. and Uganda	Double the diploid
	Dryopteris Borreri (secondary hybrids)	164	Britain	Hybrid with *D. Filix-mas*
Pentaploid	*Dryopteris Borreri* (secondary hybrids)	205	Britain	,, ,,
Incertae sedis	*Phegopteris polypodioides* Fée	90	Britain, Sweden	—

* Information added in proof on new material from Madeira.

CHAPTER 12

INDUCED APOGAMY

In contrast to the species discussed in the last two chapters, in all of which apogamy was a permanent feature of the life history, fixed and determined by a genetical mechanism carried by the nucleus, it has long been known that normal sexual prothalli in a number of species can be induced to become apogamous as an exceptional and temporary condition if normal fertilization is prevented. One method of doing this, first used by Lang in 1898, is of the simplest. The prothalli are grown on soil in covered pots standing in water. The glass cover, which may conveniently be half a Petri dish placed over the top of the pot, prevents the access of free water to the prothalli from above while permitting ample moisture for physiological purposes to reach them from below. Under these conditions no drops of free liquid are formed upon the prothalli, and the sex organs, although fully developed, cannot open. Young plants cannot therefore be formed by sexual means, though they will readily appear if the lid is removed and surface-water supplied. The unfertilized prothalli will continue to grow and may reach an unusually large size before irregularities of form become apparent, but when these begin apogamously developed sporophytes may result by methods which, morphologically, differ in many details from the sequence of events accompanying obligate apogamy, although the end-result is somewhat similar, namely, a vegetative bud with leaves, roots and ultimately a stem which may establish itself in the soil and grow on as an independent individual. Two rather different types of morphology were described by Lang, both of which have been found again by subsequent authors. In many instances a cylindrical process covered at first with archegonia may grow out from the region of the central cushion; the centre of this process may later become vascular, while leaves, roots and a stem may develop from the tip. In other cases isolated organs from sporangia to leaves and roots could proliferate from one or both surfaces of the prothallus without the intervention of any special organ, or could grow out from the apex or margins. The most surprising instance of such isolated organs were two cases (in horticultural strains of *Dryopteris* and *Scolopendrium* respectively) in which sporangia, unaccompanied by other sporophytic organs except sometimes protective scales, were borne directly on a prothallus.

A list of the species used by Lang (1898) in the course of two and a half years is given below, together with such of his morphological notes as refer to the physical characters of the apogamous developments. As will be seen, a cylindrical process precedes the formation of apogamous buds in the great majority of cases. It is perhaps to be regretted that such a large number of the strains used were horticultural varieties and not the typical wild species; this may perhaps have been of importance in determining the high incidence of apogamy. Marked differences evidently existed in the ease of induction of sporophytes in different varieties of the same species (cf. *Scolopendrium*, *Polystichum angulare* and *Athyrium niponicum*), suggesting that predisposing causes of a genetical kind

may have been present in some cases, though of a kind quite unlike those operative in the cases of direct apogamy previously discussed.

List of results obtained by Lang in 1898

Name	Form of apogamy
Scolopendrium vulgare Sm. var. *ramulosissimum* Woll.	Cylindrical process usually from the apical region of the prothallus. Tracheids in cylindrical process. Leaves, roots, sporangia and ramenta on process. Vegetative buds from tip of cylindrical process or in place of an archegonial projection.
var. *marginale*	Similar to var. *ramulosissimum* but no sporangia, ramenta or leaves found.
Nephrodium dilatatum Desv. (= *Dryopteris dilatata*) var. *cristatum gracile*	Cylindrical process usually from the under surface just behind the apex which formed a middle lobe. Tracheids in process and middle lobe. Sporangia sometimes associated with ramenta on middle lobe and process. No vegetative buds.
N. Oreopteris Desv. (var. *coronans* Barnes)	Cylindrical process from apex of prothallus. Tracheids in process. Ramenta on process. Vegetative buds rare.
Aspidium aculeatum Sw. (= *Polystichum aculeatum*) var. *multifidum* Woll.	Tracheids in prothallus. Vegetative buds rare.
A. angulare Willd. (= *Polystichum angulare*) var. *foliosum multifidum*	Ramenta on prothallus. Vegetative buds frequent.
var. *acutifolium multifidum*	No apogamy seen.
Athyrium niponicum Mett.	Tracheids in prothalloid growths from archegonial projections.
var. *cristatum*	Similar to normal form but in addition a few apogamously produced vegetative buds.
A. Filix-femina Bernh. var. *percristatum*	Cylindrical process from apex or under surface.
var. *cruciato-cristatum*	Tracheids in process.
var. *coronatum* Lowe	Continuation of process as a leaf. Vegetative buds.
Polypodium vulgare L. var. *grandiceps* Fox.	Isolated leaflike growths. Vegetative buds numerous.
Aspidium frondosum Lowe	Vegetative buds on short cylindrical processes.

In addition to Lang's paper of 1898 the earlier literature includes observations by Stange (1887) and Heim (1896), both containing observations on apogamy in species of *Doodia*. Heim's paper is of particular importance as the first description of cylindrical processes in *D. caudata*. Later, in 1908, Yamanouchi described some early stages of apogamy without cylindrical processes in '*Nephrodium molle*', but doubt was cast on his results by Black in 1909, who attempted to repeat them but failed. Later still, in 1929, Lang returned to the study of sporangia on prothalli through a repetition of the phenomenon in new material and gave some additional descriptive facts about it and about apogamy as a whole in *Scolopendrium*. Lastly Duncan, working under Lang's direction, repeated Heim's early work on *Doodia caudata*, adding some important new facts to it (Duncan, 1941). These included, for the first time, some cytological observations on apogamously produced plants, the two chromosome numbers found being given by Duncan as *c.* 65 and *c.* 130 respectively. This confirms the reality of the fact of induced apogamy, if confirmation were needed, and it also adds the last paper of importance to the subject with which I am acquainted.

The aim of this chapter will not be to add anything to the morphological aspect of the problems raised by induced apogamy, but merely to amplify the cytological knowledge relating to two historic examples already mentioned in the above lists, namely, *Doodia caudata* (Cav.) R.Br. and *Scolopendrium vulgare*, as used by previous investigators.

The genus *Doodia* consists of a group of five closely related species of small tropical ferns, spread from Ceylon to New Zealand and characterized by a soral structure closely akin to *Asplenium* and *Scolopendrium* but borne on coriaceous evergreen leaves with the outline depicted in Figs. 203 and 204. *Doodia caudata* (Cav.) R.Br. itself is native to New Zealand and Australia. It was introduced into European botanic gardens in the middle of last century and had been used for experimental purposes as early as 1887 by Stange. It is not known whether all botanic garden stocks still in cultivation relate back to a common source or whether the species may have been introduced more than once. Unfortunately, precise records concerning such matters have not as a rule been kept, and therefore the only thing which can be said with certainty about the origin of the material to be quoted below is that it came from Kew in 1938 and is identical with that used by Duncan.

The morphological part of Duncan's work had involved the induction of apogamy by Lang's method in normal prothalli of *D. caudata*, which was followed by the induction of apospory on young, detached leaves laid on soil from both apogamously produced and sexually produced plants. None of the new types of sporophyte was kept alive for long enough to reach fertility and therefore nothing was known about meiosis. Root-tip counts of apogamous plants versus sexually produced plants were, however, successful in demonstrating the gametophytic chromosome number in the apogamously produced plants. This was assumed by Duncan to be the haploid number, but, as will be shown below, this is probably not the case.

The material for the following observations has been supplied to me by Professor Lang's former research assistant, Mr Ashby, without whom the present chapter would not have been written. After Dr Duncan's departure from the Cryptogamic Laboratory in Manchester, at the end of a year's visit, the old culture pans which had been prepared for his work and which were still in being, were kept under observation by Mr Ashby, and apogamously produced plants were extracted and carefully nursed as fast as they appeared. A very beautiful specimen, detected and photographed by Mr Ashby, is shown in Fig. 202 *a*, in which one old prothallus may be seen carrying two young sporophytes. The small sporophyte on the right is apogamously produced, while the larger one on the left is sexually produced from a belated fertilization after the apogamous plant had already been initiated. Since the larger plant is also the younger, initiation of the two in the reverse order being impossible, the difference of size is very clear demonstration of the diminutive stature of the first-formed organs of an apogamous plant. This makes cytological study somewhat difficult, since the roots of such plants are of thread-like thinness. A fortunate specimen, however, gave the plate of chromosomes shown in Fig. 202 *e* and *f*, and the very great difference in chromosome number between this and a sexually produced sister can at once be seen by a glance at Fig. 202 *b–d*.

With regard to the chromosome numbers themselves, these have not yet been determined with complete finality owing to my own departure from Manchester and

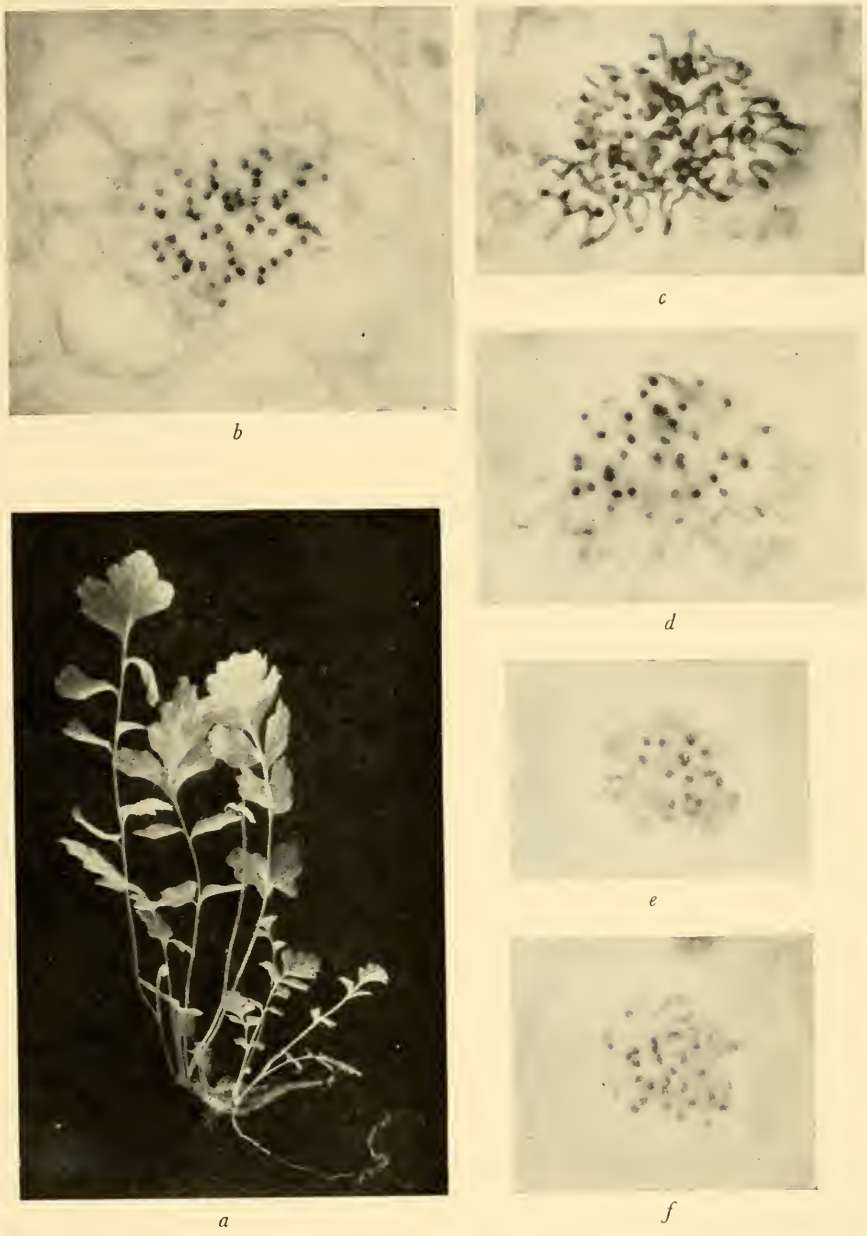


Fig. 202. Induced apogamy in *Doodia caudata* (Cav.) R.Br. *a*. A prothallus bearing an apogamous sporophyte (right) and a sexually produced sporophyte (left). Natural size. From a specimen preserved in alcohol. *b, c, d*. Different focal levels through one root tip cell of a sexually produced plant from a section. × 1500. *e, f*. Two different focal levels through one root tip cell of an apogamously produced sister plant to show lower chromosome number, from a section. × 1500.

consequent interruption of the work. My estimates agree, however, closely with those of Duncan which are certainly of the right order.

Fig. 203. Living leaves of normal *Doodia caudata* (Cav.) R.Br. of the strain used. Natural size.

By skilful cultivation, Mr Ashby succeeded where previous investigators had failed, in keeping the apogamously produced plants alive for long enough to reach maturity and Figs. 203 and 204 were obtained from fully mature fertile fronds of both types of plant

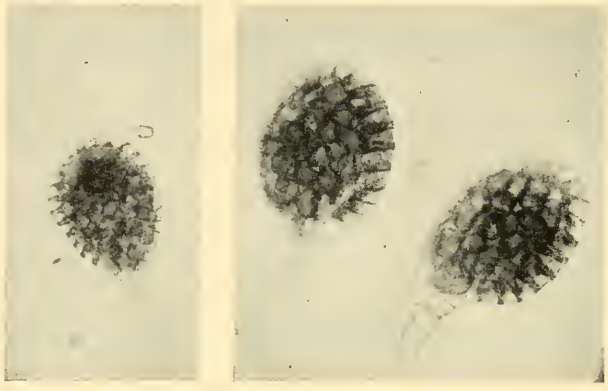
in 1947. The apogamously produced plants (Fig. 204) are still somewhat smaller than their sexual sisters (Fig. 203), though this is only apparent on fairly close inspection. On a casual glance the two types of plant are far more alike when mature than when young, and without their labels they would be difficult to distinguish apart.

Fig. 204. Living leaves of an apogamously produced plant of *Doodia caudata* (Cav.) R.Br. Natural size.

This close resemblance applies not only to the plants as a whole but also, somewhat surprisingly, to their sporangia and spores. As may be seen from Fig. 205, the sporangia of both are well filled with normal-looking spores, the only obvious difference being that those of the apogamous plant are smaller in size. Somewhat disappointingly, however, the spores of the apogamous plant have so far proved incapable of germination in spite

of their perfect morphology. They may all therefore be suspected of being genetically unbalanced.

Meiosis has been studied as far as is possible in sections in both types of plant, and the comparison, in view of their previous history and consequent peculiar relationship, is one of the principal points of interest about them. Fig. 206*a* shows the first meiotic metaphase in a normal sexually produced plant, and Fig. 207 is of diplotene or very early diakinesis in the only squash preparation which I had the opportunity of making. Neither is suitable for detailed counting, although both show clearly the perfect regularity of the division without either unpaired chromosomes or multivalent groups. Fig.

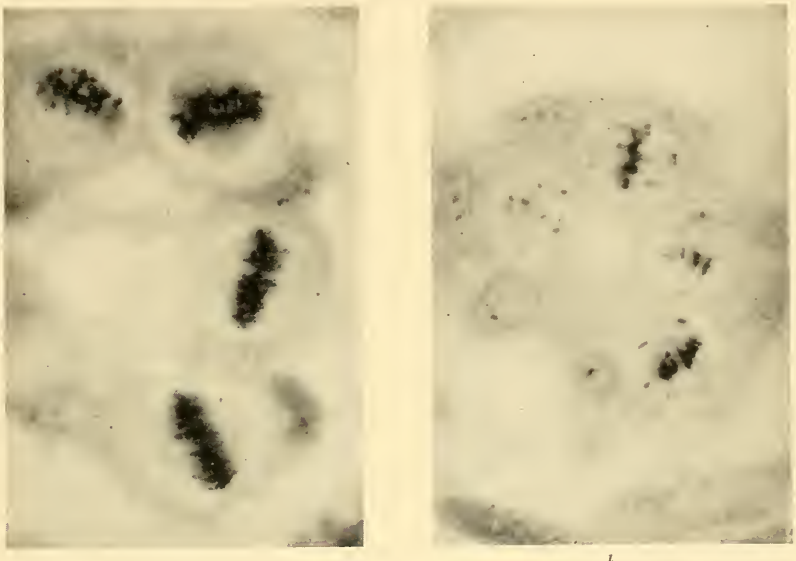


a *b*

Fig. 205. Sporangia and spores of *Doodia caudata* (Cav.) R.Br. × 100. *a*. An apogamously produced plant. *b*. A sexually produced sister plant.

206 *b*, on the other hand, shows meiosis in one of the apogamously produced plants. The much smaller size of the cells, no less than the irregularity of the metaphase plates, strikes the eye at once. This is as expected. It might, however, also have been expected that if this plant is indeed a haploid only unpaired chromosomes would be found, yet this is not the case. Univalents are certainly never absent, but they represent only a small proportion of the whole. Numerous pairs, visible on the equator even at the relatively low magnification of the photograph, are present also, and it is even possible that there are some multivalents. Pairing of this kind recalls somewhat the behaviour of the sixteen-celled sporangia of *Pteris cretica*, and the most probable interpretation would appear to be, as in that species, that the chromosome number of the apogamously produced plants of *Doodia*, though gametophytic, is not haploid. We seem to be dealing with diploid derivatives from a tetraploid stock which was itself allopolyploid. Or in other words, with a strain of *Doodia* which, beneath its regular pairing achieved by a chromosome doubling which the conditions of the experiment have reversed, contains two different sets of chromosomes between which considerable though not complete homology exists. Two gametic sets of this type are likely to be derived from distinct though related species, and we reach the somewhat unexpected conclusion that the *D. caudata* used by us and in cultivation at Kew is another case of a concealed species hybrid.

It would be a matter of considerable interest to be able to examine cytologically some freshly collected wild examples of the species, since the history of cultivated plants is such that it would be unwise to exclude the possibility that hybridization and subsequent chromosome doubling may have occurred in a botanic garden and not be a specific attribute of *D. caudata* in the wild state. Until this can be done it is scarcely profitable to study these plants in greater detail. These results are nevertheless of interest in showing, first, how an unsuspected hybrid can be detected by a technical method different from those previously employed. Secondly, it is perhaps appropriate to notice that had any of



a *b*

Fig. 206. Meiosis in *Doodia caudata* (Cav.) R.Br. from sections. × 1000. *a*. Sexually produced plant with regular pairing. *b*. Apogamously produced plant with many lagging univalents but also some pairs.

the spores borne by the apogamously produced plant been viable, which under certain conditions might conceivably occur, this species might have given one of the rare examples of an experimental series advancing in the direction of diminution of chromosomes. The fact that this possibility has not been realized, though expectation has been brought near enough for it to seem conceivable, brings out very clearly the relative irreversibility of most types of polyploidy hitherto encountered, and this irreversibility, whatever its cause, is undoubtedly a fact of evolutionary importance.

Before leaving *Doodia* it may be worth mentioning in passing that Dr Duncan's results on apospory were also repeated by Mr Ashby, though less attention was given to these and the plants have not been cultivated to maturity. The consequence of inducing apospory in an apogamously produced plant is, of course, merely to return again to a normal prothallus. The consequence of inducing apospory in a normal young plant is the production of a polyploid prothallus. One such prothallus after self-fertilization gave a sporophyte, in the roots of which over 200 chromosomes were counted. Since twice

140 should be 280, this number may be thought of as octoploid if the normal plant is a tetraploid. Unfortunately the only specimen died as a result of attempted transfer from Manchester to Leeds and nothing more can be said about it. It would be a matter of some interest to repeat the induction of such a plant in order to determine whether or not it would be capable of becoming fertile. It would be expected to show marked signs of abnormality and of sterility if the grade of polyploidy imputed to it is genuine.

With *Scolopendrium* as with *Doodia* I am fortunate in having had access, through the vigilance and private enterprise of Mr Ashby, to residual material remaining in culture from an earlier investigation, the material in this case being that relating to Lang's second paper (1929) on 'apogamy and the production of sporangia on prothalli in

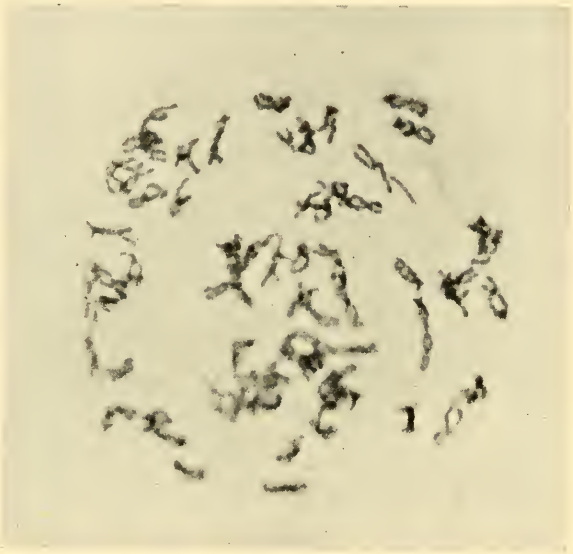


Fig. 207. Meiosis (early diakinesis) in normal *Doodia caudata* (Cav.) R.Br. to show regular pairing, permanent acetocarmine. × 1000.

Scolopendrium'. The strain of *Scolopendrium* used had originated as a stray spore of unknown origin which had germinated in the moss house in Manchester University Experimental Grounds and given rise to a plant with characteristic, heritable, leaf abnormalities of the type so often seen in horticultural strains. The leaf morphology, which can to some extent be seen in Fig. 208, could have been described as 'ramo-furcate', and in addition to the forking of the rachis and profuse branching of the leaf apex, there was a tendency to produce sori on the upper as well as on the lower surface of the leaves. The prothalli obtained from spores of this plant also showed a somewhat comparable peculiarity in the frequent production of archegonia on both surfaces, but they were otherwise quite normal and readily gave rise, when suitably watered, to crops of young plants with a morphology exactly similar to that of the original specimen. The morphological characteristics of both generations therefore appeared to be genetically determined. When water was withheld, in the manner already described, the unfertilized prothalli became very large and more abnormal in appearance. One sign of incipient apogamy

was the spasmodic appearance here and there in a culture pan of small groups of pale-coloured sporangia borne on the upper surface of some though never on all the prothalli, and at a later stage these were followed by the apogamous development of other organs from different parts of the prothalli.

The residual material remaining from Lang's work consisted of a number of adult sexually produced sporophytes of the strain and one old culture pan of prothalli, which had, however, apparently ceased to produce sporangia. In order to rejuvenate the prothallial material fresh spores of the strain obtained from one of the descendants of the original plant were sown in 1943 and kept away from free water for over a year. Late in

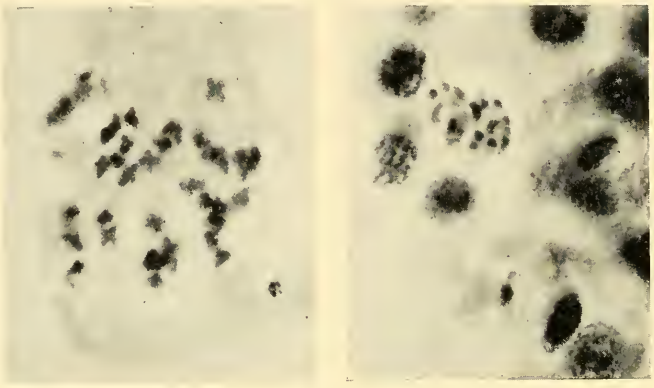
Fig. 208. Sexual and apogamous *Scolopendrium vulgare* Sm. *a*. Sister plants of the same age, of the horticultural strain used, the larger plant produced sexually, the smaller apogamously. About one-third natural size. *b*. Root-tip cell of a sexually produced plant from a section. × 1000. *c*. Root-tip cell of an apogamously produced plant from a section. × 1000.

1944 the first signs of apogamy were encountered, including both apogamous buds and sporangia on prothalli, and their production continued sparingly during 1945 when observation ceased. During this time one group of sporangia was successfully sectioned by Mr Ashby and meiosis in it encountered, while other specimens of apogamous outgrowths were lifted from the pan and grown on. One complete plant obtained in this way survived for long enough to give the photograph of Fig. 208, which was taken in the autumn of 1945. This plant was sparingly fertile for the first time in that year, producing two diminutive sori, both of which were used for cytological purposes, after which the plant unfortunately died.

The relative difference of size between a sexually produced and an apogamously produced plant of equal age of this strain of *Scolopendrium* is clearly shown by Fig. 208*a*. In the younger stages the difference is still more marked, the organs of apogamously produced plants being so small and delicate that they are difficult to handle. As expected, the cytological basis for this difference of size lies in difference of chromosome number. The sexually produced plants, in spite of their ramo-furcate and other qualities, have the normal chromosome number of the wild species and show 72 chromosomes in their roots

(Fig. 208*b*) and 36 pairs at meiosis (Figs. 209*a*, 210). In the little roots of apogamous plants there are, however, only 36 chromosomes (Fig. 208*c*).

The behaviour of these 36 chromosomes at meiosis is one of the principal points of interest in this material, and though the actual number of mother cells available has been very small indeed, being restricted to the two sori and one group of sporangia on a prothallus above mentioned, enough has been seen for a few statements to be made. From the two mother cells visible in Figs. 209*b*, and 210*b* and *c*, at the stages of metaphase and interkinesis, it is clear that meiosis is of the most irregular type possible. Chromosome pairing (Figs. 209*b*, 210*b*) is either completely absent or so slight that it cannot be detected without a larger number of cells for inspection. Chromosome distribution at



a *b*

Fig. 209. Meiosis in sexual and apogamous *Scolopendrium vulgare* Sm., permanent acetocarmine. × 1000. *a*. Sexually produced plant showing 36 pairs. *b*. Apogamously produced plant showing two mother cells and tapetal nuclei. For explanation see text and Fig. 210 *b* and *c*.

anaphase is apparently at random, and is most unequal, so that very dissimilar-sized daughter nuclei are present in the succeeding resting stage (Figs. 209*b*, 210*c*). I have not seen the second meiotic division, but the result of meiosis is total abortion of the spores.

Such behaviour might have been expected and has indeed already been looked for unsuccessfully in this and the two preceding chapters. The conclusion seems undoubtedly to be that here at last we have a genuine haploid fern, comparable to the various well-known cases of haploid sporophytes obtained in Flowering Plants but which, in the Pteridophyta, had appeared to be curiously elusive. The failure of pairing among the various chromosomes is almost certainly due to lack of homology between them, which is to be expected in a genuine monoploid set. That $n = 36$ has, indeed, been shown to be a fundamental monoploid condition in several related genera (*Asplenium*, *Ceterach*, *Scolopendrium*) makes this conclusion the more probable, and therefore for once we may discard all suspicion of harbouring a concealed hybrid or polyploid and accept the demonstration that apogamy provides that *Scolopendrium vulgare* is a simple diploid species without any ambiguity.

It would, however, be interesting to know still more about it. A repetition of these observations on normal forms of the wild species might not be impossible and would be of great value if it resulted in more abundant meiotic material. This would have more

than a merely confirmatory usefulness. It is a well-known fact that in many genuine haploids a limited power of pairing may be conferred on essentially non-homologous chromosomes by small structural lesions such as reduplications or translocations of reduplicated segments. These structural changes within chromosomes may, or may not, alter the genetical content of the nucleus as a whole, and they may or may not be detectable in the external morphology of the plants. The evolutionary importance of structural changes within the monoploid set is, however, far greater than their immediate visible effects might suggest. They introduce changes of behaviour of a wholly

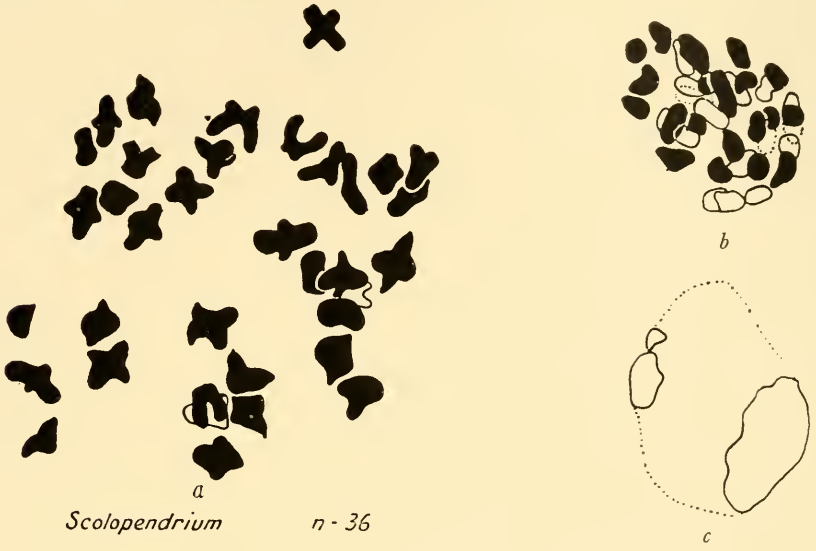


Fig. 210. Explanatory diagram to Fig. 209 *a* and *b*. × 1500.

different type from those so far studied in the Pteridophyta, and any practicable means for bringing them effectively under observation is much to be desired.

So much is this the case that it may be suggested that one of the most fruitful lines of inquiry at present outstanding in the cytology of the British fern flora is the extension of induced apogamy to other wild species besides *Scolopendrium*. That this might not be impossible is suggested by the relatively large number of species represented on Lang's original list, and if it could be done on any scale, more insight might in a short time be gained about the cytogenetic composition of our native species than would be expected to be reached in any other way. It is therefore much to be hoped that this work will be extended.

SUMMARY

The induction of apogamy in two well-known horticultural strains of *Doodia caudata* (Cav.) R.Br. (the strain used by Duncan, 1941) and *Scolopendrium vulgare* (the strain used by Lang, 1929) has been repeated by methods previously described by earlier workers with the object of including cytological observations on them. In both cases the apogamously produced plants were raised to reproductive maturity and meiosis seen. In

Doodia caudata the pairing among approximately 70 chromosomes present is so high as to suggest that though gametophytic this plant is not a haploid but a diploid derivative of an allotetraploid strain. In *Scolopendrium vulgare*, on the other hand, pairing in its 36 chromosomes is so low as to be quite absent in the very few cells which have been seen. In this case, therefore, alone among all the examples of apogamy so far examined, we seem to have a genuine haploid sporophyte. *S. vulgare* itself may therefore be accepted as a genuine diploid species without any ambiguity.

CHAPTER 13

THE GENUS *EQUISETUM*

Turning now away from the ferns to consider some representatives of the other great groups included with them in the Pteridophyta we may take as our first example of a 'microphyllous' (small-leaved) group, the genus *Equisetum*.

The very curious appearance of the Horsetails has already to some extent been illustrated in Chapter 2 and other examples will be found on p. 211 and on p. 226. So striking are they that most European languages are rich in popular names for them, and they are familiar playthings of most country-bred children, at least in the west of the Continent. Their relationship to the ferns is by no means obvious to the layman, and it rests, indeed, primarily on community of life history and on the structure of the sexual generation, details which can scarcely be observed outside a laboratory. An affinity with the Lycopods or 'clubmosses' is perhaps easier to detect, both groups possessing small leaves in contrast to the 'megaphyllous' (large-leaved) ferns, but closer inspection reveals so many differences in anatomy, morphology, form and position of the reproductive structures of both generations that the relatively isolated position of the Horsetails has long been recognized by assigning them to an independent group, the Equisetales, of equal rank to the Filicales (ferns) and Lycopodiales (clubmosses) and of an antiquity at least commensurate with these.

The Equisetales have existed almost from the earliest times at which fossil plants have been preserved. From beginnings traceable with difficulty in the rocks of the Devonian they achieved in the Coal Measure period a burst of evolutionary development which they never repeated. The Calamites of the Coal Measure forests were large trees present in great numbers, both of individuals and of species, while the variety of cone structures actually found must denote the existence of a far greater number of generic types which are lost and which greatly exceed anything that the one living genus might lead one to expect. That the range of early genera may have included *Equisetum* itself side by side with its arboreal relatives is suggested by the finding of a small fertile specimen of Carboniferous age described under the name of *Equisetites Hemingwayi* by Kidston (1892), in which the cone structure is identical with that of the living genus except for the larger size of the sporangia and scales. Unfortunately, the specimen only shows the external form of the plant and not the anatomical structure, without which the generic identity with living species is uncertain; nevertheless, the evidence is clear enough to suggest that *Equisetum* itself may extend in unbroken sequence from the Coal Measures to the present day, and if indeed it did so, we should have to regard it not merely as the sole living representative of a large and ancient group but as itself the oldest living genus of vascular plants known to science. For this reason alone a comparison of its cytological constitution with that of the recent ferns is likely to offer points of unusual interest.

In contrast to the age of the genus and the wealth of extinct species traceable at many geological levels above the Carboniferous period the existing species, some two dozen in

number, are virtually devoid of direct fossil history. Nevertheless, signs of age are not lacking from the evidence of geographical distribution. The total distribution of the living genus is almost worldwide, though Australia and New Zealand contain none and there are relatively few species in the southern hemisphere and in the tropics. With this proviso it is striking that at least half of the living species have a distribution which straddles the entire range of the genus, and all the European species are at least circumpolar. Only in America can there be found any considerable number of species which do not extend beyond that continent. These facts are perhaps most clearly revealed by a citation of the general distribution of the European species, and since only two of these are non-British and all are quoted in Hegi's *Flora*, the information easily obtainable from this source is reproduced in the following list:

Species	General distribution
E. sylvaticum L.	North and central Europe, north Spain, Balkans, north Asia, North America.
E. pratense Ehrh.	British Isles, north- and east-central Europe, Caucasus, Siberia, North America.
E. maximum Lam.	Europe (except Scandinavia and a large part of Russia), west Asia, western North Africa, North Atlantic Islands, western North America.
E. arvense L.	Europe, north Asia, North Africa, Canaries, South Africa, North America.
E. palustre L.	Europe (except for parts of the Mediterranean region), Caucasus, temperate Asia, northern North America.
E. limosum Willd.	Europe (except for parts of the Mediterranean region), north Asia, North America.
E. ramosissimum Desf.	South and central Europe, temperate Asia, India, China, most of Africa (including Madagascar), America both north and south.
E. hiemale L.	Europe (except parts of Mediterranean region), north Asia as far as Japan, North America.
E. variegatum Schl.	Europe (except for the true Mediterranean region and certain parts of the Danube countries, Russia and Denmark), Siberia, North America.
E. scirpoides Michx.	North Europe (Iceland, Spitzbergen, Scandinavia), Siberia and northern and arctic America.

Outstanding in this list are *E. arvense* and *E. ramosissimum*, which are not only circumpolar but present in the southern hemisphere also. Other interesting distributions are those of *E. maximum* and *E. scirpoides*. The former, though circumpolar in total extent, is discontinuously so to an extreme degree, being present on the west sides of Eurasia, Africa and America, but not on the east of these continents. This strongly suggests regional extinction on a large scale, an event which can scarcely be entirely recent. Direct signs of local extinction are given under somewhat different circumstances by *E. scirpoides* (Fig. 211). This is not a British plant, having on the whole a more northerly distribution, being met with right into the Arctic circle in Europe, Siberia and America. It has, or perhaps had, in addition, one outlying station in central Europe, namely, at Heiligenblut near the Pasterze Glacier in Austria. It was well known in this locality a hundred years ago, though it has not been seen recently and may have died out. Since this species is inconspicuous and of no economic or horticultural importance, it is impossible to imagine that either its introduction or its extermination, if it is in fact exterminated, in a station many hundreds of miles away from its main areas of occupation can owe anything to human interference, and it therefore seems necessary to suppose that both events are indicative of important changes of climate. *E. scirpoides* in central Europe seems, in fact, to be a relict from glacial times, and if this is correct, one must

believe that the species once had an even wider range than it shows at present. All these facts indicate a high degree of antiquity for the living species of *Equisetum*, quite apart from the fossil record of the genus.

All the European species have been available to me for study of both mitosis and meiosis. In addition, a very large Horsetail with sparsely branched shoots as thick as one's finger and reaching 15 ft. in height was sent to me from Glasnevin Botanic Garden

Fig. 211. Silhouette of a live plant of *Equisetum scirpoides* Michx. grown in cultivation but originally from Norway. Natural size.

in Dublin, and subsequently identified by Mr Alston of the British Museum as the North American species *E. robustum* A.Br. It proved easy to cultivate and in time bore cones. Lastly, sterile and fertile material of three other forms, not strictly speaking species although frequently treated as such in Floras, have been examined. These forms are *E. trachyodon* A.Br., *E. Moorei* Newman and *E. litorale* Kuhlw., and they will be dealt with separately at the end of the chapter.

A prerequisite to the effective study of any of these species is a fairly detailed knowledge of its coning habits. In a few cases cones could be obtained in culture,* but in a greater number very close observation of wild populations was needed as the only means of obtaining suitable fixations at the right time of year. Since the knowledge so gained may perhaps be of interest to field collectors, being of a kind not easily obtained from books, it may perhaps be of interest to append a few notes about it before considering the cytological results. The seasons at which successful fixations were actually taken may be listed † as follows:

Species	Locality	Month of meiosis
	Subgenus *Eu-equisetum*	
E. arvense L.	Manchester district	September
E. maximum Lam.	,, ,,	,,
E. sylvaticum L.	,, ,,	,,
E. pratense Ehrh.	Westonbirt School, Gloucestershire	,,
E. palustre L.	Manchester district	May
E. limosum L.	,, ,,	,,
E. litorale Kuhlw.	Eastern Ireland	June
	Subgenus *Hippochaete*	
E. ramosissimum Desf.	Italy (Apennines)	July
E. hiemale L.	Newcastle district	September
E. robustum A.Br.	Cold greenhouse	,,
E. variegatum Schleich	Southport sand-dunes	May
	Royal Dublin Canal	June
E. trachyodon A.Br.	Eastern Ireland	,,
E. scirpoides Michx.	Pot culture	September
E. Moorei Newman	Eastern Ireland	August

At first sight these dates may appear somewhat arbitrary, but it is not difficult to correlate them with some fairly simple general habits of the various species. Thus all those species with *deciduous* aerial shoots on which the cones are also borne will show young sporangia with maturing mother cells on the newly emerging green stems in the spring, that is, in about the month of May in Great Britain. The principal representatives of this type are *E. palustre* and *E. limosum* (cf. Fig. 5, p. 18), in both of which the young cones can indeed be found in the resting buds of the previous autumn but only in a very immature condition. The *non-deciduous* species, e.g. *E. hiemale*, *E. variegatum*, *E. Moorei*, *E. trachyodon*, and probably *E. scirpoides*—though I have not seen this non-British species in its native haunts—mature their cones later in the summer or during a longer season. Thus meiosis can be obtained under normal climatic conditions in all of these species at the end of August, though in some, notably *E. variegatum* and perhaps *E. hiemale*, a continuous succession of new shoots, some of them bearing cones, may be

* Most of the species listed may be grown easily in ordinary garden soil without any special treatment except reasonable freedom from desiccation. My own plants were grown in the ground in an unheated partially shady fern-house, the only precaution taken being to enclose them in brick compartments lined with cement to prevent the rhizomes from escaping into other parts of the house, since they become very troublesome weeds if allowed to become rampant. Under these conditions vegetative growth was luxuriant but cones were rare, probably through lack of light. The small species, e.g. *E. scirpoides* and *E. variegatum*, will, however, cone quite readily in pot culture, and *E. limosum* coned annually when grown in a large pot submerged in an artificial pond. It is probable that *E. palustre* might do the same though this species was not actually grown.

† The classification adopted here is primarily that of Milde (1867).

found from June to September. *E. ramosissimum*, which I have only seen wild in north Italy, seems to be somewhat exceptional in that there appears to be a succession of cones

Fig. 212. *Equisetum maximum* Lam. in late September, showing two cone buds and the base of a sterile aerial shoot all borne on the same node at the base of a sterile shoot of the previous year, from a living specimen. Natural size.

during a long season, not on new shoots springing up from the rhizome but on the tips of branches of different order on the aerial stems. Thus my own material was fixed in the Apennines in July in manifestly late cones borne on the tips of lateral branches, the

main branches from which the laterals were emerging having presumably been fertile earlier in the year.

Fundamentally different habits are met with in those species which bear their cones either on separate short, colourless shoots or on special colourless apical portions of green shoots. These species are *E. arvense*, *E. maximum*, *E. pratense* of the first type and *E. sylvaticum* of the second. All these four species shed their spores very early in the spring (March or April), in the first three cases before any green vegetative shoots are visible, and maturation of the spores in each is completed in the previous autumn.

Since the cones of these species are perhaps less familiar in autumn than they are in spring, a photograph, natural size, of *E. maximum*, dug up in late September, is reproduced in Fig. 212. At this time structurally perfect green spores are already present in the fat winter buds which enclose and protect the dormant cone under many sheaths of russet brown scales. The buds, two of which are seen in the figure, project an inch or more above the ground, and are as a rule to be found near the base of a standing green shoot of the current year. The base of this is seen to the left of the cone buds, and if both it and the buds are traced down to their origin it can be seen that both arise as lateral branches from the base of a still older shoot of the previous year. The habit of spring shedding of spores seems therefore to represent a delay in the liberation of spores which morphologically belong to the year in which meiosis occurs rather than to precocious development.

Meiosis takes place in these cone buds in early September, when they are already above ground but not at their full size. The cone is easily exposed by splitting the bud or by peeling off the sheathing leaves. It is soft, white and juicy when meiosis is occurring, becoming yellow with greenish sporangia when the spores are complete. In the young stages the cones seem to be very attractive to small animals which I take to be mice, since they are often found partly or completely eaten away.

Although the cone buds in all four species (*E. arvense*, *maximum*, *pratense* and *sylvaticum*) are actually above ground at the time of meiosis and may be found readily enough by careful examination of the soil surface near the base of a standing shoot, it is in practice unprofitable to do this unless a coning site has first been marked down with some accuracy in a previous spring. In every species very large areas of ground may be occupied by vigorous but sterile populations. Good coning sites seem to be topographically determined as places with rather better drainage and fuller illumination than those which suffice for vegetative growth. This is perhaps the reason why *E. arvense* is so often found to cone abundantly on railway banks. Once a coning site has been detected, fertile plants will recur there year after year with unfailing regularity, unless the ecological conditions alter. The problem of finding fertile material of *E. arvense*, *maximum*, *pratense* and *sylvaticum* is then not difficult.*

Within the cone itself the order of maturation of the various parts is the same in all species. The first sporangia to mature are those in the widest part of the cone, and de-

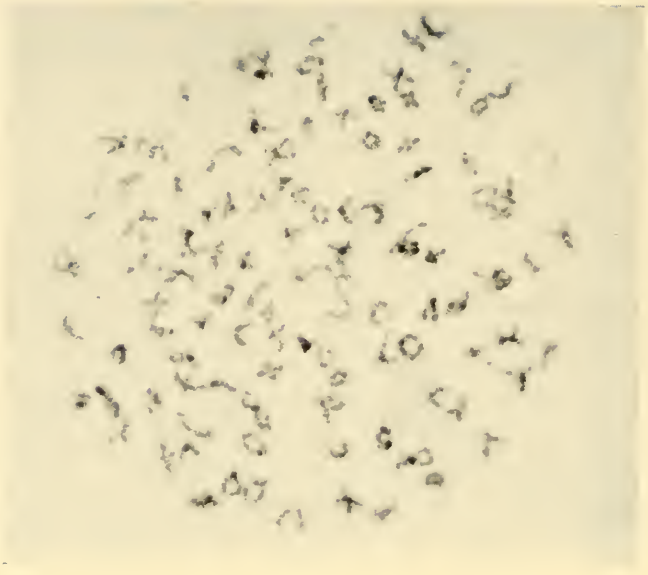
* The sites actually used for this work were: for *E. arvense*, a dump of disused builders' sand in a meadow near Stockport; for *E. maximum*, the raised bank of a canal near Stockport; for *E. sylvaticum*, a clearing in a steeply sloping wood near Stockport; and for *E. pratense*, a well-drained east-facing rock garden in the grounds of Westonbirt Girls' School, where this rare species has become established and cones annually.

velopment proceeds on the whole basipetally, although some young stages also remain at the extreme apex. This is a very different sequence of events from that followed by the leaves of a vegetative bud, and is perhaps an additional reason for thinking that the 'sporangiophores' may not be foliar in nature. Since even in the tiny cones of *E. scirpoides* a considerable number of sporangia are contained, it is usual to find every stage of meiosis in one cone, and quite a range of stages in one sporangium. The reason for this is that the mother cells do not form a uniform tissue but are separated into compact pockets of a few mother cells surrounded by plasmodial tapetum. Every cell of a pocket will be at the same stage, but adjacent pockets may be quite widely separated both in space and in stage of development. This may perhaps be seen in Figs. 5*c* and 217*b*.

All species of Horsetail respond to normal methods of modern fixation excellently and give very beautiful preparations, as the photographs in the pages which follow will perhaps testify. This is a very fortunate circumstance, because even with this advantage we step, with *Equisetum*, into a totally different order of difficulty from anything which we have so far met with in the ferns. This difficulty is reflected in the hopelessly discordant results obtainable from the literature, which may be quoted more as a warning than as an example. Thus Tischler (1935*a*) lists $2n = 24$ for *E. limosum* (Steinecke, 1932); $2n = c.\ 30$ for *E. arvense* (Lenoir, 1926); $2n = c.\ 140$ for *E. palustre* (Lenoir, 1932), while de Beer (1913) gave $n = c.\ 115$ for *E. arvense*, and very recently Hagerup has reported (see Löve and Löve, 1948) $2n = 230$ for the majority of European species.

In my experience all these records are wrong though in differing degrees, the last two being slightly too high but all the others being very much too low. The main cytological difficulties in estimating chromosome number here are three—the number itself is high, the chromosomes are very varied in size and shape, but, thirdly, their shapes are so peculiar that at first sight of meiosis they may be very misleading and may even suggest the presence of multivalent pairing, as a comparison of Figs. 213*b* or 222*c* with the photographs of polyploid *Biscutella* on p. 8 will perhaps demonstrate. The meaning of these curious appearances is only imperfectly understood, though it seems to lie in a peculiar weakness of the spiral structure in which the gyres tend to fall apart rather easily under the natural stresses of the forces at work in the cell, although as may be seen from Fig. 228, p. 230, the spiral can be quite normal in unpaired chromosomes. This type of behaviour I have seen occasionally in otherwise normal plants of *Osmunda* (Manton, 1945) under special metabolic conditions, and it is therefore unlikely to denote any very fundamental difference between *Equisetum* and other plants. The closely similar behaviour of certain Lycopods may, however, perhaps denote a measure of phyletic affinity with that group.

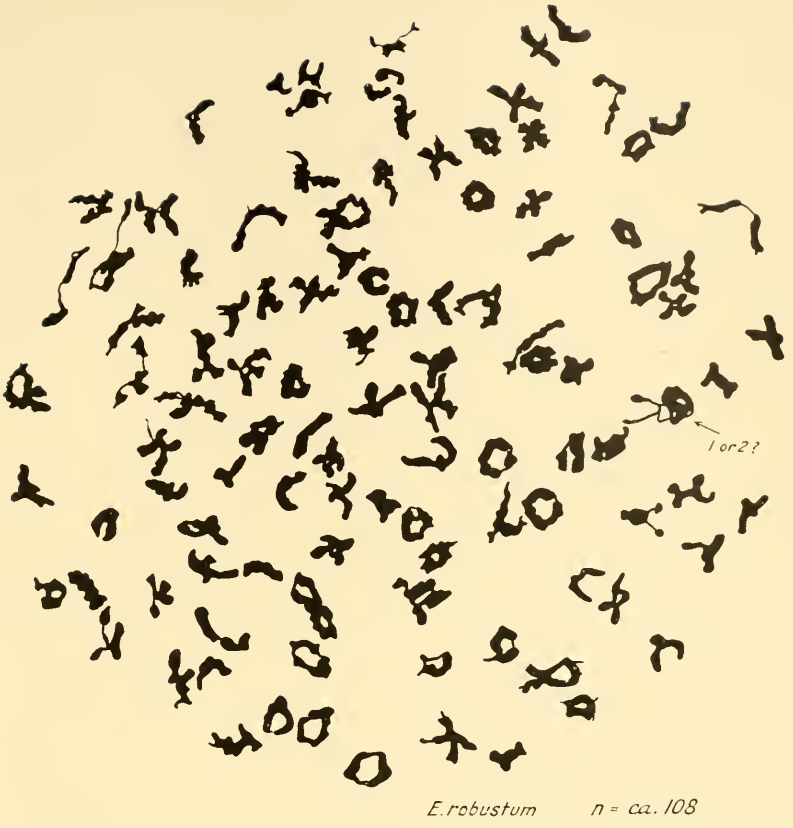
Before discussing the numerical evidence in detail it may be well to glance through an array of preparations of different stages in different species. These are contained in the photographs of Figs. 213–223. Diakinesis, metaphase 1 and anaphase 2 in four species of the subgenus *Hippochaete* are contained in Figs. 213–216. Precisely comparable preparations of two species of the subgenus *Eu-equisetum* are contained in Fig. 222*b* and *c*. A marked difference of chromosome size may be noted in comparing these two groups of figures. This difference seems to be characteristic of the two subgenera, all the forms examined of *Eu-equisetum*, namely, *E. limosum*, *palustre*, *arvense*, *pratense*, *maximum*, *sylvaticum*



a

b

Fig. 213. Permanent acetocarmine preparations of two large-chromosomed species of horsetail. × 1000. For explanatory diagrams see Fig. 214. In both $n = 108$. *a*. Diakinesis in *Equisetum robustum* A.Br. from America. *b*. First meiotic metaphase in *E. variegatum* Schleich. from Britain (Southport).

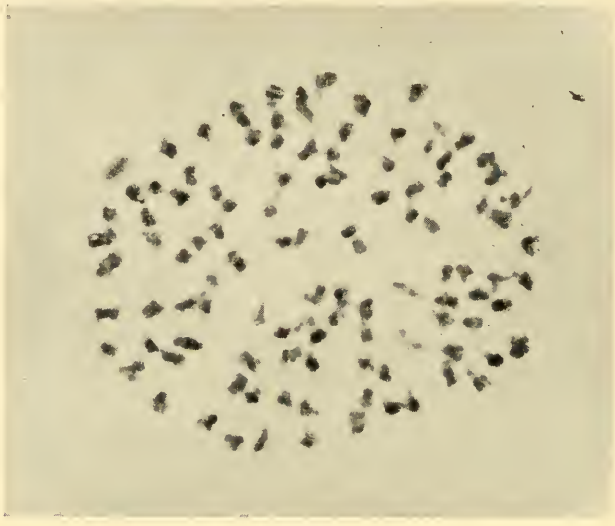


a

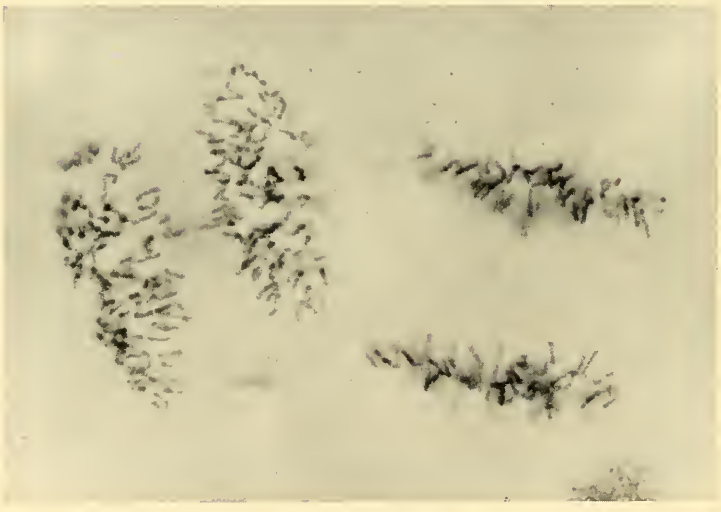
b

Fig. 214. *a*. Explanatory diagram to Fig. 213*a*. × 1500.
b. Explanatory diagram to Fig. 213*b*. × 1000.

and *litorale*, having smaller chromosomes than members of the other subgenus, which, in this study, have been represented by *E. variegatum*, *hiemale*, *scirpoides*, *ramosissi-*



a



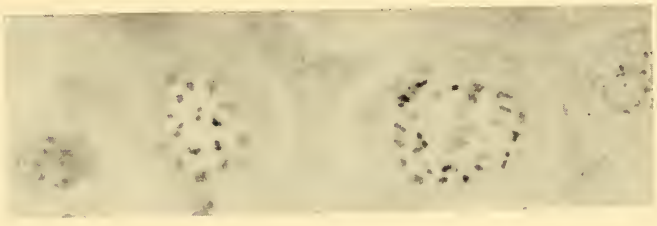
b

Fig. 215. Permanent acetocarmine preparations of two additional large-chromosomed species of horsetail. × 1000. In both n = almost certainly 108. *a*. *Equisetum hiemale* L. from Northumberland, the first meiotic metaphase. For explanatory diagram see Fig. 216. *b*. *E. scirpoides* Michx., the second meiotic anaphase to show shapes of the chromosomes at this stage.

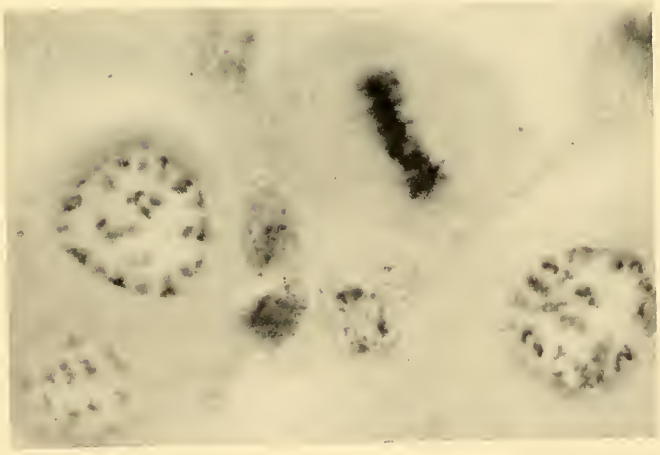
mum, *robustum*, *trachyodon* and *Moorei*. Though the subgenera are primarily distinguished by the structure of their stomata, the agreement between this character and relative chromosome size suggests that the subdivision is a natural one and of long standing.

E. hiemale $n = 108$

Fig. 216. Explanatory diagram to Fig. 215*a*. × 1500.



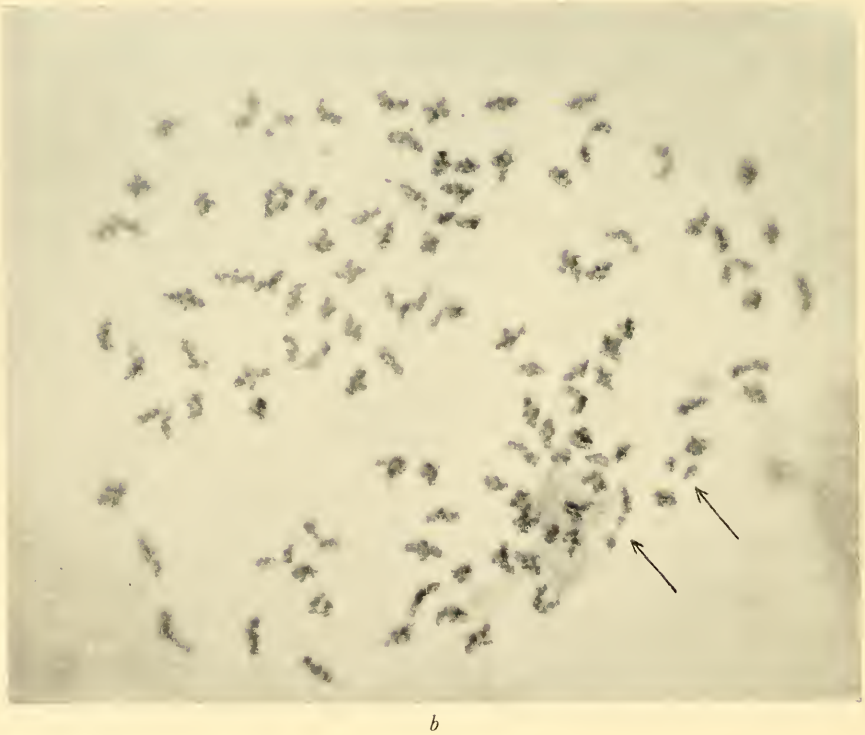
a



b

Fig. 217. Comparison of chromosome size at diakinesis in a large and a small chromosomed species of horsetail, from sections. × 1000. *a. E. arvense* L. (see further Fig. 218). *b. Equisetum robustum* A.Br. (cf. Fig. 213*a*).

a



b

Fig. 218. Meiosis in *Equisetum arvense* L. in two different techniques photographed at the same magnification. × 1000. *a*. A Feulgen squash in balsam. For explanatory diagram see Fig. 219. *b*. A fresh mount in acetocarmine. For description see text.

A further illustration of both absolute and relative sizes of the chromosomes at a comparable stage in one species of each of the two subgenera is given, from sectioned material, in Fig. 217. In the upper photograph, showing diakinesis in *E. arvense*, the chromosomes are distinctly smaller than in the lower photograph of *E. robustum*. This demonstration has been included partly for comparison with sectioned preparations illustrated in other chapters but also because *Equisetum* represents an extreme example of the extent to which chromosome size can be affected by different technical treatments

Fig. 219. Explanatory diagram to Fig. 218*a*. × 1500.

without the production of any other visible distortion. The fact that acetocarmine almost doubles the apparent dimensions is one of the many reasons for the great usefulness of this reagent, but a very misleading impression could be produced by mixing one's techniques if it were wished to do so. Thus Fig. 218 shows on the same page two cells of the same species, *E. arvense*, already illustrated in Fig. 217*a*, photographed at the same magnification (× 1000). The upper figure is from a Feulgen squash mounted in balsam. The lower figure is from a fresh acetocarmine squash photographed in that liquid. The exceptional clarity of the latter specimen was due partly to the fact that it had become spread in close contact with the glass of the cover-slip, and the optical disadvantages of observation in a liquid of low refractive index were therefore absent. The cell was also exceptional in the extreme degree of enlargement which the chromosomes had

undergone. Some of this enlargement is reversible and would have disappeared had the specimen been successfully transferred to balsam, but it unfortunately became detached and was lost in the attempt, and a second photograph more comparable to the other could not therefore be taken.

The numerical evidence from these and other preparations is as follows. All the species of *Equisetum* examined have the same chromosome number to a very close approximation. This falls certainly between the limits of 106 and 112, the haploid chromosome number being quoted. It is probable, indeed almost certain, that the chromosome number of all of them is actually 108.

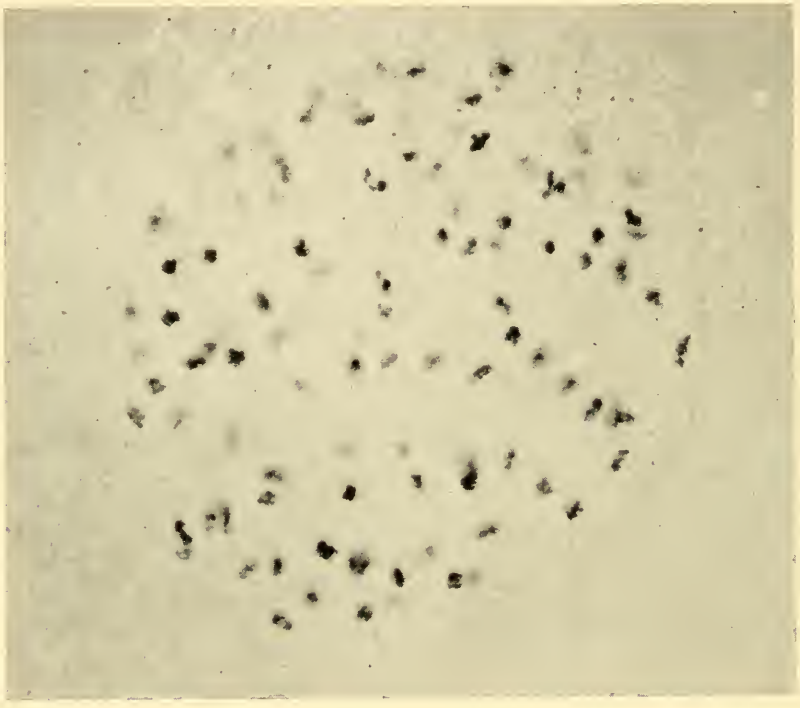


Fig. 220. Feulgen squash in acetic acid of meiosis in *Equisetum maximum* Lam. × 1000. For further information see Fig. 221.

There are, however, two difficulties to which attention should be drawn before this number is accepted. In all the large-chromosomed species, that is, in the subgenus *Hippochaete*, there is a tendency for counts to exceed this number by one or two. For these therefore the number 108 is a minimum value. In the small-chromosomed species of the subgenus *Eu-equisetum*, on the other hand, the difficulty is the other way. For these the number 108 is a maximum, and one is often tempted to wonder whether the correct value is not perhaps one or two less. The reason for this is perhaps psychological, in that large chromosomes are sometimes more easily misinterpreted than small ones in the direction of excessive subdivision, since very odd-shaped groups can deceptively resemble two pairs in accidental contact. An example of such a case is marked by an arrow in Fig. 214*a*, and a very large figure-of-eight pair on the left of the same nucleus

could be similarly interpreted; this cell could therefore possess a maximum number of 110. On the other hand, with small chromosomes of rather unequal sizes it is perhaps more likely that a tiny pair lying near another might be falsely regarded as part of it. Examples of doubt of this kind are shown by the arrows in Fig. 219, which refers to the Feulgen squash of *E. arvense* in which the maximum number is 108 though it could be 107 or 106. That the last alternative is incorrect is, however, proved by Fig. 218*b*. In this there are also two doubtful places, but the range of possible numbers is 107–110. The correct interpretation of this cell is probably that the upper doubtful place is actually one pair with the halves pushed somewhat asunder, while the lower doubtful

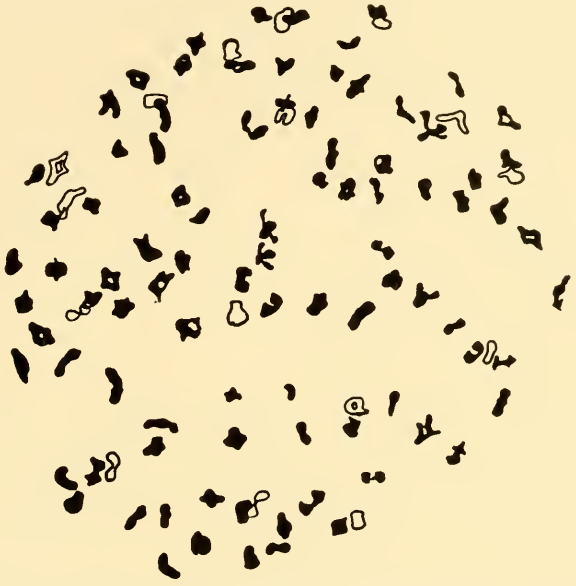


Fig. 221. Drawing of the cell of Fig. 220 made whilst it was still fresh. × 1000. In addition to the 107 bodies shown there were four small granules which could have been the arms of a dismembered pair.

place is composed of two rather more compact and smaller pairs held deceptively near one another by their attachment to the surface of the nucleolus. It must be recognized, however, that a genuine difference of one or two chromosomes between *Eu-equisetum* and *Hippochaete* would be very difficult to demonstrate and is therefore almost equally difficult to disprove. Should it exist, all these estimates will need slight revision, but if it does not the only number which will fit the facts for every species is $n = 108$.

The general uniformity of these cytological results coupled with the signs of antiquity to be found among the living species might, at first sight, lead one to suspect that evolutionary activity within the genus *Equisetum* is perhaps becoming exhausted. Direct evidence that some at least of the raw materials of evolution are nevertheless present is, however, provided by the surprising number of interspecific hybrids which have been discovered in Ireland. That this small country is specially favoured in this respect is probable from the known rarity of Horsetail prothalli in most other countries. No actual records of gametophytes exist for Ireland, but it may safely be assumed that its mild, moist, oceanic climate is likely to favour their production.

The three cases of hybrid *Equisetum* available to me were all provided either by, or with the help of, Dr Praeger of Dublin. Two of them, *E. litorale* and *E. trachyodon*, were known to be hybrids from the literature, the third, *E. Moorei*, was expected to be a pure

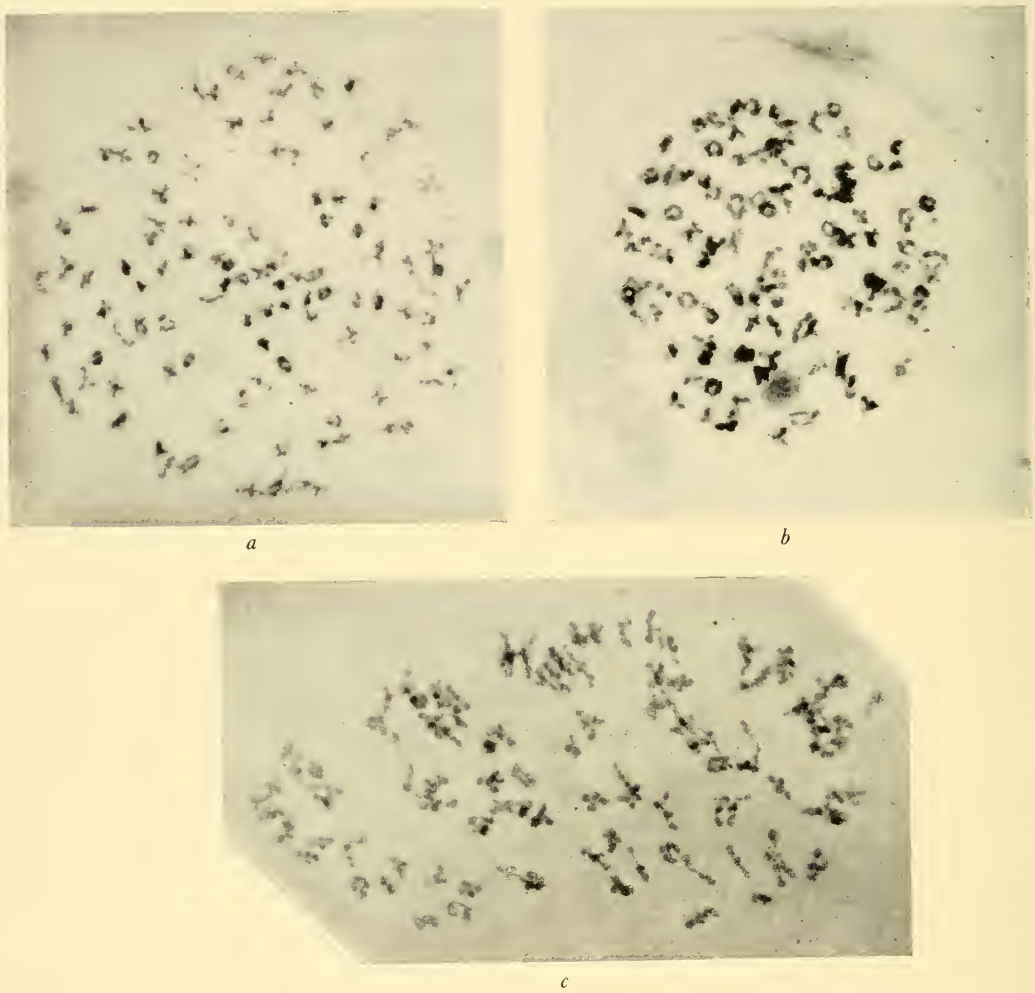


Fig. 222. Two additional small-chromosomed species of horsetail for comparison of sizes and shapes with Figs. 213 and 215. × 1000. *a*. *Equisetum limosum* L. diakinesis in a Feulgen squash. For explanatory diagram see Fig. 223. $n = 107–108$. *b*. *E. palustre* L. diakinesis in acetocarmine showing more usual size appearance with this technique in contrast to the exceptional degree of enlargement shown in Fig. 218*b*. *c*. *E. limosum*, metaphase for comparison with the large-chromosomed species shown in Fig. 213*b*.

species or subspecies, and it was with considerable surprise that unmistakable cytological signs of hybridity were discovered in it.

It will be convenient to consider *E. litorale* first. This plant (Fig. 224), first found in central Europe, was regarded as a probable hybrid between *E. arvense* and *E. limosum* by Milde in 1867 on the ground both of its intermediate morphology and its abortive

spores, and although Milde began to doubt this diagnosis in later life owing to the increase in the number of recorded localities, his original reasons are still valid. The abortive spores in themselves suggest hybridity even more clearly to us than to a botanist in Milde's day, and the morphology of the plant is not only intermediate between the two suspected parents but ranges rather widely between the two extremes in different localities, thus suggesting that it is either re-formed from time to time from different strains or that a limited degree of fertility exists in it and therefore some genetical segregation.

I am indebted to Dr Praeger for supplying me with the distribution map shown in

Fig. 223. Explanatory diagram to Fig. 222*a*.

Fig. 225, which indicates that *E. litorale* is scattered widely over Ireland with a frequency which bears some relation to the closeness of sampling; the abundance of localities in the Belfast area, for example, being probably diagnostic of the alertness of the Belfast Natural History Society rather than denoting an actual preference for this region. Living material from two sources was sent to me. In one, which came from Galway Bay, partially expanded cones much resembling *E. arvense* but with wholly aborted spores were too old to investigate. The second specimen came from eastern Ireland and grew for many years in cultivation, but gave only sterile shoots much resembling *E. arvense* in appearance. A visit to the wild locality of this material in June 1938 with Dr Praeger showed it (Fig. 224) to be in habitat an aquatic plant as is *E. limosum*, though with a solid branched stem like *E. arvense*. The cones were, however, borne exclusively on the ends of the current green shoots as in *E. limosum*. From these, successful fixings

Fig. 224. Silhouette of a pressed fertile shoot collected in June of the Irish material of *Equisetum litorale* Kuhlw. Natural size.

Fig. 225. Map of the distribution of known localities of hybrid horsetails in Ireland, from information kindly supplied by Dr Praeger.

were obtained, and the section shown in Fig. 226 at once gives confirmation of the diagnosis of hybridity and a reason for the abortion of the spores. Fig. 227*a*, from an acetocarmine preparation, adds details of the pairing and shows again the large number of univalents.

In the hope of synthesizing *E. litorale*, prothalli of the two parent species were grown in 1939, and insemination from young males of *E. limosum* into old females of *E. arvense* was watched under the microscope. Unfortunately, the difficulties of culture were not successfully surmounted for the later stages and all the inseminated prothalli died from fungus attack. If these cultural difficulties could be overcome the synthesis of *E. litorale* ought to be possible.

E. trachyodon A.Br. is known in Floras as a very rare European plant in which defective spores were detected as long ago as 1864 by Duval-Jouve. It is best known from the

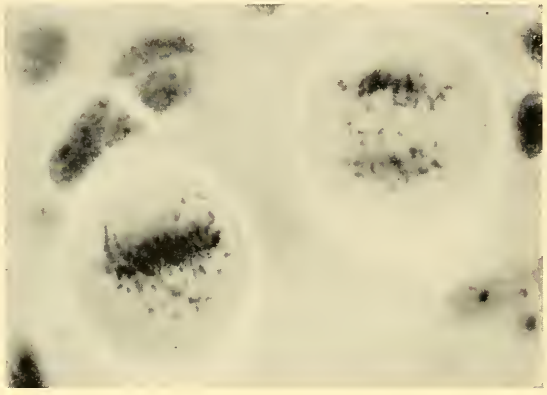


Fig. 226. Meiosis in *Equisetum litorale* Kuhlw. in a section showing lagging unpaired chromosomes. × 1000.

Rhine valley and from Ireland, although single stations are now on record for a few other countries, e.g. Scotland and Latvia (Kupfer 1929). In contrast to *E. litorale*, *E. trachyodon* is very uniform morphologically wherever it is found, although Milde was able to detect a few minor differences between the Irish and Rhineland specimens. As in *E. litorale* the spores of *E. trachyodon* are completely aborted, and the means for its very wide distribution are at present quite unknown.

As in the previous case, my material of *E. trachyodon* came from Ireland. I visited an east Irish locality with Dr Praeger in June 1938, and fixed wild cones, but this particular plant cones also readily in cultivation and abundant material is easily obtained. The principal results are perhaps sufficiently shown by Figs. 227*c* and *d*, representing diakinesis and metaphase (or anaphase) respectively. The almost complete absence of any sign of pairing is the most characteristic feature, although a solitary bivalent can be seen on the spindle in Fig. 227*d*.

Failure of pairing on this scale is the obvious explanation for abortion of spores, and it is not, in fact, more extreme here than in some other well-authenticated wild hybrids, notably some of the triploid ferns such as the *Polypodium* of Fig. 139. It should,

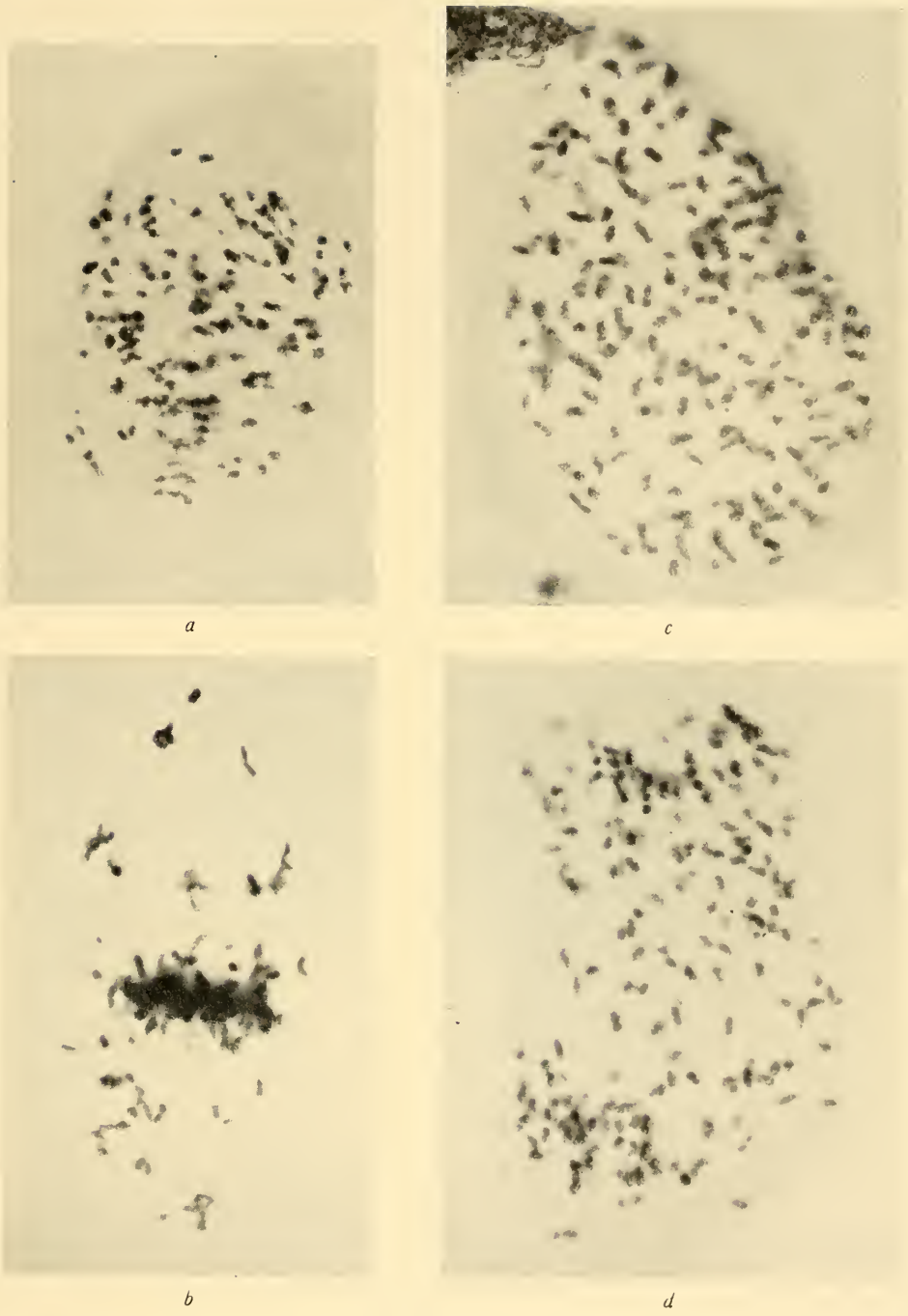


Fig. 227. Meiosis in three hybrid horsetails in permanent acetocarmine mounts. × 1000. *a. Equisetum litorale* Kuhlw. first meiotic metaphase showing pairs and univalents. *b. E. Moorei* Newm. the same showing pairs, univalents and multivalents. *c. E. trachyodon* A.Br. diakinesis showing complete failure of pairing. *d.* The same at anaphase of the first meiotic division showing one pair on the equator and the remaining unpaired chromosomes distributing themselves at random to the poles.

however, be noted that complete failure of pairing can sometimes be produced by other means, either metabolically or owing to an asynaptic mutation. If the latter were the case here, however, it is difficult to see why an asynaptic mutant should be the only representative of a species to survive and positive evidence for the former is wholly lacking. Indeed, in an attempt to alter the grade of chromosome pairing experimentally, a piece of the plant was transferred to a hot greenhouse and maintained at tropical

Fig. 228. Spiral structure at anaphase of the first meiotic division in *Equisetum trachyodon* A.Br. × 2000.

temperatures (70° F.) for over two years without the slightest effect. A hybrid origin therefore remains the most probable explanation for it, and the most probable parents in that case are *E. variegatum* × *E. hiemale.**

In spite of the absence of experimental proof of the suggested parentage of *E. trachyodon* there would probably be little reason to look elsewhere for a mode of origin but for the circumstance that the third case of an apparent hybrid, *E. Moorei*, seems to relate to the same two species, although it is itself quite unlike *E. trachyodon* in habitat and appearance. *E. Moorei* is a maritime plant of sandy soil found for about 100 miles along

* A relationship with the south European *E. ramosissimum* has sometimes been suggested, which I was at first inclined to disregard since this species was thought not to be British. I have however recently been informed by Mr. A. H. G. Alston of the British Museum that an authentic specimen of *E. ramosissimum* has recently been found in Norfolk and it may therefore perhaps also have existed in Ireland in former times. If this were so it would greatly assist the problem of finding suitable parents for both *E. trachyodon* and *E. Moorei* in that country.

the coast of Wexford (*E. trachyodon* is a plant of river margins). It has relatively stout erect green shoots more like those of *E. hiemale* than of *E. variegatum*, though with characteristic details of the surface markings of its own. It cones in August. Fig. 227*b* shows metaphase I in *E. Moorei*. The very irregular pairing, including multivalents and univalents, is very striking. This, together with abortive spores, is a clear indication of hybrid origin, and though speculation might be possible to explain the coexistence of both *E. Moorei* and *E. trachyodon* in Ireland, it is more profitable to defer this until a hybrid of appropriate kind has been synthesized. Until this has been done it is perhaps best to confine our present conclusions to the statement that *E. Moorei* is not a good species in the usual sense of the word and to commend it to Irish botanists for closer scrutiny.

The detection of three species hybrids among little more than a dozen representatives of the genus is a surprisingly large number, especially when the rarity of prothalli is remembered, and it suggests fairly clearly that speciation can still occur. The complete absence of polyploidy is, however, noteworthy, and the extreme uniformity in chromosome number found throughout the genus is also in striking contrast to the behaviour of ferns. At the same time a haploid number as high as 108 cannot be thought of as primitive, though we are never likely to know by what stages or from what simpler state it was evolved. As we find them now, the Horsetails give the impression of being a very ancient and a very stable group, still able to make new species by genetical means though doing so only slowly, but long out of the habit of giving rise to new generic types. The very stereotyped morphology is perhaps not so much primitive as specialized upon an archaic pattern, and the general cytological condition is perhaps also best interpreted as ancient rather than simple.

SUMMARY

Mitosis and meiosis in all the European species of *Equisetum* and of one American species have been seen. In all, the haploid chromosome number is either exactly or approximately 108, though a slight possibility remains of a minor numerical difference between species of the two subgenera, *Eu-equisetum* and *Hippochaete*. There is a difference of chromosome size between these subgenera, the latter having the larger chromosomes. Three Irish forms, namely, *Equisetum litorale*, *E. Moorei* and *E. trachyodon*, have been shown to have the meiotic behaviour of hybrids. Spiral structure of chromosomes has been demonstrated in *E. trachyodon*. The list of species and hybrids examined is:

Subgenus *Eu-equisetum*

Species	Source	*n*
E. arvense L.	Manchester	Probably 108
E. maximum Lam.	,,	,,
E. sylvaticum L.	,,	,,
E. pratense Ehrh.	Hort.	,,
E. palustre L.	Manchester	,,
E. limosum L.	,,	,,
E. litorale Kuhlw.	Ireland	Irregular meiosis

Subgenus *Hippochaete*

Species	Source	*n*
E. ramosissimum Desf.	Italy	Probably 108
E. hiemale L.	Durham	,,
E. robustum A.Br.	Hort.	,,
E. variegatum Schleich.	Southport	,,
Large form	Dublin	,,
E. scirpoides Michx.	Norway	,,
E. trachyodon A.Br.	Ireland	Irregular meiosis
E. Moorei Newm.	Ireland	,,

CHAPTER 14

THE PSILOTALES

The Psilotales are a microphyllous group without close relatives and without known ancestry. They are microphyllous in the sense that they are certainly not megaphyllous like the ferns, though it is an open question whether in fact they possess leaves at all, the curious little appendages borne on the aerial portions of their stems being so unlike leaves in the ordinary sense that they are perhaps not of this nature. *Psilotum* and *Tmesipteris* are the only known genera, each containing only one, or at most two or three species, none of which is native to Europe or nearer to our shores than the tropics and the southern hemisphere. Nevertheless, so important are they in all morphological discussion of the Pteridophyta that they cannot be omitted. In their complete absence of roots and in other relatively simple features they are now generally regarded as in all probability the most primitive of living vascular plants, and if any relationship can be established with other members of the Pteridophyta it is likely to be with the long-extinct Psilophytales, themselves the simplest vascular plants known to science, rather than with any more recent group.

Fortunately, though not native to Europe, examples of both genera are available in botanic gardens and, in addition, I was in the uniquely favourable position of having been entrusted in 1939 with the cytological examination of both sporophytes and gametophytes collected in New Zealand by the late Dr Holloway of the University of Otago and described by him in the *Annals of Botany* of 1938 and 1939. There were special peculiarities about Dr Holloway's prothalli which made a cytological study of them desirable, and the cytological findings were published in detail in 1942 (Manton) to preserve them from risk of loss by enemy action. The observations recorded in that context are still the most important contribution that I personally have to make to knowledge of this group, but through the kindness of Professor H. N. Barber of Tasmania, who has communicated privately some additional observations from Australian material with permission to quote them, a few additional facts can now be supplied. Since I understand that Professor Barber is also himself engaged on further study of both genera on native Australian material, the account to be given below need only be regarded as an interim statement, of value in the present connexion for comparative purposes with the other great groups, and as neither final nor exhaustive in itself.

Members of the genus *Psilotum* are found in the tropics right round the globe, though they are familiar objects in most botanic gardens since they are fairly easy to grow and, in addition, reproduce themselves by bulbils which are very easily transported. They are therefore liable to spring up unexpectedly as weeds in tropical glasshouses where pot plants are grown, and may have been repeatedly introduced to Europe along with other plants. This is partly the reason why a place of origin can rarely if ever be assigned to botanic garden material, and even in Japan, the only country in which they appear ever to have been extensively grown for horticultural purposes (Okabe, 1929), it cannot be

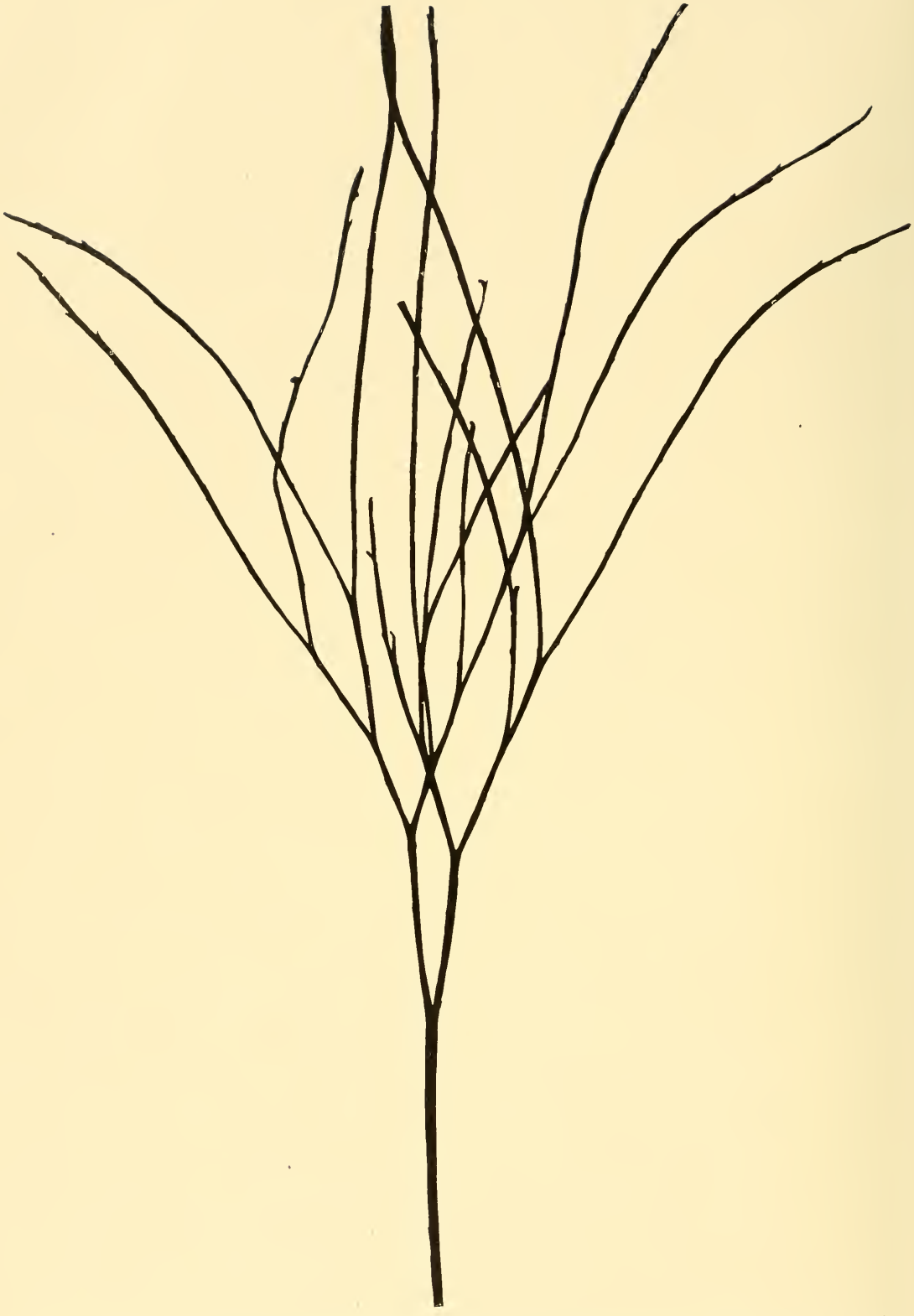


Fig. 229. Shoot of tetraploid *Psilotum nudum* (L.) Beauv. (*P. triquetrum* Swartz) of unknown wild origin, grown at Kew. From a herbarium specimen from which the sporangia fell off as a result of drying. Natural size.

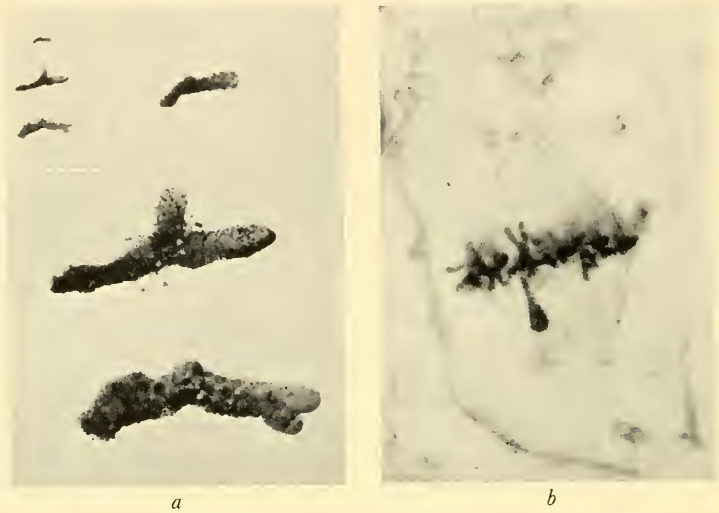
certainly known that all strains are of Japanese origin. For this reason the wild material to be described below is of particular interest.

Of recognized taxonomic species there are two: *P. flaccidum* Wall., Fig. 230, a rather uncommon pendulous form with flattened stems and *P. nudum* (L.) Beauv. (*P. triquetrum* Swartz), Fig. 229, with stiff, erect stems, which is much more frequent. To this species Dr Holloway's prothalli belonged, and since the prothalli were both the starting point and the centre of interest of my personal connexion with the whole group, it will perhaps be appropriate to start with them.

The sexual generations of the Psilotales have so far only been found in the southern hemisphere and by very few observers. The first gametophytes of *Psilotum* were detected near Sydney, Australia, by Darnell Smith and by Lawson, both of whom published notes about them in 1917, though not all stages required for a complete description of the life history were present. They were apparently not found again until Holloway did so on Rangitoto Island, Auckland, New Zealand, where numerous examples were obtained, many of them bearing young plants, on each of two visits made with an interval of seven years between. These prothalli were the basis of Holloway's paper of 1938 and of my own paper of 1942. In the meanwhile Holloway had also completed the life history of *Tmesipteris* by finding prothalli and young plants of this genus also in New Zealand (Holloway, 1917, 1921). By the activities of this one worker the main developmental facts for both genera of the Psilotales have therefore been elucidated, though the cytological interpretation is still somewhat imperfect, as will shortly be seen.

Fig. 230. Part of a diploid shoot of the pendulous *Psilotum flaccidum* Wall. of unknown wild origin, grown at Kew, from a herbarium specimen. Natural size.

The prothalli of both *Psilotum* (Fig. 231) and *Tmesipteris* are small cylindrical structures of a few millimetres or perhaps centimetres in length in the largest specimens, living underground with the aid of a symbiotic fungus. Branching is dichotomous and growth is by an apical cell as in the subterranean rhizomes of the sporophyte, which they indeed closely resemble except for the numerous sex organs which they bear over their surface. An additional point of resemblance met with in the Rangitoto material of *Psilotum* was the presence of vascular tissue. This was found only in the largest prothalli and it varied in extent. In the simplest cases the centre of the prothallus was occupied by a group of elongated thin-walled cells free from fungus, as may be seen in Fig. 232*a*. In other cases an endodermis could be seen surrounding such cells, and



a *b*

Fig. 231. The vascular prothalli of *Psilotum nudum* from Rangitoto Island (see text). *a*. Intact prothalli preserved in 70% alcohol. ×4. Inset natural size. *b*. A dividing cell from a prothallus from this locality, after Manton (1942). ×1000.

in yet other cases the central strand contained actual tracheids with lignified walls. Some of these can be seen in the longitudinal view photographed by Mr Ashby from one of Holloway's original preparations reproduced in Fig. 233. The prothallial vascular strand was liable to be interrupted at intervals by the invasion of the entire central tissue by the symbiotic fungus, in which case the central cells remained undifferentiated, and in the smaller prothalli, which formed the bulk of the collection, they were quite absent. They were, however, obtained from different parts of the island on each of Dr Holloway's two visits, and could therefore not be dismissed as local malformations, and it was their presence which prompted Dr Holloway to seek for a cytological investigation.

The results of such an investigation carried out on material supplied by Dr Holloway in alcohol after fixation in acetic-alcohol were that all sizes of prothalli from Rangitoto Island were diploid, and that the sporophytes on the island to which they were no doubt related were tetraploids. In addition to being tetraploids, which would not in itself explain the presence of vascular tissue in the prothalli, a very characteristic and

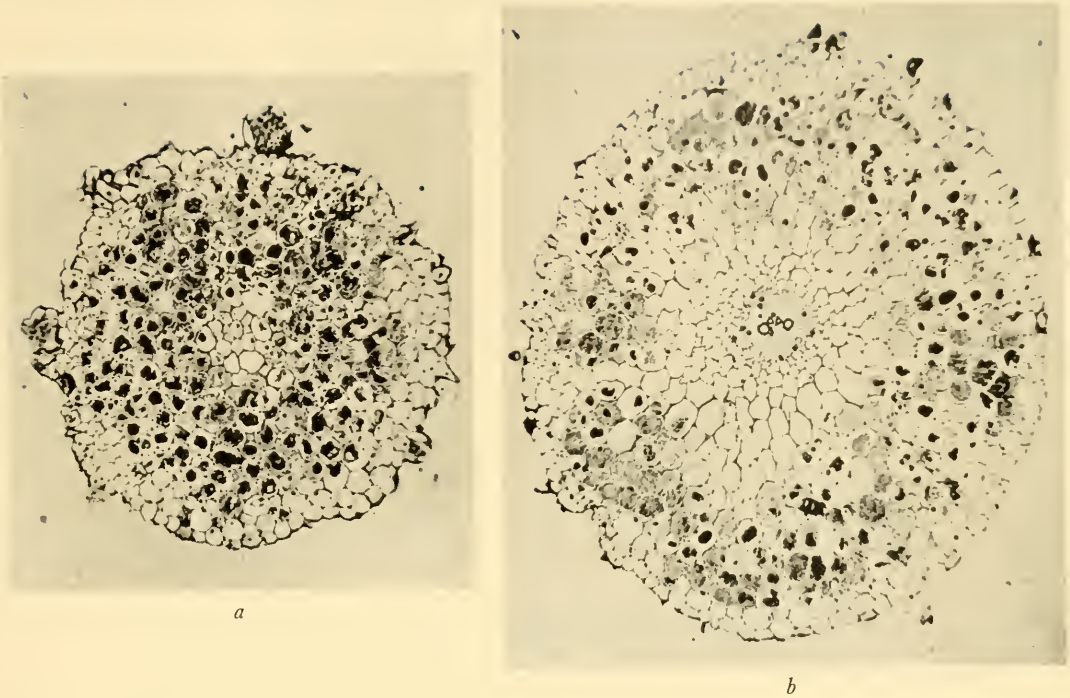


Fig. 232. Cross-sections stained in haematoxylin and Bismark brown to show a comparison between a prothallus and a rhizome in *Psilotum nudum* (L.) Beauv. (*P. triquetrum* Swartz). × 40. *a*. A diploid prothallus from Rangitoto Island showing central area free from the endophytic fungus though this specimen is without actual tracheids. Note projecting antheridia. *b*. Transverse section of a tetraploid rhizome from Malay showing fungus and central vascular tissue.

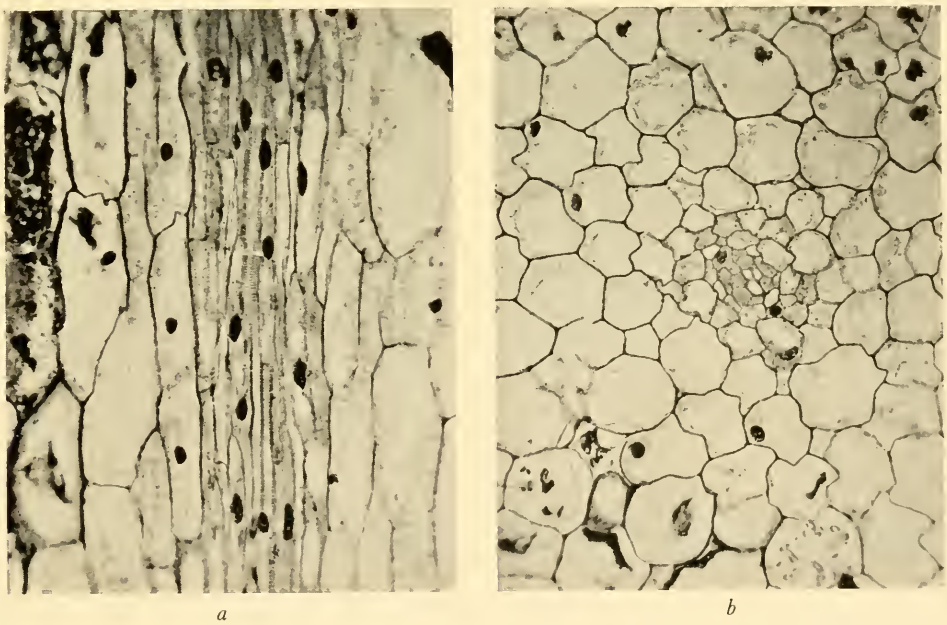


Fig. 233. Vascular prothallus from Rangitoto Island to show detail of central conducting strand. *a*. Longitudinal section. *b*. Transverse section. Both × 100.

unusual malformation of the spindle was found at meiosis in many of the spore mother cells, which resulted in tripolar or quadripolar figures at the first division (Fig. 234*c*) and from six to eight nuclei at the end of the second. This abnormality was thought to be metabolic rather than cytological in origin, since it is not a normal attribute of tetraploidy as such, but it confuses the interpretation of the prothallial structure by introducing a possible source of genetical abnormality into their make-up. This risk may be

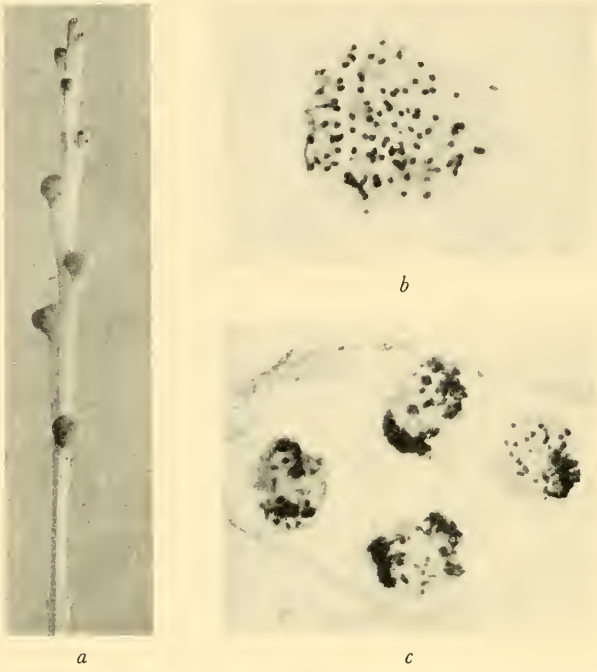


Fig. 234. Tetraploid sporophyte of *Psilotum nudum* (L.) Beauv. (*P. triquetrum* Swartz) all after Manton (1942). *a*. Tip of a fertile twig of a sporophyte from Rangitoto Island decolorized in alcohol. × 2. *b*. Mitosis in a tetraploid rhizome from Malay in which a chromosome count ('over 200') was made. × 1000. *c*. Group of four mother cells showing irregular meiosis with a tripolar spindle from the Rangitoto material. × 500.

thought of as more apparent than real, since spores as irregular as those observed are most unlikely to have been viable, and reproduction of the species could easily be confined to the normal mother cells which were also present. The fact of polyploidy, however, at once disclosed the need for further investigation of both generations on wild material.

It is at this point that Professor Barber's recent observations are of special interest. He reports that material growing wild near Sydney, Australia, is also tetraploid. This means that Darnell Smith and Lawson's (1917) source of prothalli is likely to be of the same general type as that on Rangitoto Island and a true haploid is still to seek. Further, Professor Barber made the interesting observation that meiotic irregularities involving tripolar spindles were also encountered by him at Sydney on wild material fixed after a spell of unusually cold weather, but that the same material after transfer to the laboratory,

where it was merely kept in water for a spell of some weeks, was still in meiosis at the end of the time but showed perfectly normal spindles. This confirms the suggestion made in relation to Rangitoto that the meiotic irregularities found there are metabolic in origin.

Tetraploid sporophytes are thus now known from New Zealand and Australia; they may be suspected to be present in parts of Japan from the work of Okabe (1929), and a rhizome count reported from Malay (Manton, 1942) was also of this nature. The only certain diploid sporophyte so far referable to an exact locality is one from Ceylon (Manton, 1942). It is obvious, however, that very large tracts of the earth's surface are entirely unexplored, and it is still too soon to know whether the diploid or the tetraploid sporophytes will have the wider distribution.

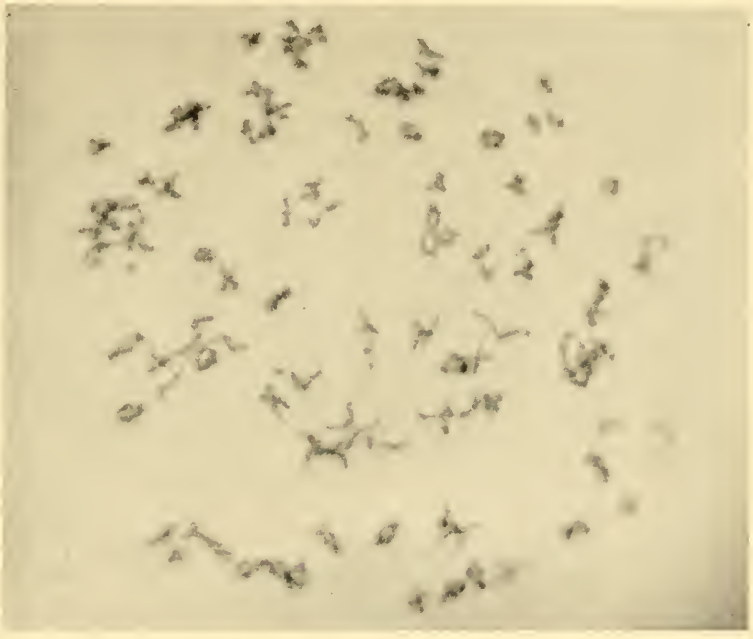
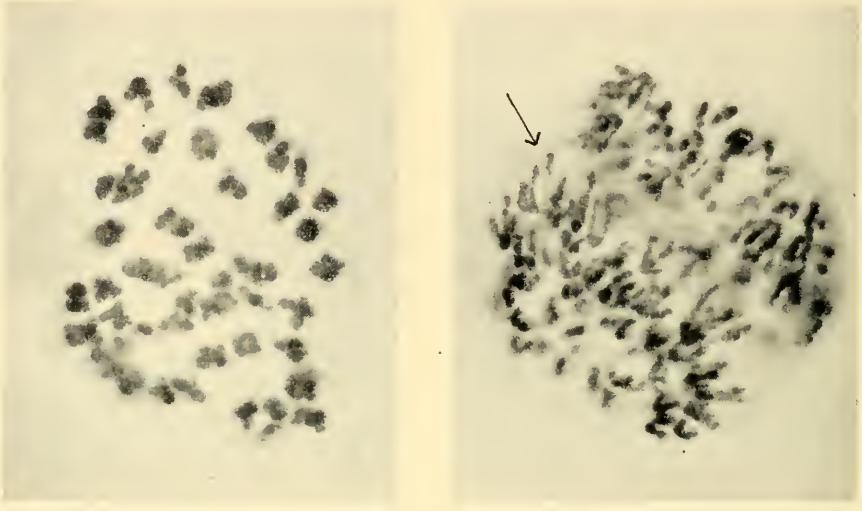


Fig. 235. Meiosis in tetraploid *Psilotum nudum* (L.) Beauv. (*P. triquetrum* Swartz) from Kew, permanent acetocarmine. × 1000. From the specimen of Fig. 229, after Manton (1942).

From what has been said it will be obvious that in *Psilotum* the fact of polyploidy is of far greater interest than the actual details of the chromosome numbers, and for this reason it has been discussed first. It now remains to add the numerical details as far as these are known, which, unfortunately, is still only imperfectly. My own information is still exactly as in 1942. The only diploid sporophyte available to me alive was a plant of *P. flaccidum*, of unknown wild origin, growing at Kew (Fig. 230, p. 235). In this there are not less than 52, nor more than 54, chromosome pairs at meiosis (Figs. 236*a*, 237), though I was unable to decide between these two numbers. This is the only record of *P. flaccidum* so far available, but in the best modern study of *P. nudum* (*P. triquetrum*) known to me (Okabe, 1929) the haploid number for horticultural strains in Japan is given as 52. This number is therefore undoubtedly very near to the truth. Fig. 235 shows meiosis in a tetraploid sporophyte, also of unknown wild origin, growing at Kew in

which the doubled number of rather smaller chromosomes is visible. Since it is profitless to attempt exact enumeration in a tetraploid without having a diploid of the same species for comparison, I have made no further attempts at greater precision. There is, however, a strong suggestion in Fig. 235 of the presence of some multivalents, and Professor Barber informs me that the same may be seen in Australian tetraploids. It is therefore possible that in this particular species we are dealing with an autopolyploid series.

This is as far as knowledge of *Psilotum* can at present be carried, and there is clearly scope for much further work by anyone with direct access to wild material. The same



a *b*

Fig. 236. Meiotic metaphase and anaphase in *Psilotum flaccidum* Wall., permanent acetocarmine. × 1000. *a*. After Manton (1942), explanatory diagram in Fig. 237. *b*. Showing traces of spiral structure.

is also true of *Tmesipteris* (Fig. 238). This has been available to me only in Botanic Garden material growing on the stem of a tree fern at Glasnevin in Dublin and believed, though not certainly known, to have come from Tasmania. Since Tasmania is now the centre of Professor Barber's activities, who may be expected shortly to unravel the whole situation, description of my results can be brief. Fig. 239*a* shows a somatic division in a tapetal cell, and Fig. 239*b* is a mother cell at the first meiotic division. The number of chromosomes is horrifying, and I do not propose to attempt an exact enumeration. It is sufficient to state (Fig. 240) that the sporophytic number is between 400 and 500, and that the corresponding gametophytic number is somewhat over 200. That this is also a case of polyploidy is known from an earlier description of *c.* 100 at meiosis by Yeates (1925) and from Professor Barber himself, who has detected (personal communication) two chromosome numbers in Australian material, of which 'somewhat over 200' is the higher. With this intimation that polyploidy exists in both genera, we may therefore safely leave the matter in Professor Barber's hands.

Two things may, however, perhaps be added by way of comment. Both *Psilotum flaccidum* and *Tmesipteris* display the same type of curious chromosome shape as that already seen in *Equisetum* and referable to the same cause, namely, a characteristic

laxity of the spiral structure, traces of which are nevertheless detectable at anaphase in the former species (Fig. 236). These appearances, which will be met with again in *Lycopodium* and *Isoetes*, may be of little phyletic significance; on the other hand, they may perhaps add one slight contribution to the other reasons for believing these various genera to be related.

Secondly, with regard to the details of chromosome number, it is much to be desired that the demonstration of the basic haploid for the group should be made clear with complete finality, since in the present state of knowledge comparisons are suggested

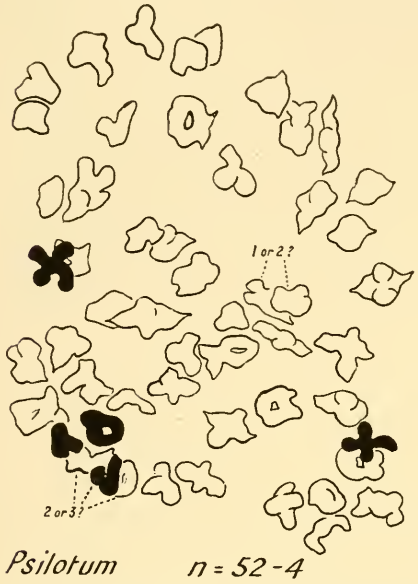


Fig. 237. Explanatory diagram to Fig. 236*a* from Manton (1942). × 1500.

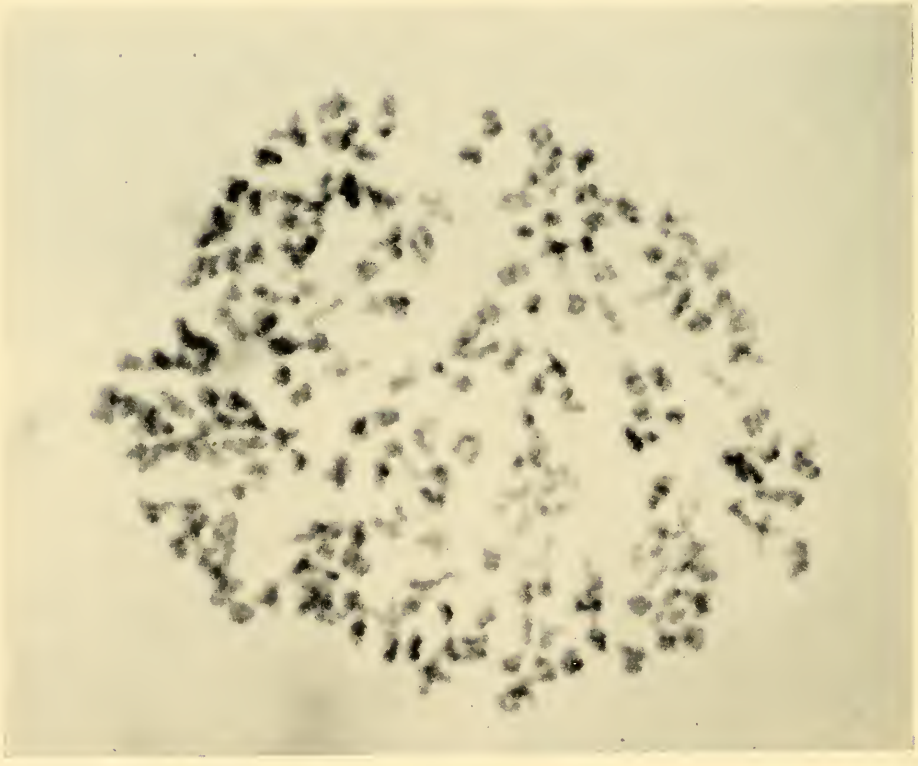
Fig. 238. Small shoot of *Tmesipteris tannensis* (Spreng.) Bernh. of unknown wild origin from a live specimen grown in the Botanic Garden at Glasnevin, Dublin. Natural size.

which, if based on erroneous information, may be very misleading. If the lowest gametic number were indeed to be 54, it is tempting to draw a comparison not only with the 108 of *Equisetum* but also with $n=9$ of *Selaginella* which will be seen in the next chapter. It is conceivable that all these high numbers are the end-products of very ancient polyploid series which relate back to simple beginnings even in those groups such as *Equisetum*, in which no direct traces of polyploidy at present remain. Further discussion of this is not possible without facts of irreproachable accuracy to go upon; the matter is, however, worth mentioning here in order to act perhaps as a spur to a favoured observer who, with wild material accessible to him, may perhaps be tempted thereby to make the very considerable effort required to remove our present doubts.

SUMMARY

Summing up the facts for the Psilotaceae, even in their present imperfect state, it has now been shown that polyploidy exists in each of the two living genera of *Psilotum* and *Tmesipteris*, the lowest number now known at the base of the series being of the order of 50 odd in *Psilotum* and 100 odd in *Tmesipteris*. The fullest information at present

a



b

Fig. 239. Cytology of the Dublin specimen of *Tmesipteris tannensis* (Spreng.) Bernh. Permanent aceto-carmine. × 1000. *a*. Mitotic metaphase in a tapetal cell with $2n$ = over 400. *b*. Meiosis in the same with n = over 200.

available refers to tetraploid *Psilotum* which has now been obtained from New Zealand, Australia, Malay, and perhaps Japan, while a corresponding diploid has only so far been found in Ceylon, though it may easily be present in other parts of the world. A char-

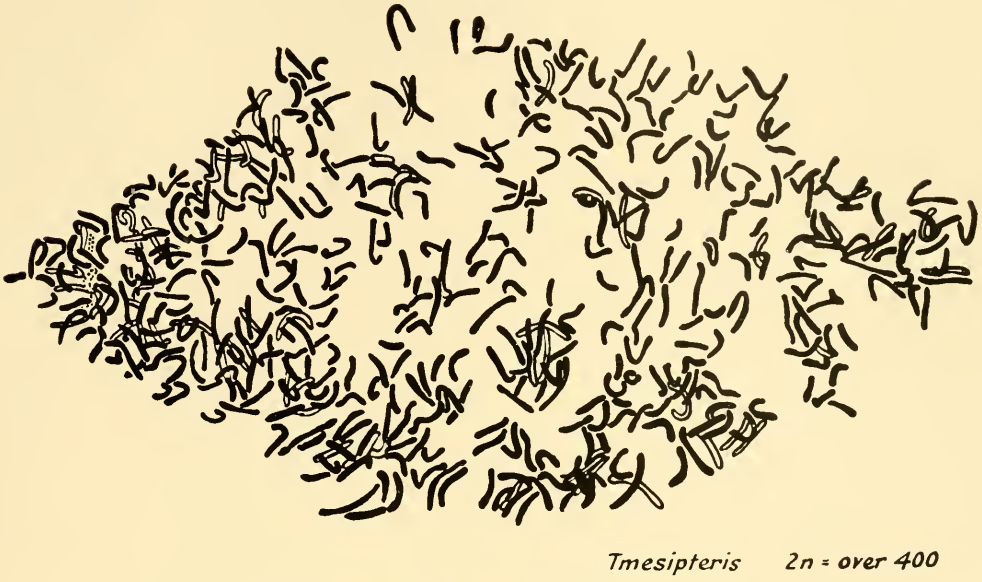


Fig. 240. Explanatory diagram to Fig. 239*a*. × 1000.

acteristic aberration of the spindle reported from Japan, New Zealand and Australia has been shown by Professor Barber of Tasmania to be of metabolic origin in unfavourable environmental conditions. The causes of the presence of anomalous vascular tissue in the diploid gametophytes described by the late Dr Holloway from New Zealand are still uncertain, and a genuine haploid prothallus is still undiscovered in this genus. Further study of both genera and of all species in them is much to be desired.

CHAPTER 15

THE LYCOPODS (CLUBMOSSES)

The next great group, and the one to which the name microphyllous particularly applies, albeit with reservations in the case of *Isoetes*, is that of the true Lycopods. These consist of three exceedingly dissimilar genera, *Lycopodium*, *Isoetes* and *Selaginella*, all of which are cosmopolitan and of one additional monotypic genus confined to Australia and New Zealand, *Phylloglossum*. Further reference to *Phylloglossum* will, for the moment, be omitted, since the centre of interest throughout this book is among wild European plants wherever possible. The contents of this chapter will, therefore, be confined to the British representatives of *Lycopodium*, *Isoetes* and *Selaginella*, slightly supplemented, in the case of *Selaginella*, by examination of two non-British wild European species.

The British Lycopods comprise only nine species, namely, three of *Isoetes*, five of *Lycopodium* and one *Selaginella*. All are small in stature and quite insignificant components of the vegetation, but in structure, life history, ecology and past history they combine more points of interest, and raise more fundamental botanical problems than can be found at one time in any other group. They are, moreover, each and all sufficiently localized and distinctive to constitute landmarks in the recollection of every field botanist or nature lover who has made their acquaintance by his own exertions.

Fig. 241. Silhouette of *Lycopodium clavatum* L. from Borrowdale. Natural size.

Taking the genus *Lycopodium* first as perhaps the best known to the average field naturalist, we have five British species, all of them to be found among mountains and most of them confined to such regions. *L. clavatum* (Fig. 241), '*the* Clubmoss' *par excellence*, is also to be met with on heaths, where its creeping branched stem, densely clothed with little leaves and rooted at intervals, may cover many yards of ground. Very similar in habit, though more restricted in range, is *L. annotinum* L. (Fig. 251), locally abundant in the Scottish Highlands but very rare in England, the best known locality being one hillside in the Lake District where I suspect that it is not always fertile (see below). *L. alpinum* L. is somewhat more specialized in structure, having a subterranean, colourless, creeping stem from which little tufts of aerial branches, closely pressed to the ground, arise at

intervals. It occurs characteristically on mountains above the tree line. *L. inundatum* L. is probably the most difficult species to detect, since the length of its stem is to be measured in inches and not feet, although its leaves and solitary cone are in themselves as large as in the others. It is scattered over the country at various altitudes but always in boggy places and often in quite small colonies.

L. Selago L. completes the list. This very conspicuous little plant, with the habit of an erect dwarf bush covered with spreading spiny leaves, is the most easily found of all our British species at an appropriate altitude, which is usually slightly lower than that required by *L. alpinum.* It differs from all the others in ways which appear to be morphologically primitive. The radially symmetrical erect stem and the absence of specialized cones are two of the more important points, and in these respects its nearest relatives among living species are to be found in the tropics, e.g. *L. squarrosum*; though if fossils are also related, a point which must not be too readily assumed, *L. Selago* seems to show more features of (perhaps superficial) resemblance to very ancient types such as *Drepanophycus* (Devonian) or *Baragwanathia* (Silurian) than do any of the other species listed. The interest of the relatively primitive construction in *Lycopodium Selago* is somewhat enhanced by a geographical distribution which, at present, is virtually worldwide, and by an abundance of individuals which is made possible by a very characteristic vegetative reproductive mechanism. Detachable bulbils are borne in place of some of the leaves on zones of the stem, alternating with zones in which the leaves bear axillary sporangia, and these bulbils germinate very readily to produce new plants.

The sexual generation of the genus *Lycopodium* was for long a matter of speculation, for it is exceedingly difficult to detect in nature, and the spores, though produced in such abundance as to be sold commercially as 'Lycopodium powder', will only germinate under very special conditions and even then only after a lapse of several years. The early germination stages of *L. inundatum* were, however, seen in 1858 by de Bary, and adult prothalli of the same species were found wild in 1887 by Goebel in Germany. Prothalli and young plants of *L. annotinum* were found in Switzerland by Fankhauser in 1873 and again in Germany by Bruchmann in 1884, after which this last indefatigable and gifted observer proceeded to discover the prothalli of all the other European species (Bruchmann, 1898) and at a later stage germinated their spores (Bruchmann, 1910). All these specimens were of continental origin and most of them were from the Thuringerwald and the Harz Mountains where, to quote Bruchmann, they are not so much rare as very local in their occurrence. Prothalli have, however, also been seen in Great Britain by W. H. Lang, who discovered those of *L. clavatum* in Scotland in 1899 and of *L. Selago* on two occasions subsequently (Lang, personal communication). I am indebted to Professor Lang for permission to reproduce an old photograph taken at the time of the discovery of the prothalli and young plants of *L. clavatum* (Fig. 242) which will serve to illustrate the type of structure. The gametophytes are colourless subterranean organisms living saprophytically with the aid of an endophytic fungus as in *Psilotum* (see previous chapter), but with a characteristic shape which differs considerably from those of that genus. There is therefore no risk of confusion with other organisms even if the identity were not attested by the presence of young sporophytes. In practice, however,

it is most unlikely that prothalli would be found at all without the index of juvenile sporophytes to draw the searcher's attention to a suitable spot.

These facts regarding the discovery of the sexual generation have been given in some detail, partly for their own interest in giving a picture of the genus, but also in relation to the cytological observations on *Lycopodium Selago* which will be described below. As far as field observations go that species differs in no essential way from the others. Gametophytes are equally rare in all, being apparently produced sporadically in response to locally favourable conditions, and the main colonization of territory is carried out by vegetative growth in the creeping forms, or by bulbils in *L. Selago*.

Fig. 242. *Lycopodium clavatum* L. Young sexually produced plant (right) and a prothallus (left). Natural size. From a photograph kindly supplied by Prof. Lang (cf. Lang, 1899).

Difficult as it is to grow the prothalli and spores under artificial conditions, the culture of the sporophytes is scarcely less so with the one exception of *L. Selago*, which will grow readily in pot culture. I have also myself succeeded in keeping *L. annotinum* alive for several years, but in no case are cones normally produced in culture in the British species. This may suggest that they are not perhaps ideally suited to cytological study, and it may be said without fear of contradiction that for this purpose they are the most awkward genus of Pteridophytes in the whole of the British flora. It is not merely that the appropriate seasons for their study are short and the plants relatively inaccessible, but fixation presents acute difficulties which, added to the extremely peculiar shapes of the chromosomes and other attributes which will shortly be mentioned, may make even a successful preparation very difficult to interpret.

The easiest species to study, however, somewhat surprisingly, and perhaps fortuitously, proved to be *L. inundatum*. This rather uncommon species was sent to me by post from Aviemore (Scotland), in perfect condition, in July 1936. It had been packed tightly in a tin amongst moist moss, and was still in full meiosis when received. It gave the preparation shown in Figs. 243 and 246*a*, in which the antenna-like form of the chromosomes is strikingly displayed. Their number is, however, not in doubt: $n = 78$.

The next species to give a result was *L. clavatum*. This is rather later than most in maturing its spores, and the end of July is more favourable than earlier in the month, both in Scotland and in the Lake District. At the beginning of July, at which season my own material was obtained in the Lake District, meiosis can only be found in the largest cones available, and farther north, in Scotland, this date is definitely too soon. Once material of the right age has been obtained the cytological observations are fairly straightforward. Fig. 246*d* shows diakinesis sufficiently well spread to enable one to dispense with

a diagram; there are 34 pairs of chromosomes. Fig. 246*e* (at a higher magnification) shows the first meiotic metaphase for which a diagram is supplied in Fig. 244, again showing $n = 34$. Confirmation of this number to the extent which is possible from sections has been made on roots of both Swiss and British material, in each of which $2n = 68$ as nearly as can be determined.

Fig. 243. Explanatory diagram to Fig. 246*a*. × 1500.

L. annotinum, the next species, is rare in Britain as already pointed out. My material of it is less complete than could be wished since the Scottish localities were inaccessible during the war and in its only English station* it appears to be receding for climatic reasons. It was not fertile when I visited the Lake District in June 1939, and my

* I am informed by my colleague, Dr Sledge, that the species has been found in one other place, in Yorkshire.

observations on British material are therefore confined to roots. Fortunately, these were unusually clear and they have been supplemented by roots from a specimen fixed in Switzerland (Fig. 251) and also by a few cells in meiosis obtained at Storlien in Sweden in July 1948. The meiotic material is somewhat scanty and requires confirmation. One cell, however, is illustrated in Figs. 247*a* and 248, and the chromosome number appears to be $n = 34$ as in *L. clavatum*. This is in close agreement with the evidence from roots (Fig. 247*b*) in Britain in which also $2n =$ probably 68. It is therefore certain that *L. annotinum* both in Britain and on the Continent is very similar to *L. clavatum* and is probably identical cytologically with that species.

This cannot be said of the other two species. *L. alpinum*, in spite of the high altitude of its habitats, matures its cones some weeks before those of *L. clavatum* in the same district, and June would be the best month to seek for it. In the first week of July 1944, only a few residual cones

Fig. 244. Explanatory diagram to Fig. 246*e*. × 2000.

Fig. 245. Explanatory diagrams to Figs. 246*b* and *c*, for description see text. × 2000.

were still young enough to be used, while *L. clavatum* at the same date had scarcely begun meiosis. Figs. 245 and 246*b* and *c* show two stages in spore mother cells of *L. alpinum*. At the metaphase of the second meiotic division (Figs. 245*a*, 246*c*) there appear to be 25 split chromosomes, of which one half-chromosome in the middle of the field appears to have become separated rather widely from its fellow in the making of the preparation. This specimen is of importance because, unsuited as

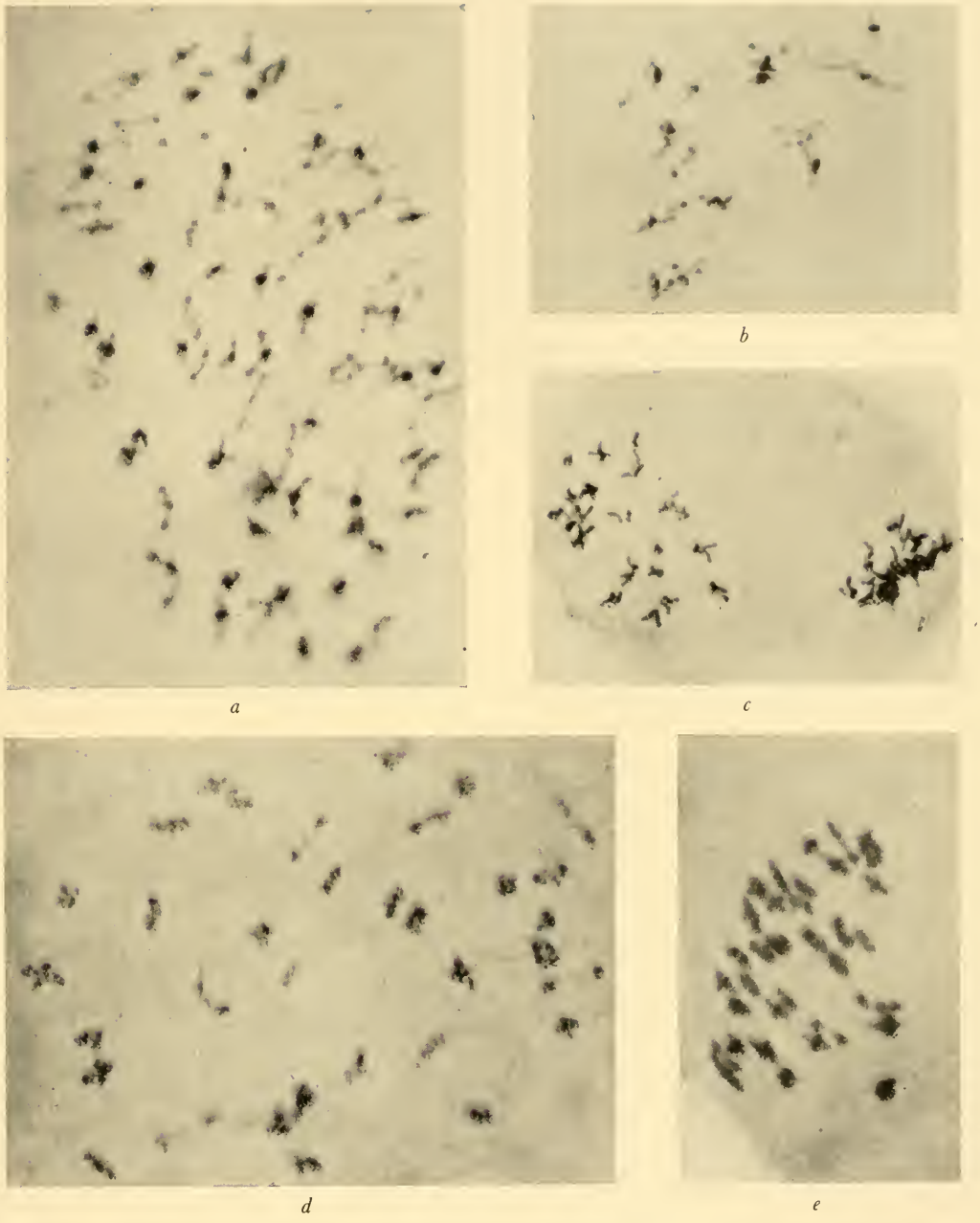
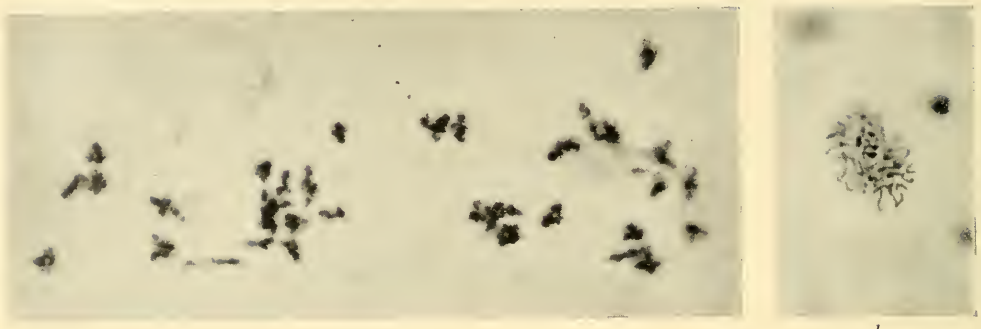


Fig. 246. Meiosis in British species of *Lycopodium*, permanent acetocarmine. All except *e*, × 1000. *a*. *L. inundatum* L., first meiotic metaphase. $n=78$. *b*. The same in *L. alpinum* L. For explanatory diagram see Fig. 245*b*. *c*. The same, at the second meiotic metaphase. For explanatory diagram see Fig. 245*a*. *d*. Diakinesis in *L. clavatum* L. $n=34$. *e*. The same at metaphase. × 1500. For explanatory diagram see Fig. 244.



a *b*

Fig. 247. Chromosomes of *Lycopodium annotinum* L. × 1000. *a*. Meiosis in a Swedish specimen in balsam after acetocarmine. For explanatory diagram see Fig. 248. n = probably 34. *b*. Root-tip section showing mitosis in a British specimen, for comparison of chromosome size with other plants.

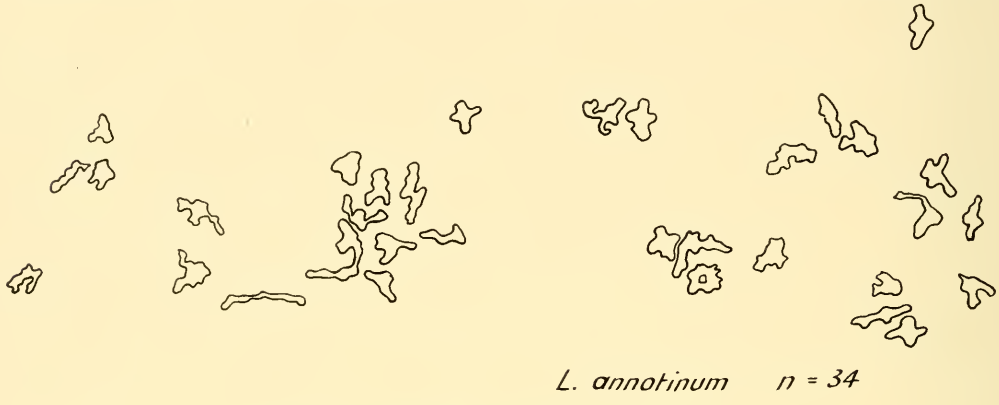
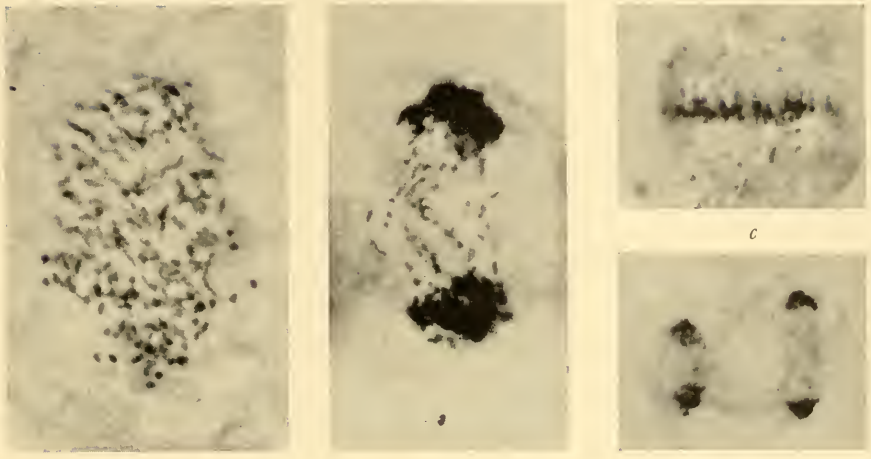


Fig. 248. Explanatory diagram to Fig. 247*a*.

is the second division for accurate counting in almost every member of the Pteridophyta, the first division in this particular species seems to be worse. Diakinesis is unusable owing to diffuseness of chromosome outline, which is more extreme in *L. alpinum* than in *L. clavatum*, though it is also apparent (Fig. 246*d*) in that species. At metaphase (Fig. 246*b*), on the other hand, the despiralization of the chromosomes is so extreme that the task of disentangling them is almost insuperable. This figure is an unusually successful attempt at doing so, which at first counting gave 23 or 24 pairs. It is, however, just possible that the cell is incomplete. The chromosome number must therefore be left uncertain as not less than 24 nor more than 25.

L. Selago remains, and here the cytologist's troubles reach a climax in spite of the ease of cultivation and other advantages which one might expect would facilitate the task. In actual fact this species is, in my experience, the worst cytological object that I have ever encountered, and in the unequal contest between cytologist and plant, the plant has in this case so far won handsomely. The reason is that fixation of roots is virtually hopeless by all the older methods, and at meiosis, even with modern methods, the combination of high chromosome number with extreme irregularity of pairing produces a most intractable situation. Simple chromosome enumeration becomes virtually impossible at



a *b* *c* *d*

Fig. 249. Meiosis in *Lycopodium Selago* L., permanent acetocarmine. *a*. Polar view of the first metaphase. × 1000. For explanatory diagram see Fig. 250. *b*. Side view of the first anaphase showing numerous lagging univalents. × 1000. *c*. Side view of the first metaphase showing pairs and lagging univalents. × 500. *d*. Side view of the second anaphase showing lagging half-chromosomes derived from split univalents. × 500.

any stage, and, without accurate knowledge of chromosome number, detailed analysis of meiosis cannot be carried out. It is obvious that nothing less than a prolonged special study and probably the application of new technical methods will be needed to break this deadlock, and only an approximate result can be given here. Demonstration of the qualitative side of irregular pairing is, however, not difficult. Fig. 249*c* shows the first meiotic metaphase, at a low magnification, in a squash preparation in which a cloud of univalents are conspicuous objects. Laggards at anaphase, i.e. the univalents which are splitting, are equally conspicuous in Fig. 249*b*, and they appear again at the end of the second meiotic division in Fig. 249*d*. A fuller demonstration of the qualitative side of pairing is scarcely required, and it should perhaps also be pointed out that these figures do not represent one plant at one season but are from several places in several years, Fig. 249*b* being from a Scotch plant in 1936, Fig. 249*c* and *d* from a Welsh plant in 1938 and Fig. 249*a* from a Lake District plant in 1944. Irregular pairing must, therefore, be recognized as a characteristic feature of *L. Selago* over much, though not necessarily all, of Great Britain.

L. Selago

Fig. 250. Explanatory diagram to Fig. 249*a*. × 2000. Paired chromosomes in black, univalents in outline.

Compared with this fact the interest of the actual chromosome number is far less, and the imperfection of the assessment of it is perhaps not such a serious deficiency at the present stage of the inquiry as might have been anticipated. An approximate analysis of a polar metaphase in a squash preparation is contained in Fig. 250. Multivalents, if present, cannot easily be recognized or allowed for, but ignoring their possible existence, the analysis records approximately 113 pairs and 37 univalents. The sporophytic chromosome number of *L. Selago* cannot therefore be less than 260.

Fig. 251. Silhouette of *Lycopodium annotinum* L. from Switzerland. Natural size. This specimen provided a root-tip count.

The interpretation of these facts is not at once obvious. In the British species of *Lycopodium*, with the exception of *L. annotinum*, which almost certainly links up with *L. clavatum*, the cytological evidence as a whole can only underline their complete dissimilarity from one another, and one must recognize in them the representatives of phyletic lines which have been so long separated that their cytological connexion, if it ever existed, has become completely obscured. They seem now to be far more different from each other than are the genera or even groups of genera of the Polypodiaceous ferns. This is perhaps a sign of antiquity. Yet suddenly, in *L. Selago*, we find a species which is behaving like a hybrid that has succeeded in covering up a defective meiotic process by a highly successful reproduction by means of bulbils. Can this really be the case? To investigate it further we need to assemble observations on meiosis not from Britain only but from all over the world. It may be that the British plants are peculiar,* or it may be that under certain conditions failure of pairing may be induced from metabolic causes and not from lack of homology among the chromosomes. In that case the undoubted gametophytes which have been found might result from local strains or under metabolic conditions in which pairing was not irregular. Before the idea of hybridity can be accepted some explanation for the sexual prothalli must in any case be found. It would be unwise, at this stage, to prejudge the issue as to what this explanation might be, but if the signs of hybridity are borne out by further study, one may be quite certain, first, that the parent species, wherever they may once have been, are unlikely now to be

* As this chapter goes to press mention can be made of one observation on a non-British plant. At Storlien in Sweden unpaired chromosomes were seen again in the summer of 1948.

Fig. 252. *Isoetes acustris* L., two growth forms from Lake Windermere, from pressed specimens. Natural size.

in Britain, and secondly, that *L. Selago*, if it is a hybrid, is likely to be the most ancient impure species that cytology has so far detected.

Leaving *Lycopodium* we come to the two heterosporous* genera *Isoetes* and *Selaginella*. The aquatic Quillworts, *Isoetes lacustris* L. (Fig. 252) and *I. echinospora* Durieu, are not unfamiliar inhabitants of the pure waters of our glacial lakes and mountain tarns, and their conditions of culture are fortunately of the simplest. It is only necessary to put a little garden soil at the bottom of an inverted bell-jar, which is then filled up with water,

Fig. 253. Explanatory diagram to Fig. 256*a*. × 2000.

and *Isoetes* will grow indefinitely in a laboratory if kept near a cool north window. In addition to the two species recognized taxonomically, there are undoubtedly many true-breeding strains characteristic of different lakes, which form local inbreeding communities. Maximum stature appears to be partly under genetical control and ranges from the fairly small plants with leaves 4 in. or so long, characteristic of Windermere, to immense plants with leaves over a foot in length such as the var. *Morei* Moore found in Loch Bray in County Wicklow in Ireland. These size differences are constantly maintained in culture and do not appear to be environmentally induced growth forms.

* By heterosporous is meant the production of spores of two sizes, the large, or megaspores, being few in number and heavily stored with food for the production of exclusively female prothalli; the small, or microspores, being formed in greater numbers destined to produce only diminutive male prothalli. The microspores of *Isoetes* and *Selaginella* are the equivalent of the pollen grains in the higher plants.

A viviparous form in which vegetative buds replace sporangia is also known from Windermere and no doubt from other places.

Material kept in culture for many years and used for cytological study of root tips has been as follows:

I. echinospora, normal form from County Kerry in Ireland.

I. lacustris, normal form from Windermere (Fig. 252); viviparous form from Windermere; var. *Morei* from County Wicklow, Ireland, with very long leaves; form with leaves intermediate in length, Wales.

All these plants proved to be cytologically indistinguishable with more than 100 chromosomes in their roots. Examination of meiosis was therefore confined to material from the most easily accessible wild locality, namely, the *I. lacustris* colonies in Windermere.

As in many, or perhaps all, heterosporous Pteridophyta the maturation of megasporangia tends to precede that of microsporangia. In *I. lacustris* the former takes place in July, but the latter, which is greatly to be preferred for cytological purposes, only at the end of August. Figs. 253 and 256*a* show a metaphase plate obtained at this season from Windermere, and the peculiar shapes of the chromosomes already seen in *Lycopodium* and *Equisetum* are again displayed. For this reason, as in the other cases, accurate counting is difficult. The cell in question contains not less than 54 pairs, nor more than 56, and this, unfortunately, is as near to accuracy as has so far been attained.

The other British species, *Isoetes hystrix* Durieu, is both so different and so unfamiliar that a word of description about it may not be out of place. This minute terrestrial species has its headquarters geographically in the Mediterranean regions, where it is sparingly met with in isolated small colonies, spread from the south of France to North Africa, occupying sites of an extremely xerothermal character. It is confined to areas which are regularly moistened with flood water in winter, during which season it vegetates freely and may become totally submerged. In summer, on the other hand, the sites dry out completely, and all the plants inhabiting them pass the hot season in a dormant and desiccated condition, either as subterranean geophytes (*Isoetes*) or as seeds (*Juncus capitatus*). The 'Isoetetalia', as these localities are called in ecological language, have been studied several times on the Continent by Braun-Blanquet and his school, though I am not aware of any similar study of the only two existing British sites, which are in Guernsey and on the Lizard respectively. That *Isoetes hystrix* is present in Guernsey has been known since 1860. The Lizard site, though detected a century ago, was so difficult to find again that the record was disbelieved until 1933, when plants of *Isoetes* were re-found there in some quantity by R. Melville of Kew whilst searching for *Juncus capitatus*. I am indebted to Dr Melville for instructions as to how to reach the Lizard site, and I have also been able to collect material from Guernsey, on both occasions the season being early April, at which time the vegetative season is almost at an end and meiosis of the last microspores in progress. By June all traces of the leaves have vanished from above ground, but, curiously enough, in the skilful hands of Mr Ashby, *Isoetes hystrix* from both localities proved surprisingly easy to cultivate. Intact sods containing it, together with other geophytes such as *Scilla verna*, were lifted in 1938 and 1939 and transferred to small pots in the roof greenhouse of Manchester University where

they have been maintained ever since. In order to respect their evident need for a dry dormant period, they are dried out annually and placed in a cupboard for some months, the season chosen being the winter instead of the summer in order to give them the benefit of such sunshine as is available in Manchester. Under this treatment they have

a

b

Fig. 254. *Isoetes hystrix* Durieu. *a*. Pot of plants from the Lizard, Cornwall, growing in cultivation. Half natural size. *b*. Two wild specimens pickled as found, the left-hand plant from the Lizard, the right-hand plant from Guernsey. (Note the double crown in the latter case.) Natural size.

increased very greatly in size, as the photograph of Fig. 254*a* taken in 1945 will show. This is half natural size, and yet the plants in it resemble the full stature of the original wild specimens which are shown, pickled at the time of collection, in Fig. 254*b*. In this, the left-hand specimen is a plant from the Lizard, as are those of Fig. 254*a*; the right-

hand specimen of Fig. 254*b* is from Guernsey and was especially selected as an abnormally large plant with a branched stock.

The remarkably disjunct character of *Isoetes hystrix* localities makes it certain that in each must be a little inbreeding community, isolated for long periods of time, and it is

Fig. 255. *Isoetes hystrix* Durieu growing in cultivation to show characteristic differences of habit in material from different sources. The tall left-hand plant came from Morocco, the right-hand plant from the Lizard. About a quarter natural size.

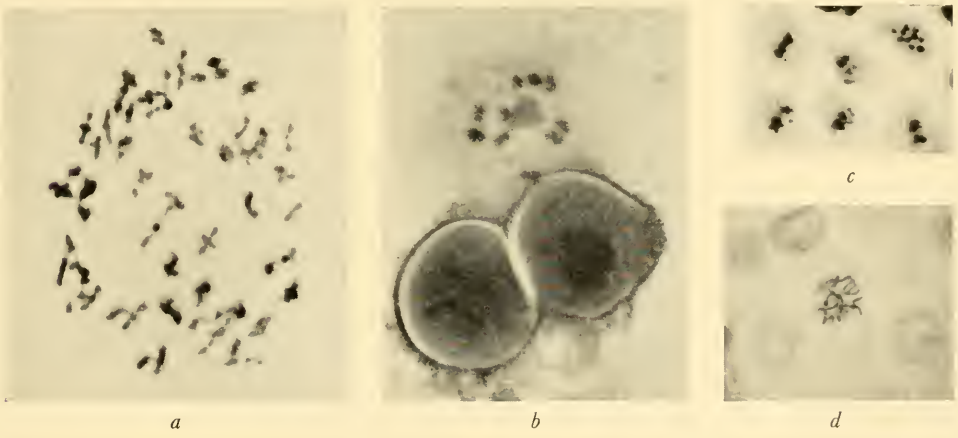


Fig. 256. Chromosomes of *Isoetes*. All × 1000. *a*. *I. lacustris* L. first meiotic metaphase, in permanent acetocarmine. × 1000. For explanatory diagram see Fig. 253. *b*. *I. hystrix* Durieu from the Lizard, diakinesis in permanent acetocarmine. × 1000. $n=10$. *c*. The same, showing metaphase in a section. × 1000. *d*. Root of *I. hystrix* from Morocco in a section, stained with Feulgen's method, to show chromosome size. × 1000. $2n=20$.

therefore to be expected that local populations should show genetically based differences of the same type as those already met with in the aquatic species, and this is undoubtedly the case. A detailed comparison between the populations of Guernsey and of the Lizard has not been made owing to the inaccessibility of Guernsey during the war years. At the time of my visit to that island in 1939, only a token collection of live plants had been

brought back, which was shortly reduced to a single plant by inadvertence, thereby providing too scanty a basis for comparison. Fig. 255, on the other hand, shows an old pot plant from the Lizard (right) growing beside an old pot plant from Morocco (left) provided

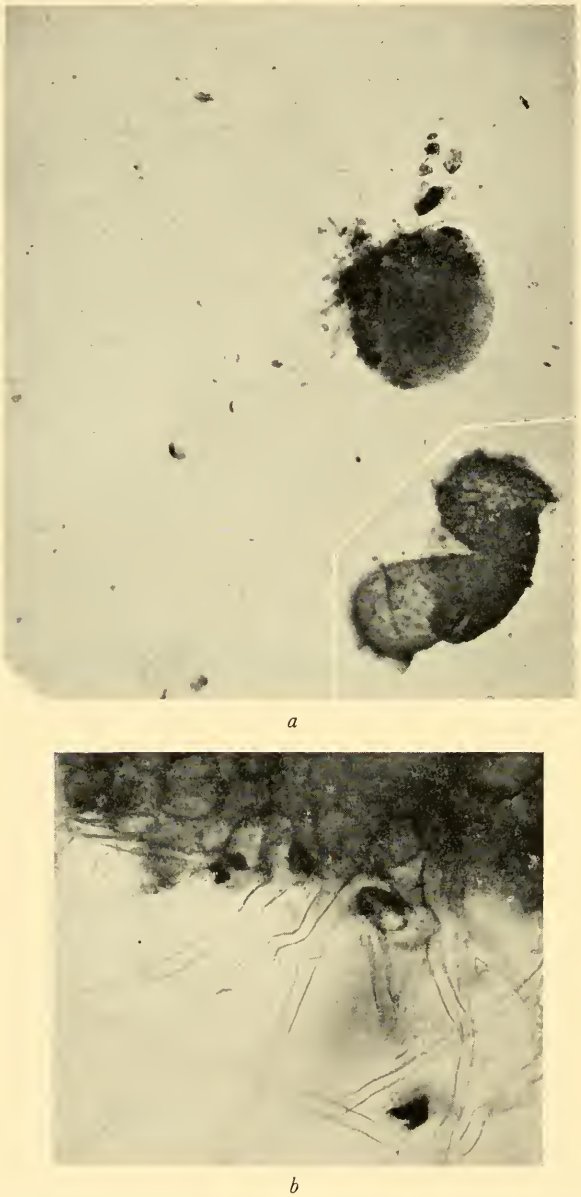


Fig. 257. Prothallus of *Isoetes hystrix* Durieu from a glycerine jelly mount. *a*. Germinated megaspore showing rhizoids and detached spore coat (inset). × 50. *b*. Rhizoids enlarged × 500.

some years before by Professor T. G. B. Osborn. Two specimens from Morocco were kept alive in Manchester for many years by means of the cultural treatment described above, and both differed strikingly and constantly from the British plants both in stature and

mode of growth of the leaves. In British material the leaves grow spirally and are pressed down to the ground. In the African specimens they are stiffly erect and considerably longer. Whether this means that *I. hystrix* should be split into more species than one is, however, for taxonomists to say.

Fig. 256*b–d* gives the cytological facts for the African and British material. Fig. 256*c* shows microspore mother cells in *I. hystrix* from the Lizard, fixed in the field and subsequently sectioned and stained in haematoxylin. Fig. 256*b* shows a squash preparation of the same material at the same magnification: $n = 10$. The diminutive chromosomes and their low number make a very striking contrast with everything which we have hitherto seen, and the comparison of chromosome size can equally be made from the root of the Moroccan plant (Fig. 256*d*), in which approximately 20 somatic chromosomes are visible, at the standard magnification (1000 diameters), used almost throughout this book.

Before leaving *Isoetes* it may be of interest to add a note about the prothalli of *I. hystrix*, since, as far as I am aware, these have never before been seen. They were detected by Mr Ashby in the material from Morocco, and I am indebted to him for the photograph in Fig. 257. A germinated megaspore from which the spore coat has become detached is shown in Fig. 257*a*, and the great length of the rhizoids is a striking characteristic (see also Fig. 257*b*). Since rhizoids are absent from the aquatic species and are as a rule poorly developed in the otherwise not dissimilar prothalli of *Selaginella*, their presence here in association with the rather extreme habitat conditions of the species may perhaps be a point of ecological importance.

The second heterosporous genus, *Selaginella* (Fig. 258), need not detain us long, since we have only one species in Britain, *S. spinulosa* A.Br., relatively common and vigorously fertile in all our mountain districts. The only cytological difficulty it presents is due to its small size, but roots can be fixed at any time and meiosis can be obtained in July. The chromosomes are, however, minute. Their number (Fig. 259*a*) appears to be 9 at meiosis and 18 (Fig. 260*c*) in roots. These numbers were found again in the other two European species. *S. helvetica* (L.) Link was fixed in the field in Switzerland in July 1938, and root tips gave the result shown in Figs. 259*e* and 260*d*. *S. denticulata* (L.) Link was collected in North Italy in the same year by Professor Lang, who brought it alive to Manchester, where it coned in cultivation. Fig. 259*d* shows the side view of metaphase in a megaspore mother cell in which the diminutive size of the group of chromosomes is so extreme that they are scarcely visible. Their number, however, appears to be 9 in this cell and 18 as before in roots.

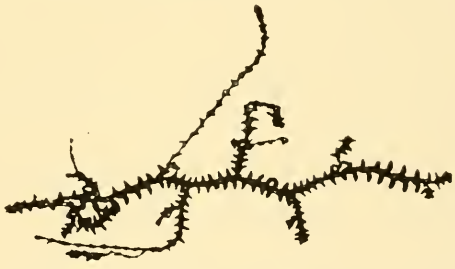


Fig. 258. *Selaginella helvetica* (L.) Link. Silhouette of a dried specimen of the plant used, from Switzerland. Natural size.

This low chromosome number, comparing closely only with *Isoetes hystrix* among all the living Pteridophyta so far studied, is a matter both of surprise and of importance. The genus *Selaginella* is very large, with over 800 species, most of which are tropical. The three European species, however, belong to widely different sections of the genus, *S. spinulosa*

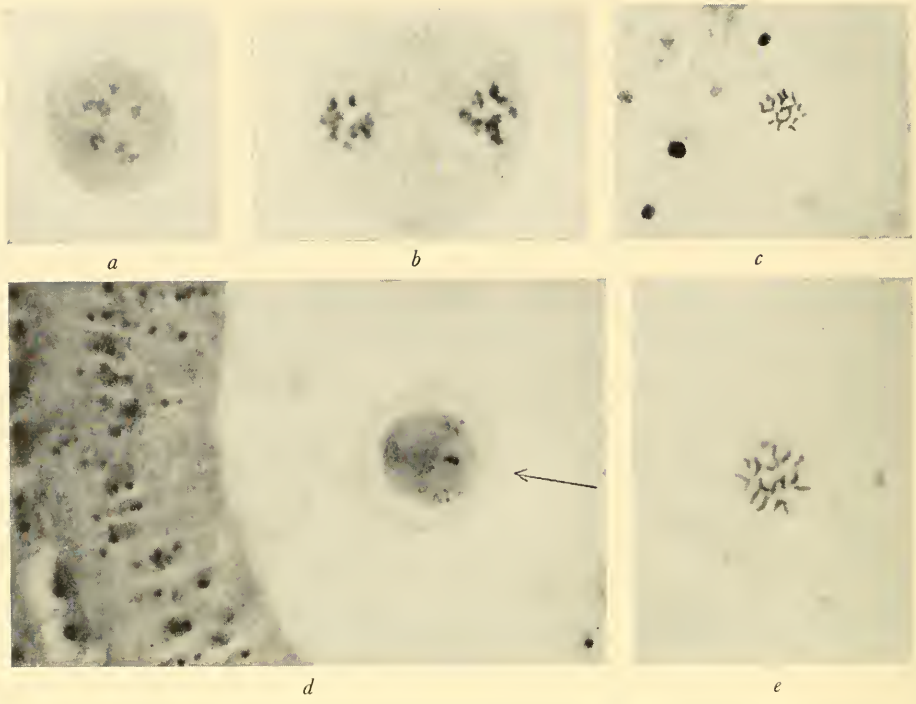


Fig. 259. Chromosomes of *Selaginella*. *a*. *S. spinulosa* A.Br. diakinesis in fresh acetocarmine. × 1000. For explanatory diagram see Fig. 260*a*. $n=9$. *b*. The same, metaphase of the second meiotic division, permanent acetocarmine. × 1500. For explanatory diagram see Fig. 260*b*. *c*. The same, mitosis in a root. × 1500. For explanatory diagram see Fig. 260*c*. $2n=18$. *d*. *S. denticulata* (L.) Link. Part of a section through a megasporangium with the single spore mother cell which it contains at metaphase of the first meiotic division. The diminutive plate of tiny chromosomes lies in the plane of the arrow. × 1000. *e*. *S. helvetica* (L.) Link. Mitosis in a root. × 1500. For explanatory diagram see Fig. 260*d*. × 1500. $2n=18$.

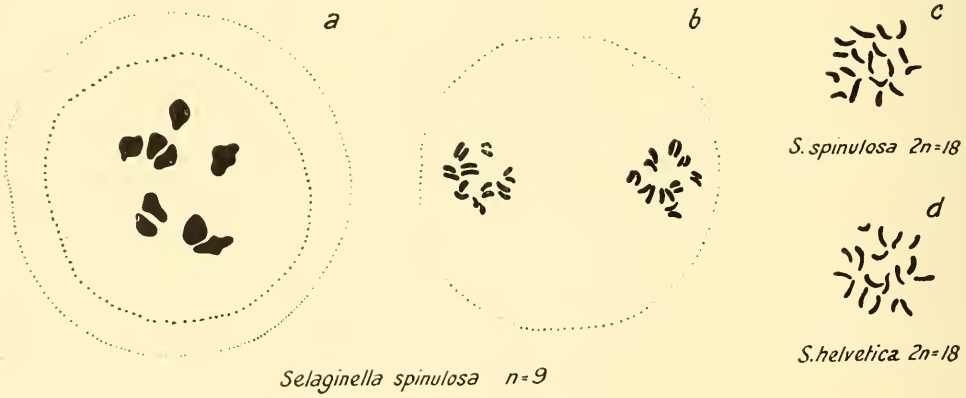


Fig. 260. Explanatory diagram to Fig. 259 *a*, *b*, *c*, *e*. All × 2000.

being radially symmetrical, with uniform leaves, and no rhizophores, the other two species being of the dorsiventral type with rhizophores. Their very close cytological similarity must mean that considerable uniformity is likely to prevail throughout the genus, and yet in spite of this, or even perhaps because of the low chromosome number, far more active speciation seems to have been at work here than in most other living members of the group. Why such a low chromosome number should have persisted here and not elsewhere in the Pteridophyta is a question which we are not likely to answer, but the fact that it has done so is of importance to this inquiry as a clear indication that the high chromosome numbers found elsewhere and increasingly so in the most ancient groups are, in a sense, secondary. The Pteridophyta seem almost certainly to have possessed in the past, and to have retained in these two genera, the simple nuclear state that the modern Flowering Plants still for the most part display. In this one particular of chromosome number the heterosporous members of the Lycopodiales seem to have retained a primitive condition, though little else about them would suggest that they are simple.

SUMMARY

In a review of all the British species of *Lycopodium* and *Isoetes* and of all the European species of *Selaginella* special morphological interest attaches perhaps to the new observations on the prothalli and growth forms of the terrestrial species of *Isoetes* (*I. hystrix*). Special points of cytological interest are the wide diversity and peculiar shapes of the chromosomes of the species of *Lycopodium* together with the very remarkable display of unpaired chromosomes suggesting hybridity in *L. Selago*. *Selaginella* and *Isoetes hystrix* are exceptional among all known members of the Pteridophyta for the small size and low number of their chromosomes. The other cytological facts provided in the chapter are contained in the following list:

Species	Source	$2n$	n
Isoetes hystrix Durieu	Cornwall	20	10
	Guernsey	20	.
	Morocco	*c.* 20	.
I. lacustris L.	Windermere	Not less than 100	54–56
I. echinospora Durieu	West Ireland	Not less than 100 (probably identical with preceding)	.
Selaginella spinulosa A.Br.	Lake District	18	9
S. helvetica (L.) Link	Switzerland	18	.
S. denticulata (L.) Link	Italy	18	9
Lycopodium inundatum L.	Scotland	.	78
L. clavatum L.	Lake District	68	34
L. annotinum L.	,,	*c.* 68	.
	Sweden	.	34
L. alpinum L.	Wales	Probably 48	24–5
	Lake District	.	.
L. Selago L.	,,	'260' (see text)	Irregular
	Scotland	.	,,
	Wales	.	,,
	Sweden	.	,,

CHAPTER 16

THE ANCIENT FERNS

We have almost reached the end of this inquiry, and now it only remains to supplement the account of the modern ferns given earlier in the book by adding some facts for the ancient ones, choosing, as before, those groups with British representatives. In this case there are three. Of the Eusporangiatae, thought to be the most ancient of all the living ferns by Bower, we have the Ophioglossaceae, represented by the Adder's Tongue (*Ophioglossum*) and the Moonwort (*Botrychium*). Of the Osmundaceae, placed by Bower on the border between the Eusporangiatae and the more modern Leptosporangiatae, we have *Osmunda*, and as an example of a Leptosporangiate group more primitive than the Polypodiaceae we have the Hymenophyllaceae or Filmy Ferns, of which there are three British representatives distributed among the two* genera which it contains, namely, *Trichomanes* and *Hymenophyllum*. Taking these groups in the order mentioned we may start with the Ophioglossaceae.

Bower's reasons for regarding the Eusporangiatae, represented in Britain by *Ophioglossum* and *Botrychium*, as the most ancient of all living ferns are purely morphological and depend on the fact that in the relatively massive construction of all parts of the plant, especially of the sporangia and sex organs, these genera contrast strongly with the undoubtedly modern Leptosporangiate ferns in features which Bower interprets as primitive. Direct fossil record of these genera is wholly lacking, but granted the correctness of the reasoning, which there seems no reason to doubt, it is certainly easier to derive the living genera in imagination from certain extinct groups of the Carboniferous Period known as the Coenopterideae than from any living ferns.

The Eusporangiatae in the world as a whole are commonly subdivided into two main groups, the Ophioglossaceae and the Marattiaceae, the latter consisting of a few genera of archaic tree ferns, all of which are tropical, and for this reason excluded from this survey at its present stage. The Ophioglossaceae contain only three genera, *Ophioglossum*, *Botrychium* and the tropical *Helminthostachys*, and therefore only the last will be excluded.

The British species of *Ophioglossum* are two, *O. vulgatum* L., the Common Adder's Tongue, widely spread over the whole country in moist pastures and probably more abundant, because of its inconspicuousness, than is generally supposed, and *O. lusitanicum* L., a much smaller Mediterranean species with a well-known British locality on the island of Guernsey. Material of both these species has been available to me, and for convenience *O. lusitanicum* will be taken first.

The appearance of this little plant at the end of its vegetative season (late April in Guernsey) may be seen in Fig. 261*a*. It is a tiny fern forming a dense, close sward in which some characteristic little bulbous Monocotyledons, notably *Scilla verna* and

* It should perhaps be noted here that Copeland (1947) replaces this simple classification of the Hymenophyllaceae by no less than thirty genera, most of which are confined to the southern hemisphere.

Romulea columnae, are also present. On the island of Guernsey, from which my material comes, *Ophioglossum lusitanicum* is a markedly xerothermal plant, in some ways resembling in its requirements *Isoetes hystrix*. It is found in a few shallow hollows near the summit of cliffs on the south coast of the island, which enjoy very full insolation and almost complete protection from north, west and east. It is not surprising, therefore, that in cultivation it seems to appreciate an annual period of desiccation as does *I. hystrix*, and, in fact, it has been given treatment exactly corresponding to that described on p. 256 since 1939, when it was first collected, and no plants have been lost by death since that date. Owing to neglect during the war, however, and to an accidental shortening of the growing season for several successive years, sporangia have ceased to be produced. For this reason the cytological study has been less complete than would otherwise have been possible.

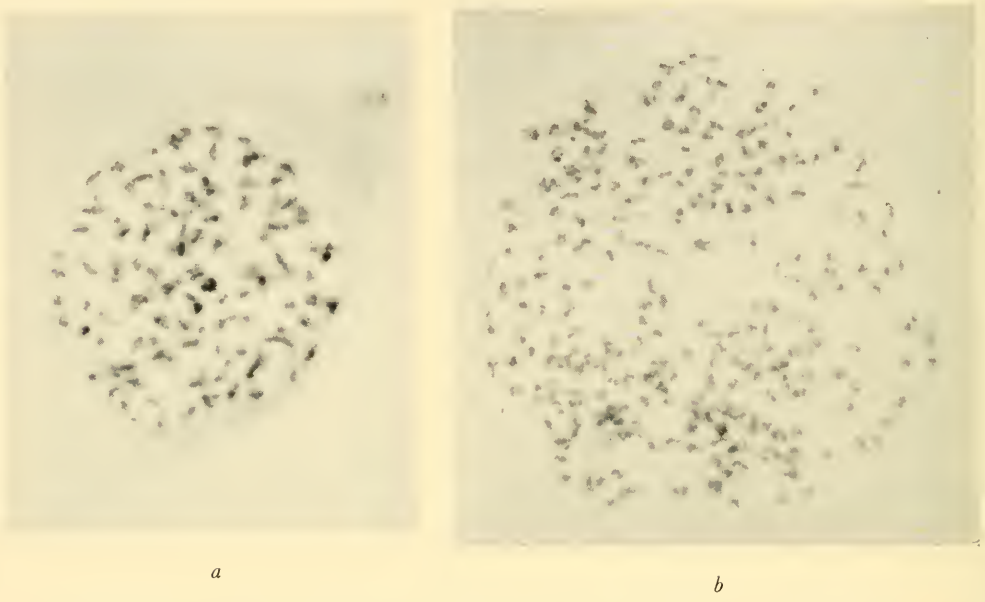
Sections of roots show at once what has been encountered so often in the Pteridophyta, namely, that the chromosomes are so numerous that mitotic figures are virtually useless for evaluating them. Fig. 262*a*, however, shows meiosis with an explanatory diagram in Fig. 263*a*. It was obtained in 1940 from the first crop of fronds to be produced in cultivation. Though good enough for an approximate count, the preparation is unfortunately not quite adequate to establish the gametic number with final accuracy. The approximate value is, however, unquestionably of the order of $n = 128$, though the number of cells available is too small to exclude a possible margin of error of one or two chromosomes. Since the difference between 128 and, say, 126 is of importance in interpreting the whole position, as anyone who has followed the description of *Cystopteris* will understand, it is desirable not to prejudice the argument at this stage by claiming a premature finality. The haploid number for *Ophioglossum lusitanicum* must therefore for the moment remain in the uncertain position of $n = c.$ 128 or, more precisely, 'not less than 125 nor more than 130'. Even so, the reader may feel that here are quite enough chromosomes for such a tiny plant.

Fig. 261. *a*. *Ophioglossum lusitanicum* L. from a dried specimen from the Guernsey locality. Natural size. *b*. *Ophioglossum vulgatum* L. from Yorkshire, from a dried specimen. Natural size.

O. vulgatum, the Common Adder's Tongue, is not infrequent in moist meadows near Manchester, and I was fortunate in having been able to collect fairly abundant material

of both generations from this region. Since the prothalli have not, so far as I am aware, been described previously from England, though they have doubtless been found by other workers, it may perhaps be of interest to give some biological notes about them.

Prothalli of *O. vulgatum* (Fig. 264) were found by me on many occasions in the summer of 1941 between the months of May and September in a field near Adlington in Cheshire, which had been long under grass and from which turf had been cut a few weeks before the search began. Attention had been attracted to the field by the circumstance that young *Ophioglossum* fronds with severed petioles were found in the cut turf which had been laid as a lawn in a neighbouring garden. When the site of the cutting was visited



a *b*

Fig. 262. Meiosis in British *Ophioglossum* in balsam after acetocarmine. × 1000. For explanatory diagrams see Fig. 263. *a. O. lusitanicum* L. *b. O. vulgatum* L.

some fully expanded fertile fronds of *Ophioglossum* were found to be growing in the undisturbed grass at the edge of the bared patch. Study of the rest of the field showed that local small colonies of the fern were scattered about in various parts, too far away from each other to be easily the result of passive vegetative expansion by means of the root buds, and the possibility of inoculation having occurred by germinated spores seemed favourable. To search for these, sods of the soil from which the turf had been removed were dug out from the immediate neighbourhood of the untouched adult plants and carefully crumbled to pieces over a sheet of white paper. As it turned out, prothalli were detected surprisingly easily, in most cases at a depth corresponding to about 5 in. below what must have been the original surface of the grass before the turf had been removed.

The prothalli of *Ophioglossum* are little, contorted, wormlike objects, very like those of *Psilotum* referred to in Chapter 14, though without any trace of vascular tissue. A group

of larger and smaller ones is shown, natural size, in Fig. 264, and the larger ones can be seen to be branched. Some sections through fertile regions are shown in Fig. 266, and the

O. lusitanicum n = c. 128

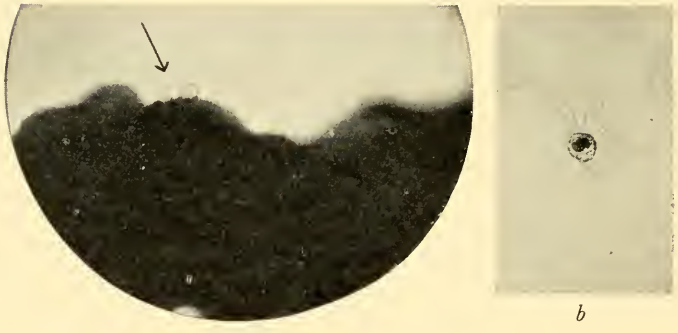
Ophioglossum vulgatum n = c. 256

Fig. 263. Explanatory diagrams to Fig. 262. × 1500.

soft undifferentiated central tissue can easily be seen. As in *Psilotum*, the prothallus lives saprophytically with the aid of an endophytic fungus, and in most details my own observations merely confirm the very excellent general description given by Bruchmann in

1904. The sunken sex organs are well seen in Fig. 266, with some additional details of the opening of archegonia, etc., depicted in Fig. 265. As was observed by Bruchmann, June is the first month in which mature sex organs can be found, but from June till September it is easy to observe both the liberation of spermatozoids and the opening and impregna-

Fig. 264. Prothalli of *Ophioglossum vulgatum* L. found in Cheshire and preserved in alcohol. Natural size.



a *b*

Fig. 265. Sexual reproduction in living prothalli of *Ophioglossum vulgatum* L. in the month of July. *a*. Part of a prothallus in water showing two of the neck cells of a freshly opened archegonium opposite the arrow. × about 100. *b*. A spermatozoid recently emerged and killed in iodine to show the cilia. × about 200.

tion of archegonia. Some observations to illustrate this are contained in Fig. 265, in which Fig. 265*b* shows a newly emerged male gamete killed with a drop of iodine and photographed at once, and Fig. 265*a* a living prothallus with the neck of a newly opened archegonium projecting beyond the solid tissue. The size of the neck cells in relation to the whole prothallus can be judged by noticing the white patch on the lower side of the picture which marks the other side of the prothallus. A short time after

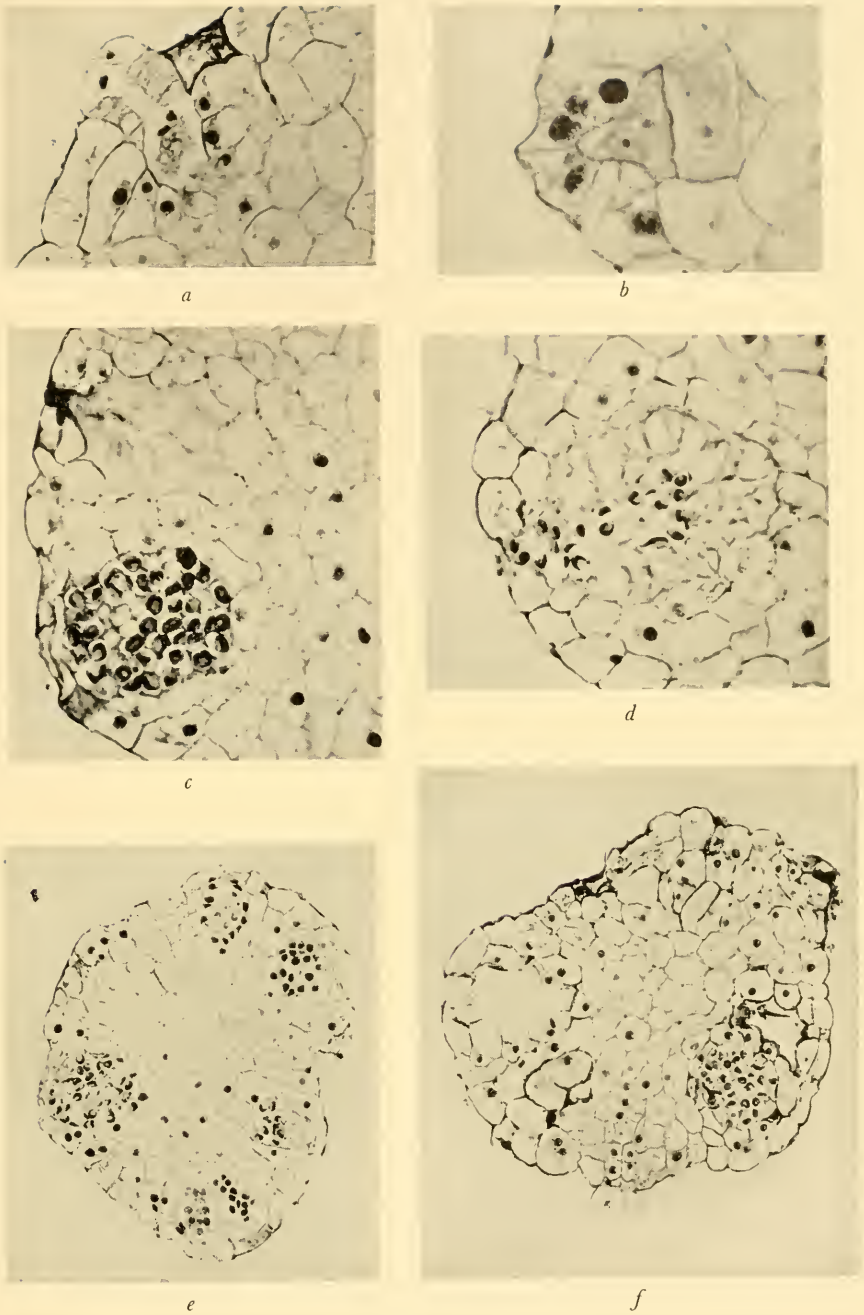


Fig. 266. Some anatomical details of the prothalli of *Ophioglossum vulgatum* L., from sections. *a*. A recently impregnated archegonium with two spermatozoids in contact with the egg (somewhat shrunk on fixing). × 200. *b*. Immature unopened archegonium. × 400. *c*. Part of a transverse section with two antheridia, the upper one empty, the lower containing young spermatozoids. The dehiscence cell appears dark in both. × 200. *d*. The same with spermatozoids emerging. × 200. *e*. A complete transverse section through the fertile region of *c*, showing various stages of antheridia. × 100. *f*. The same, showing the archegonium of Fig. 265*a* (pointing downwards). × 100.

opening, the neck cells of the topmost tier generally seem to loosen from each other and are thrown off, after which the neck closes whether fertilization has occurred or not. An early stage in the process of fertilization is illustrated in Fig. 266*a*, but no developing embryos were seen. The signs of nuclear fusion in the first few days after insemination were, however, sufficiently clear to leave little doubt that the gametes are fully functional.*

This fact is of some importance, for otherwise the question might have been raised as to whether the nuclear condition subsequently found in the sporophyte is compatible with effective sexuality. The reader will by now have become somewhat acclimatized to a record of high chromosome numbers, especially after seeing *Equisetum* and *Tmesipteris* (Chapters 13 and 14). In *Ophioglossum vulgatum*, however, we have an even more extreme case. As Figs. 262*b* and 263*b* will show, the chromosomes are smaller than those of *O. lusitanicum* and about twice as numerous. There is no sign of multivalents, so that we need not ask whether this is merely a polyploid strain. The number is difficult to assess with final accuracy, but allowing the maximum margin of uncertainty that the observations require, it may be said with confidence that in *O. vulgatum* the gametic chromosome number is not less than 250 nor more than 260, and that the correct figure lies somewhere between these limits. This is the highest chromosome number yet discovered in a wild species in the plant kingdom, for it means that there are more than 500 chromosomes per cell in the sporophytic tissue.

It is so probable from these approximate figures that *O. vulgatum* has exactly twice the chromosome number of *O. lusitanicum*, that this may be safely assumed without further demonstration, and it is here that the range of possible numbers already mentioned for the latter species becomes of importance. We are clearly dealing with the upper members of a polyploid series, the lower members of which are unknown, and we cannot at the moment diagnose the fundamental chromosome number until either the lower members are found or greater accuracy can be introduced into our study of the higher ones. If, for example, the correct figures for the two British species could be shown to be exactly 128 and 256 respectively, we should be unquestionably dealing with a polyploid

* In an attempt to observe the cytological details of fertilization a number of small prothalli were inseminated on 21 June 1940, and fixed at intervals thereafter. The presence of opened archegonia was checked in each case by microscopic examination at the time of insemination. The first specimen, which was fixed within an hour of the start of the experiment, namely, at 7 p.m. on 21 June, contained the archegonium with the still open neck canal photographed in Fig. 266*a*. Three hours later a second specimen showed an archegonium with a male nucleus somewhat swollen but definitely inside the egg. On the following day at 1 p.m. a third specimen was fixed, which on sectioning showed a female nucleus with a crescentic reticulated mass spread partly over its surface, which resembled very closely a fertilization stage seen in *Equisetum sylvaticum*, so that it can safely be interpreted in the same way. Unfortunately, this specimen had been cut by the microtome knife rather inconveniently, the centre of the crescent being in one section and the two arms in the next, so that a convincing photograph is difficult to obtain. A fourth specimen fixed at the end of a week showed no trace of unabsorbed male nuclei but also no trace of division of the egg. The only difference between this and the behaviour of *Equisetum* or *Dryopteris*, in both of which I have successfully followed the details of fertilization, is the much greater slowness of *Ophioglossum*. The late fusion stage described after 18 hours is reached in three in *Equisetum*, and in *Dryopteris* the first division of the egg occurs after a week. It is therefore probable that had observations been continued for longer the cleavage stages might have been seen. As it is, there is only proof of nuclear fusion.

series on the number 8, very familiar in the flowering plants though not so far encountered in the ferns; in that case we might either hope to find, or could postulate the former existence of, species with the intermediate numbers 8, 16, 32, 64. If, on the other hand, the correct numbers for the British species are 126 and 252 respectively, we have an extension of the position already encountered in *Cystopteris*, in which the immediate monoploid number was 42, though this might in turn be referable back to a basic haploid of 7. Since present accuracy does not enable us to distinguish between these two possibilities, we must leave the matter *sub judice* at this point. It is evident, however, that the two British species of *Ophioglossum* have opened up some very interesting problems in a very interesting genus which could profitably be further pursued in other parts of the world.

In comparison with *Ophioglossum*, the one British species of *Botrychium* (Fig. 267) offers little of special interest from a cytological point of view. From the technician's standpoint it is more difficult to handle than *Ophioglossum*, in that its seasonal periodicity is less convenient. In both species of *Ophioglossum* meiosis takes place when the fertile fronds are well above the ground and the sporangia easily seen and tested. In *O. vulgatum* itself the fertile spike is then about 2 cm. long, and the month in the north of England is June. The Moonwort, *Botrychium lunaria* (L.) Sw., matures its spores much earlier when the fronds are in the act of piercing the ground and whilst the sporangia are still closely enveloped by the unexpanded sterile portion. This means that they are very difficult indeed to find in the wild until they are too old, unless very exact knowledge of localities has been assembled in a previous year. On the other hand, cultivation presents difficulties, but under suitable conditions sods containing plants can be maintained alive through at least one season, and this, in my experience, is the less difficult way of obtaining fixations.

Figs. 268 and 269 show meiosis in material of *B. lunaria* obtained from Teesdale in summer but

Fig. 267. *Botrychium lunaria* (L.) Sw. from Teesdale. Natural size.

fixed in cultivation in April of the following year. The chromosomes are fairly large and very distinct (Figs. 268–269). Their number is $n = 45$. This number has not so far been found elsewhere in the ferns, a fact which is of no special importance. Further comment must, however, await the study of a greater number of species.

Turning now to the Hymenophyllaceae we have three British species to consider. The Killarney Fern, our only species of *Trichomanes*, is almost confined to the very moist conditions of the west of Ireland from whence it derives its name. The two species of *Hymenophyllum*, the Filmy Ferns of our mountains and of a few lowland localities such as the Common at Tunbridge Wells from which *H. tunbridgense* has long since disappeared,

Fig. 268. Meiosis in *Botrychium lunaria* (L.) Sw., permanent acetocarmine. × 1000. For explanatory diagram see Fig. 269. $n = 45$.

Fig. 269. Explanatory diagram to Fig. 268. × 1500.

are widespread and not difficult to find, though *H. unilaterale* is the more abundant of the two in most parts of England. Both rank among our smallest vascular plants though they may each cover extensive surfaces with a dense mat of saturated, translucent foliage on suitable shaded rocks or even, at the extreme west of our islands, on the bare ground.

Considering the very delicate nature of their leaves all British members of the Hymenophyllaceae are surprisingly hardy in cultivation though they are not always fertile under these conditions, and fixations should therefore, if possible, be made in their native haunts. I was unable to do this with *Trichomanes*, but I was fortunate in receiving some very fertile leaves of Irish origin from the Botanic Garden at Glasnevin in Dublin, one of which is represented in Fig. 270. The chromosomes of this species appear in Figs. 271 and 272. They are fairly large and their number is $n = 72$. The longevity of this leaf was so astonishing, it being remembered that the lamina is only two cells thick, that the facts are worthy of mention. It remained shut up in a tin box for four weeks in total darkness after the fixings had been taken, at the end of which time it was still fresh and green and the sori had increased considerably in size by comparison with the other specimen which had been pressed at once.

Fig. 270. *Trichomanes radicans* Sw., from a pressed specimen of the leaf used, supplied by Dublin Botanic Garden and probably of native Irish origin. Natural size.

With regard to *Hymenophyllum*, *H. unilaterale* Bory was fixed in the field in Borrowdale in the Lake District in early July. Its chromosomes are shown in Fig. 274*a* and they are very surprising. In spite of the minute size of the plant they are the largest individual

Fig. 271. Meiosis in *Trichomanes radicans* Sw., permanent acetocarmine. × 1000. For explanatory diagram see Fig. 272. $n = 72$.

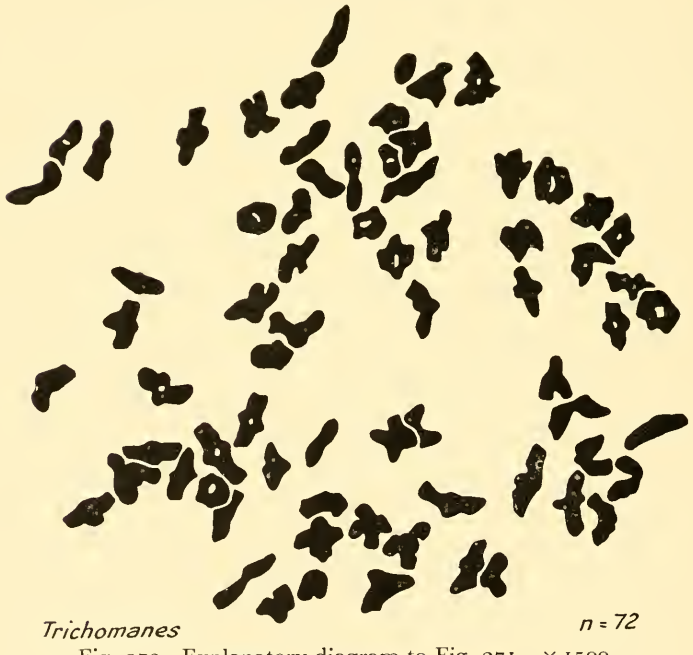


Fig. 272. Explanatory diagram to Fig. 271. × 1500.

chromosomes yet recorded in the Pteridophyta and their number is only $n = 18$. The same kind of surprise can be felt in viewing *H. tunbridgense* (L.) Sm. (Fig. 274*b*). This was fixed in the moss house of Glasgow Botanic Garden which seems to suit it unusually

well, for, in a rather dry July, it was fruiting far more luxuriantly there than in the local wild habitat near the shore of Loch Lomond. The chromosome number is even lower than that of the other species and there is a greater range of dimensions. As Fig. 274*b* will show, *H. tunbridgense* has $n = 13$, with some individual chromosomes as large as those of *H. unilaterale* but others quite small.

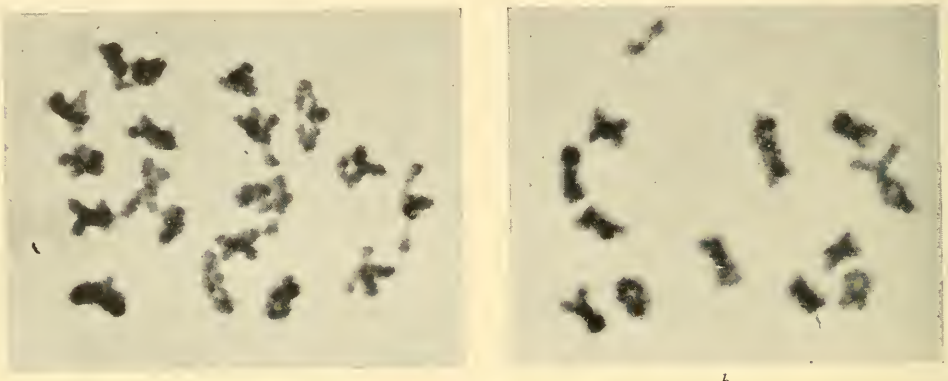
The significance of these two numbers and of the range of chromosome sizes in the last species cannot be known unless other species can also be studied. It is obvious that the two species themselves are quite distinct, in spite of a superficial resemblance caused by a general community of habit and habitat which tends to obscure their morphological differences at a first acquaintance. Which of the two chromosome numbers should be thought of as the more primitive cannot yet be known, though the relation between $n = 18$ (*H. unilaterale*) and $n = 72$ (*Trichomanes radicans*) may suggest that the former number at least is rather deeply seated in the group. It is therefore possible that the present state of *Hymenophyllum tunbridgense* may be the more derived condition, in which case it would be the only instance which has so far come before us in which we can imagine that evolution might have entailed a reduction of chromosome number.

Fig. 273. *Hymenophyllum tunbridgense* (L.) Sm., silhouette of a living frond of the strain used, grown in cultivation but obtained from Loch Lomond, Scotland. Natural size.

Be that as it may, it is clear that we have here a quite unusually interesting group for further study, though such study should preferably be carried out in the southern hemisphere where the majority of living species are to be found. That they would make wonderful cytological material for other purposes, if only their habitat requirements were less inconvenient, is perhaps indicated by the demonstration of spiral structure in *H. tunbridgense* at anaphase of the first meiotic division, which is appended in Fig. 275. Recollection of the spiral may perhaps diminish the surprise at first caused by the large size of the chromosomes, though a problem of great interest is, nevertheless, present. Bulk for bulk it looks as though a single chromosome of *H. tunbridgense* is several times larger than the whole nuclear apparatus of, say, *Selaginella*, and yet who will say that the one is more effective than the other? The extreme contrast between *Hymenophyllum*, *Ophioglossum* and *Selaginella* may indeed impress forcibly upon us how superficial are the comparisons which we have been making. *Selaginella* alone should warn us how minute is the quantity of genetical material actually sufficient for the development of an organism, and the enormous numbers of chromosomes in, say, *Ophioglossum*, no less than the massive size of the chromosomes in *Hymenophyllum* must be only of secondary importance to them, interesting as these characters may be as a guide to developmental changes in the past.

Restraining ourselves, however, from premature discussion of philosophic questions we may pass on to our last and in some ways most important group, the Osmundaceae.

Osmunda regalis, the only European member of it, has already been described in some detail in Chapter 3. A single species, however, is insufficient basis for discussion of a group, and in this one instance it seems desirable to break our general rule of avoidance of botanic gardens in order to supplement it. This is perhaps less harmful here than it



a *b*

Fig. 274. Meiosis in British *Hymenophyllum*, permanent acetocarmine. × 1000. *a*. *H. unilaterale* Bory. $n=18$. *b*. *H. tunbridgense* (L.) Sm. $n=13$.

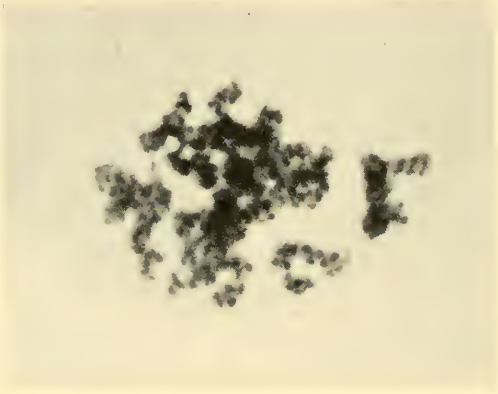


Fig. 275. *Hymenophyllum tunbridgense* (L.) Sm. Spiral structure of chromosomes at anaphase of the first meiotic division. × 2000.

might be elsewhere, because all members of the group are so distinctive and possess such well-defined geographical ranges that confusion between them is virtually impossible, even in the absence of all records of the actual sources of collections.

The living Osmundaceae consist of the three genera, *Osmunda*, *Todea* and *Leptopteris*. The first contains about ten mainly temperate species, collectively distributed over a very large part of the earth's surface. The second, *Todea*, is monotypic, consisting only of *T. barbara* (L.) Moore (sometimes known as *T. africana* Willd.), a subtropical plant

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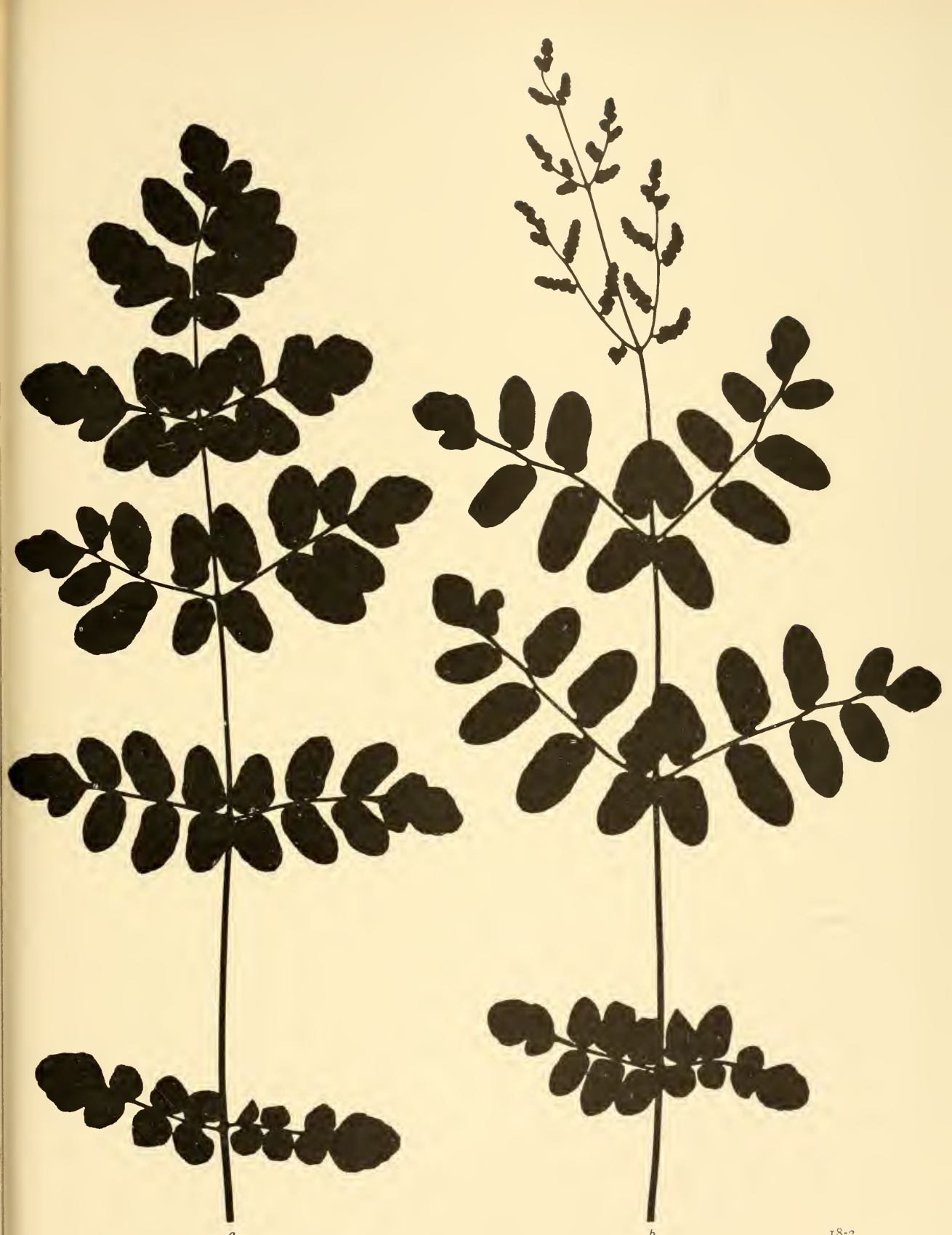
Fig. 276 (opposite). *Osmunda palustris*. Silhouette of living leaves from a horticultural source. *a*, sterile; *b*, fertile. Natural size.



a

b

of South Africa and Australia. *Leptopteris*, which resembles *Todea* in many ways but differs from it by the fact that the leaf texture is 'filmy' as in the Hymenophyllaceae, is confined to New Guinea, Polynesia and New Zealand and contains about seven species.

This very modest array of living forms is in striking contrast to the wealth of the fossil record. The most important information regarding this is still to be found in the classic researches of Kidston and Gwynne-Vaughan (1907–14), who described the structure of all anatomically preserved specimens known at that time, and very few have been added since. Stem and petiole structures, so like those of the living *Osmunda* that they could be thought of as cogeneric with it, have been found in many countries and at many geological levels, as the following list of '*Osmundites*' will show:

Table of species of Osmundites *with anatomically preserved structure known to Kidston and Gwynne-Vaughan*, 1907–14

Horizon	Species
Tertiary of Paraguay	*Osmundites Carnieri*
Tertiary of Spitzbergen	*O. Spetsbergensis*
Miocene of Hungary	*O. Schemnitzensis*
Eocene of Britain	*O. Dowkeri*
Lower Cretaceous of British Columbia	*O. Skidegatensis*
Upper Jurassic of South Africa	*O. Kolbei*
Jurassic of New Zealand	*O. Dunlopi*
,, ,,	*O. Gibbiana*

At a still earlier level, other stems and petioles have been found, so like those of *Osmunda* and *Osmundites* that they must certainly be referred to the same group though not to the same genus, since they differ from all the living and late fossil genera not only in being larger but also in being in certain respects more complex. In the Permian of Russia alone no less than four distinct genera have been described (*Zalesskya*, *Thamnopteris*, *Anemorrhoea* and *Bathypteris*). Further back still the resemblance of Osmundaceous sporangia to certain unattached fruit bodies of Carboniferous age may perhaps indicate an ancestral type in the Coal Measures.

A direct record of this kind is something unexampled elsewhere in the ferns, and it is, indeed, only to be compared with *Equisetum* among living vascular plants. This is the reason for the special importance of the group in the present context, for, perhaps even more than in *Equisetum*, we know that it, and perhaps even certain species in it, have existed unchanged for very long periods of time, although its most important evolutionary outburst was at the beginning.

All the species available in cultivation in the botanic gardens of Europe have been examined, and, in addition, I have received one specimen of *Osmunda javanica* direct from Malay, though this will not be separately described since it differed in no way from the form of the species available at Kew. It will be convenient to deal with the genera in the order listed above, and the species in the genera in the order of their geographical distribution.

All the available species of *Osmunda* are represented in Fig. 277 except for *O. regalis*, which can be seen on reference back to Fig. 22*a*, p. 36. The chromosomes of the two North American species, *O. cinnamomea* L. and *O. Claytoniana* L. (= *O. interrupta*) are shown in Fig. 277*a* and *c*. Two species or forms closely resembling *O. regalis* but smaller,

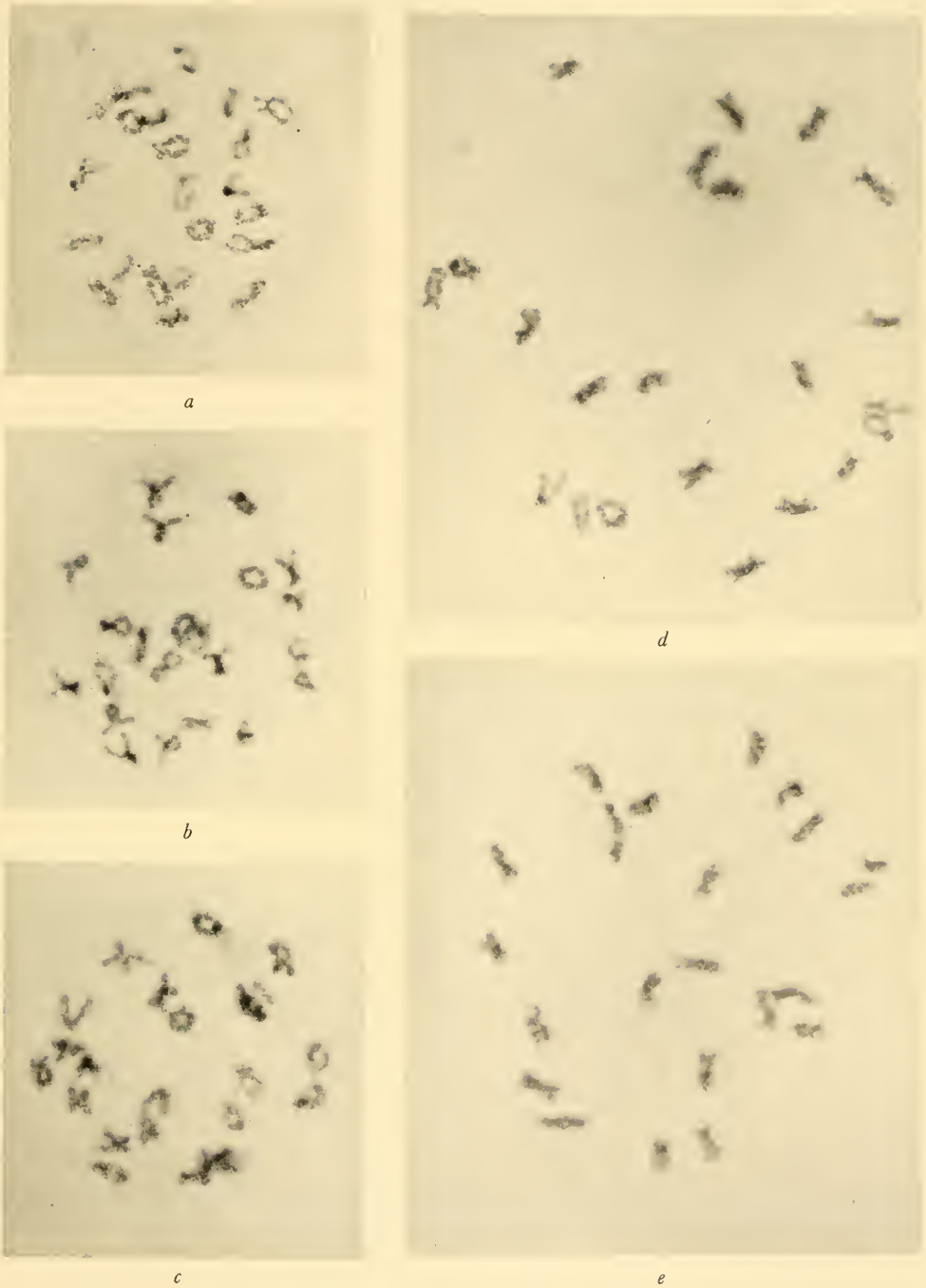


Fig. 277. Meiosis in species of *Osmunda*. × 1000. *a* in fresh acetocarmine, all the others in balsam. $n=22$ throughout. *a*. *O. cinnamomea* L. *b*. *O. gracilis* Hort. *c*. *O. Claytoniana* L. *d*. *O. palustris* Hort. *e*. *O. javanica* Bl.

and believed to have come in the first instance from South America, are *O. gracilis* Hort. and *O. palustris* Hort.* (Fig. 276), the first a hardy deciduous plant and the second a stove evergreen. Their chromosomes are visible in Fig. 277*b* and *d*. Lastly, *O. javanica*

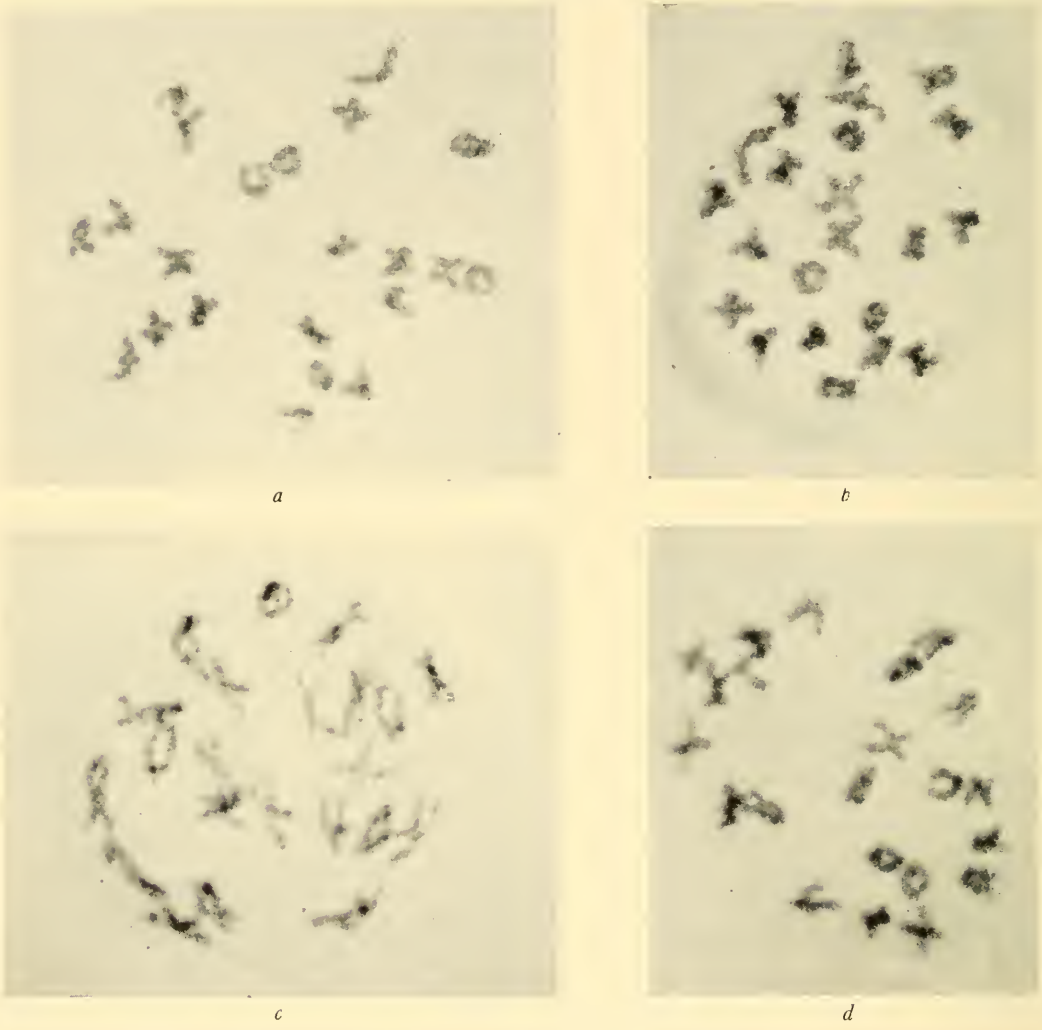


Fig. 278. Meiosis in *Todea* and *Leptopteris*, in permanent acetocarmine. × 1000. $n=22$ throughout. *a. Todea barbara* (L.) Moore. *b. Leptopteris Frazeri* (Hk. et Grev.) Presl. *c. L. hymenophylloides* (A. Rich.) Presl. *d. L. superba* (Col.) Presl.

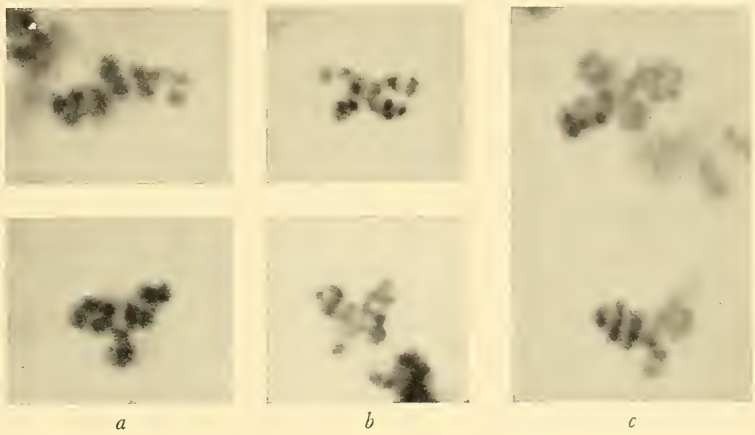
Bl., the most genuinely tropical species, will be found in Fig. 277*e*. It is obvious at a glance that all these species are very similar cytologically. All have a chromosome number which is identical with that of *O. regalis*, namely, $n=22$. The only detectable difference to the eye is indeed the slightly smaller chromosome size in the last

* The origin of this well-known horticultural plant is somewhat obscure, since it seems to have been confused at a very early date with *O. gracilis* Link with which it is not identical. If it does not come from South America its origin is unknown.

three species and the rather more diffuse outline which seems to be characteristic of *O. javanica*.

The available species of *Todea* and *Leptopteris* are grouped together in Fig. 278. *Todea barbara* (L.) Moore is in Fig. 278*a*. The other three figures are of three species of *Leptopteris*, namely, *L. Frazeri* (Hk. et Grev.) Presl, *L. hymenophylloides* (A. Rich.) Presl, and *L. superba* (Col.) Presl. The close agreement of all these species also, both with each other and with *Osmunda*, is very evident. Again, they all have $n=22$ as regards number, and the only detectable difference in this case is a slightly larger size of the chromosomes in *Leptopteris*.

That the resemblance between the Osmundaceous genera concerns not only their chromosome number but also some features of their chromosome structure is indicated by the few observations on spiral structure with which this chapter may conclude. In



a *b* *c*

Fig. 279. Spiral structure in three genera of Osmundaceae. ×3000. In each case two chromosomes from one species are shown. *a. Todea barbara* (L.) Moore. *b. Osmunda gracilis* Hort. *c. Leptopteris superba* (Col.) Presl.

Fig. 279*a* there is *Todea barbara*, in Fig. 279*b* *Osmunda gracilis* and in Fig. 279*c* we have *Leptopteris superba*, all differing characteristically in chromosome size according to the specific differences already noted but alike to a striking degree in the pitch of coil, the number of gyres per chromosome and so on.

One is left with the impression that the Osmundaceae alone, of all the ferns considered, are a genuinely primitive group which has remained primitive because it has changed very little since a remote geological period. It is not merely a case of a resemblance to an archaic ancestor shown by a form which has become specialized. The primitive character is expressed in morphology, in anatomy and in the relatively simple chromosome complement. This may perhaps suggest that the longevity and stability of the family may in part be the expression of the stability of its nuclear construction.

This idea will be touched on again in the next chapter. In the meanwhile we may offer one last comment on the possible significance of the chromosome number itself. In the Flowering Plants, as explained in Chapter 2, the commonest haploid numbers are of the order 7, 8, 9 and 11, though unexpected jumps to 22 occur in several places in the

Cruciferae and some other numbers can be found. In the Pteridophyta such low numbers have very rarely been encountered, but instead we find polyploid series on 37 or 41, prime numbers which cannot possibly be primitive. Only in the microphyllous groups, though in most of these indirectly, are we sometimes led to consider 9 or 10 or their multiples. In the ancient ferns, however, there are signs of these numbers again. *Ophioglossum*, as we have seen, may be polyploid on 8, *Hymenophyllum* and *Trichomanes* bear a quite definite relation to 9, and the Osmundaceae are similarly related to either 11 or 22 as the case may be. The importance of these groups lies at least as much in this fact as in the interest which they excite for their own sakes. No matter how imperfectly, they demonstrate with sufficient clearness that even the Pteridophyta must have started life in the distant past from cytologically simple beginnings. And before a general interpretation of our data can be even attempted this is a fact which we very much need to know.

SUMMARY

The Ophioglossaceae, Hymenophyllaceae and Osmundaceae have been examined, the first two in their British representatives only, and the last also from botanic gardens. *Ophioglossum* is remarkable as the highest chromosome number yet recorded in the plant kingdom. *Hymenophyllum* is remarkable for having the largest chromosomes in the ferns and the lowest chromosome number known in the group. The Osmundaceae stand out as the only group which seems to be genuinely primitive in all respects.

List of new chromosome numbers introduced in the chapter

	2*n*	*n*
OPHIOGLOSSACEAE		
Ophioglossum lusitanicum L.	.	125–130
O. vulgatum L.	.	250–260
Botrychium lunaria (L.) Sw.	.	45
HYMENOPHYLLACEAE		
Trichomanes radicans Sw.	.	72
Hymenophyllum unilaterale Bory	.	18
H. tunbridgense (L.) Sm.	.	13
OSMUNDACEAE		
Osmunda regalis L. (Chapter 3)	44	22
	66	Irregular
	88	44
O. cinnamomea L.	.	22
O. Claytoniana L.	.	22
O. gracilis Hort.	.	22
O. palustris Hort.	.	22
O. javanica Bl.	.	22
Todea barbara (L.) Moore	.	22
Leptopteris Fraseri (Hk. et Grev.) Presl	.	22
L. hymenophylloides (A. Rich.) Presl	.	22
L. superba (Col.) Presl	.	22

CHAPTER 17

CONCLUSIONS

The facts are now before us and though minor conclusions have been drawn as they arose in chapter after chapter it is perhaps worth the attempt to stand back a little to survey the whole before ending. The survey will be partial and incomplete as are the facts to be summarized but within the multiplicity of details which have at times perhaps seemed over elaborate a kernel of continuity can nevertheless be extracted which may be worth looking for. Before doing so, however, it will repay us to forget for a moment the microphyllous and ancient groups which have occupied the last few chapters and to think again of the more modern ferns dealt with earlier in the book, in order to consider a little more closely the British fern flora as a sample of the world's vegetation.

As we have seen, there are some 47 species of leptosporangiate ferns in the British Isles, which were analysed in detail in Chapters 4–8. Polyploidy and hybridization were met with so abundantly that we were at once able to conclude that the Filicales have indeed utilized the same types of evolutionary mechanism as the Flowering Plants. As the narrative unfolded in species after species, another impression was also conveyed, namely that many of the polyploid changes encountered seemed to be of relatively recent date and sometimes only partly fulfilled. Time after time, e.g. in *Polypodium*, *Cystopteris*, *Asplenium*, *Dryopteris*, we were forced to discriminate, often with difficulty, between populations in various grades of polyploidy and others, of lower chromosome number, still present in the same geographical area. In many cases the lineal relationship between low-numbered and high-numbered forms could be clearly demonstrated though, in some, the low-numbered forms were either imperfectly known or missing. Throughout, the general impression was gained as of a wave (or epidemic) of polyploidy which has affected the flora as a whole and which has recently resulted in the partial replacement of low-numbered species by their higher-numbered descendants, a process which is perhaps still continuing. This confronts us at once with the question as to whether the British fern flora is to be regarded as a fair and representative sample of the ferns of the world or whether it is in fact only typical of the vegetation of this part of Europe.

This is where a statistical comparison with the Flowering Plants is perhaps of importance. As was foreshadowed by Tischler (1935) and more recently worked out in greater detail by Löve and Löve (1943 and 1948) there exists a statistically significant correlation between latitude and frequency of polyploidy if comparisons are made within the rather limited area of Western Europe.* From this, Löve and Löve conclude that polyploidy as such is an adaptation to cold.

* The very fragmentary information quoted by these authors from Sicily and Timbuctoo must here be discounted as too unreliable to bear the weight of statistical comparison.

TABLE 8. *Statistical frequency of polyploidy among Flowering Plants in different regions of north-western Europe, after Löve and Löve*, 1943

	Km.²	Latitude	Percentage of flora determined	Percentage of polyploids		
				Monocots	Dicots	Total
Schleswig-Holstein	20,000	54–55° N.	92·0	63·1	43·5	48·7
Denmark	44,000	54½–58° N.	87·7	70·1	45·8	52·1
Sweden	449,000	55½–69° N.	81·5	74·6	45·9	53·7
Norway	323,000	58–71° N.	83·6	74·3	45·9	54·1
Finland	383,000	60–70° N.	80·7	77·4	45·4	54·6
Faeroes	1,400	*c.* 62° N.	85·0	76·1	51·7	60·7
Iceland	106,000	63½–66½° N.	80·0	84·3	53·4	63·5
Spitzbergen	63,000	77–81° N.	—	95·2	68·4	77·4

TABLE 9. *Statistical frequency of polyploidy among the leptosporangiate ferns of the British Isles and Madeira*

	Km.²	Latitude	Percentage of flora determined	Total number of		Percentage polyploidy
				Diploids	Polyploids	
British Isles	316,000	50–61° N.	100	22	25	53
Madeira	635	32° N.	91	22	16	42

Leaving this last conclusion aside for a moment we may compare the British ferns with Löve and Löve's data for Flowering Plants. These are summarized in Table 8, in the form presented in their 1943 paper which for the present purpose is the more convenient to quote. The top part of Table 9 gives the comparable facts for the British ferns compiled with the sole assumption that the three cases for which only non-British specimens have been available (*Cystopteris montana*, diploid *D. dilatata*, *Asplenium Adiantum-nigrum* var. *acutum*) will actually be found here in the form predicted. With this assumption, the total frequency of polyploidy among British ferns is of the order of 50 per cent, and though the exact figure obtained (Table 9) should not be taken too seriously since it might easily have been slightly different had the search for relict diploids been less thorough, or had others been found. As an order of magnitude it nevertheless compares closely with Löve and Löve's estimate for Flowering Plants in general and for Dicotyledons in particular at comparable latitudes in Scandinavia.

Does this, however, mean that polyploidy as such is geographically determined either in ferns or Flowering Plants as an adaptive response to cold? I personally think that it does not, but rather that the historical incidence of recent glaciation has produced this appearance locally and incidentally. If comparisons are conducted not within the glaciated territories but outside them a somewhat different picture emerges which, when it can be more fully seen, will almost certainly solve this particular problem. The island of Madeira is an excellent starting point for such a comparison and though the facts can at present only be quoted in a preliminary form they will be briefly introduced at this point because they provide a valuable corrective against the hasty acceptance of what may prove to be an incomplete, and therefore misleading, explanation.

CONCLUSIONS

The fern flora of Madeira contains 42 known species of leptosporangiate ferns, 38 of which have been studied cytologically at the time of writing (Manton, unpublished). Madeira itself is a mountainous island off the coast of Africa placed in a subtropical latitude quite outside the area of glaciation and which has therefore enjoyed a vegetation-cover without interruption since it emerged from the sea in Tertiary times. Of its 42 species of ferns 22 have been attributed to species also present in Britain, the remainder being species characteristic of warmer, in some cases of tropical, climates. A few are endemic.

If now the British species are examined in Madeira, Löve and Löve's generalization is borne out since out of 22 species only about 7 are polyploid, i.e. one-third instead of half the relevant part of the flora. On the other hand, if we include the non-British species as well, the total incidence of polyploidy though slightly less than in Britain (Table 9) has another, perhaps even more significant, difference, which is not shown in the Table. In Britain (the Ophioglossaceae apart) the *grade* of polyploidy is in most cases that of tetraploid with only two cases of hexaploids in *Cystopteris* and *Polypodium* respectively. In Madeira on the other hand, among the non-British species so far analysed there are two octoploids and a decaploid clearly recognizable as such by the fact that they belong to well-known British genera: the species in question are *Asplenium aethiopicum* (Burm.) Bech. (=*A. furcatum* Thunb.) $n=144$ (octoploid), *Polystichum falcinellum* (Sw.) Pr. $n=164$ (octoploid) and *Adiantum reniforme* L. $n=150$ (decaploid). Further, while in Britain the polyploids are in most cases still in geographical contact with lower-numbered relatives (from which circumstance they are thought to be recent), in Madeira these high chromosome numbers all belong to undoubtedly ancient and isolated types totally devoid of local relatives and in some cases, notably *Polystichum falcinellum* and *Adiantum reniforme*, either endemics or nearly so. These must therefore be ancient species with a long past history which, for this reason, is no longer spread before us as in so many of our own native species.

My personal conclusion from this is that polyploidy as such is not in itself either ancient or modern or an adaptation to cold or any other single climatic or ecological factor but that it is correlated rather with climatic or geographical upheavals however caused. Under stable conditions, the natural spread of species is probably accompanied by some, though perhaps infrequent, polyploidy as new species come into contact with old ones and hybridize with them. In a relatively undisturbed flora the incidence of polyploidy might therefore be expected to be low. Under changing climates or topography, on the other hand, the opportunities for hybridization and therefore for allopolyploidy can hardly fail to be increased. The nature of the significant climatic or topographical changes may be very varied and include perhaps cold, heat, drought, inundation, mountain building, volcanic action, changes in the distribution of land and sea or any other vicissitude which may affect the whole earth or portions of it. All of these may be expected to leave their mark on the evolution of vegetation.

If this is so, the high polyploids of Madeira may perhaps record world events of a different type and older than the Ice Age whereas the polyploids of Britain and of north-western Europe in general may still bear in their distribution and physiological

preferences the imprint of the most recent geological event which has engulfed this area, namely a repeated succession of glacial and interglacial periods.

If this is even partially true we are forced to the conclusion that, in the statistical attributes of their cytology, the British ferns are *not* a representative sample of the ferns of the world but that in details they are typical only of the vegetation of Europe in this particular age and latitude.

This need not, however, embarrass us unduly, for the microphyllous groups and therefore the Pteridophyta as a whole can scarcely be subjected to statistical treatment on our present limited knowledge, and discussion of them must therefore be confined to those qualitative features which even a small or imperfect sample displays quite clearly. Leaving this digression on the British ferns aside, we may therefore now proceed to a more general discussion of the larger group of which the Filicales, though a part, are only one among many. Lest, however, the reader should at this point expect the impossible and look for a general discussion of all the varied aspects of a complex and rapidly growing subject in a manner proper only to our imaginary historian of Chapter 1, it may be well to recall the rather limited terms of reference defined at the beginning of Chapter 2 and within which the inquiry has been conducted. At the present stage it would hinder and not help us to attempt to equate the results arrived at with all the current views on general evolutionary topics which have been voiced from time to time by students of other groups of plants or of animals. The remainder of this chapter will therefore contain only a summary discussion of the facts presented in the book itself and of the conclusions directly arising from them. The further evaluation of these, and in particular their assimilation into the general body of knowledge which is mainly based on the Flowering Plants, although it must eventually be attempted, would require far more than the end of a chapter and may fittingly be left to the future.

The first general conclusion from the work as a whole is perhaps the justification of the method. Cytogenetics when applied with care and with modern techniques is at least as informative in the Pteridophyta as in any other group of plants, and it is quite certain that important new light will be shed on many hitherto insoluble problems of taxonomy and of phylogeny when further work has been done. It may, however, be well to warn a beginner yet once more against over-confidence and the hope of quick results. The problems are legion and may be pursued in almost any country, but they cannot be solved quickly, and unlimited care must be urged upon any would-be investigator in the verification of specimens, in the recording of their places of origin and in the full authentication of cytological observations, or, in a group as difficult as this is technically, great harm may be done by the perpetuation of the types of error which have dogged investigators with very few exceptions in the past and which once recorded in print may be very hard to eradicate.

A second conclusion is that the Pteridophyta as a whole while employing many of the same evolutionary mechanisms as those of the Flowering Plants, have in some respects proceeded further than the Flowering Plants, as their longer history had led us to expect. The very high chromosome numbers recorded in every major group are likely in themselves to be a sign of antiquity, though the continued presence, even in the most ancient groups, of some genera and species with low numbers seems to

indicate that in the distant past the cytological state of the Pteridophyta as a whole must have been more like that of the Flowering Plants of to-day than is now the case.

Of the various types of mechanism enumerated in Chapter 2, all have been seen to be operative, though the degree of completeness in our knowledge about them varies very greatly, as was to be expected. It may be of interest to pass each briefly in review.

(1) *Hybridization* has been met with unexpectedly often and in a great variety of groups. Outstanding examples were *Equisetum litorale*, *trachyodon* and *Moorei*, perhaps *Lycopodium Selago*, and the various ferns, the latter ranging from frequently reformed hybrids such as *Asplenium germanicum*, *Polystichum illyricum*, *Dryopteris uliginosa* and so on, to very ancient hybrids now ranking as species owing to their loss of sexual reproduction, such as *Pteris cretica* and the other apogamous ferns.

(2) *Polyploidy* is present in almost bewildering profusion and has reached levels not yet touched by any other group of plants. It is only necessary to recall the 205 chromosomes of pentaploid *Dryopteris Borreri*, the approximately 204 of tetraploid *Psilotum*, the 216 of *Equisetum* (the sporophytic and not the reduced numbers are, of course, now being quoted), the 222 of hexaploid *Polypodium*, the 252 of hexaploid *Cystopteris*, the 400 odd of *Tmesipteris*, the 500 odd of *Ophioglossum vulgatum*, to realize how much further these nuclear processes have gone here than in the mere 81 or 120 chromosomes which constituted the maximum numbers quotable in the Cruciferae. The most probable conclusion to draw from this fact is, as already suggested, that high chromosome numbers are not primitive but a sign of antiquity. The undoubted tendency for outstandingly high numbers to accumulate most conspicuously in the most ancient groups (Psilotales, some Lycopods, *Equisetum*, *Ophioglossum*) will then become intelligible and may further be found to denote a measure of senility in these groups.

It is not always possible to assess with certainty the *grade* of polyploidy involved. In favourable cases where the monoploid state is a prime number, as in *Dryopteris* ($n=41$) or *Polypodium* ($n=37$), we may diagnose, say, hexaploidy with certainty, but in many other instances we are clearly dealing with only the upper members of series whose bases have been lost. In these we can infer the existence of polyploidy and some facts about it only by the indirect evidence of the arithmetical attributes of the numbers (*Cystopteris*, *Ophioglossum*), or the uncertain evidence of comparisons external to the groups (*Equisetum*). By all these means, however, a strong suspicion is raised that the rather rigid limitations on nuclear increase encountered in the experimentally induced autopolyploid series of *Osmunda*, which ended in sterility at $4n$, and in similar series in other parts of the plant kingdom (see Chapter 3), has been far exceeded by the degree of polyploidy actually achieved in nature. The reason for this is uncertain, but it may at least suggest to us that there are some effects of longevity which are not exactly reproduced when these processes are imitated in the laboratory.

With regard to the *type of polyploidy* it is noticeable how little sign of autopolyploidy has been discovered except in the artificially induced series of *Osmunda* (Chapter 3). *Psilotum* is the only clear case in which simple autopolyploidy is suspected on positive grounds. It should, however, be remembered that the only positive criterion for the diagnosis of autopolyploidy is multivalent pairing, and we do not know for certain whether in the course of thousands or millions of years this power might not become lost.

(3) *Allopolyploidy*, i.e. polyploidy imposed upon hybridity, is surprisingly common, and although, in the Pteridophyta, the best authenticated examples such as the Male Fern or *Polystichum aculeatum* have all so far been found among ferns, it is most unlikely that the phenomenon is confined to these but rather that full demonstration in the microphyllous groups is more difficult. In the Polypodiaceae, however, as we have seen, the evidence from the British flora is sufficiently advanced to permit, for the first time, of a statistical estimate of frequency. Within the 50 per cent of polyploids which characterize the whole fern flora (Table 9) we have diagnosed or given reasons to suspect a hybrid origin in at least eleven cases, i.e. *D. Filix-mas*, *Polystichum aculeatum*, perhaps the ancestor of the *D. spinulosa* or *D. dilatata* complexes, perhaps *D. Villarsii*, at least one and possibly more species in each of *Asplenium*, *Cystopteris* and *Polypodium*, and perhaps *Woodsia alpina*, *Phegopteris* and *Dryopteris Borreri*. This number is almost certainly an underestimate but it amounts to about a quarter of the total flora.

(4) *Aneuploidy* is also very conspicuous though less frequent. As in the Cruciferae it tends to characterize the relation between genera or groups of genera rather than between species. A partial exception is at first sight suggested by *Isoetes* and *Lycopodium* (see Chapter 15), though the true meaning of the facts here may merely be that the greater antiquity of the Lycopods relative to the ferns has resulted in a shift in the phyletic value of the systematic units named by taxonomists, so that a species in, for example, *Lycopodium*, is really the equivalent of a genus among ferns. As in the Cruciferae the numerical order of chromosome numbers involved in effective aneuploid changes is far lower than those involved in polyploidy, though the actual numbers are considerably above those of the Cruciferae. The commonest monoploid numbers among ferns fall (Chapters 4–11) between 29 and 41, as opposed to 6 to 11 in the dicotyledonous family, again perhaps the result of antiquity.

(5) *Genic mutations* must also be important, perhaps indeed of primary importance, since they are presumably involved as a principal factor in the formation of many, if not all, of the residual half or three-quarters of total species in which allopolyploidy is not involved. It may, indeed, be suspected that a most essential clue to the causal aspects of evolutionary mechanism must lie in the analysis of the nature of interspecific differences between species in which *no* gross cytological differences can be detected. In the Pteridophyta this subject is at present a closed book, and it is perhaps the largest topic in which the study of this group at present lags greatly behind what has been learnt from some of the more favourable Flowering Plants.

(6) Another subject on which our knowledge is deficient is the phyletic significance of *chromosome shape*. In the course of this book spiral structure of chromosomes has been demonstrated incidentally in *Hymenophyllum*, *Equisetum*, *Psilotum*, *Todea* and *Leptopteris* in addition to *Osmunda* in which it was already known, thereby increasing the comparative interest of the Pteridophyta as cytological objects. The peculiarities of texture encountered in the chromosomes of many of the microphyllous groups which result in their very unusual shapes is, however, a new phenomenon, and one which well deserves further study from the point of view of pure cytology.

Changes of chromosome shape are less easily observed in this group than in many others, though the genus *Hymenophyllum* would be admirably suited to their study if more

species could be investigated and especially if breeding work could be carried out. Here, if anywhere in the Pteridophyta, we might hope to learn something about the mechanism involved in aneuploid changes.

(7) *Chromosome size* was commented upon in Chapter 16, but the possible significance of *changes* of size, especially in association with advancing polyploidy, should perhaps be listed here. It can frequently be observed (*Ophioglossum* is a good example) that if related species are compared, the one with the higher number will often possess the smaller individual chromosomes and the reverse has not been found. This suggests that diminution of chromosome size must often either accompany or follow the incidence of polyploidy, and since our experience with *Osmunda* gave no indication of the former, it seems possible that the order of events is the latter. The interest of this observation is in its possible relation to the paradox enumerated under item (2) above, namely, that the grades of polyploidy occurring naturally seem incommensurate with the limits encountered in artificial series. It seems probable that some nuclear or physiological readjustments must occur with the passage of time to restore the power of an organism to sustain a repetition of polyploidy on a scale which would be impossible otherwise. We know nothing of the nature of such readjustments, but diminution of chromosome size is perhaps one.

This enumeration of evolutionary mechanisms expresses the factual basis for the comparison of the Pteridophyta with the Cruciferae, though it does not wholly exhaust the general conclusions which can be drawn, some of which will next be discussed.

An observation which is strengthened by the facts in both groups is the difference in evolutionary effect of aneuploid and polyploid changes. The latter make species only. The former make species also in the first instance, but such species seem usually to be potential genera or larger groups, since they have not been encountered except in their descendants which are thus designated. Why this should be so is by no means self-evident, though we may suspect that reproductive isolation of a more effective kind than is achieved by polyploidy may have something to do with it (cf. Manton, 1932), though this cannot be the whole story. The fact, however, forces us to realize that the fate of a species may depend as much on its method of origin as on any other circumstance, a conclusion which is perhaps in itself of some importance.

An observation which emerges far more clearly from the Pteridophyta than from the Cruciferae is the apparently high *survival value* of the high chromosome numbers. Their accumulation in the most ancient groups was not unexpected, but the tendency of the low-numbered forms to die out first is not so easily explained. That this is the position is, however, suggested in group after group. It is only necessary to recall the numerous cases among ferns (*Cystopteris*, *Polypodium*, *Dryopteris*, *Asplenium*) in which polyploids are abundant, but the related diploids have to be looked for with care, to be satisfied that a wave of polyploidy is affecting our flora, which may or may not be exceptional and an effect of recent glaciation as discussed on p. 283, but which now gives the impression of a recent replacement of older, low-numbered, species by newer descendants of higher chromosome number. There are exceptions of course, but the really glaring exceptions such as *Selaginella* ($n=9$), terrestrial *Isoetes* ($n=10$), *Hymenophyllum* ($n=13$ and 18) and *Osmunda* ($n=22$), stand out as much by the relative absence of polyploidy as by

the persistence of low chromosome numbers, and they only serve to enhance the generality of the rule that where polyploidy is extensively practised, the low-numbered species are the most likely to die out first. This, as we have seen, may mean that in a really ancient group such as *Equisetum*, the Psilotales or *Ophioglossum*, the origin of a series may become effaced, and it can then only be reconstructed in imagination from indirect evidence.

An unequivocal reason for this behaviour is not easy to diagnose, though suggestions about it can of course be made. The mutational character of allopolyploid species, i.e. their sudden appearance, may have something to do with it. This may perhaps be specially important if, as must often have happened, the two parent species have been brought into juxtaposition by some unusual circumstance such as changing climate, which may destroy old habitats together with their inhabitants and liberate new ecological sites for colonization, provided only that suitable colonists, not too closely bound to the older conditions, can step in while the opportunity is open. Under these circumstances, the possible advantages of mutational change (saltation in the sense of the old mutation theory), as opposed to the slower process of adjustment to changing conditions by means of natural selection acting on the raw materials (e.g. polygenes, major genes or recombinations of biotypes) provided by *genic* mutations, are obvious, and the effect may well be decisive in determining which forms can survive. Another possible explanation for the replacement of low- by high-numbered species is hybrid vigour in the latter; but this is almost certainly to be discounted in view of the immense lapses of time which must be involved in the changes which we are considering.

That to a limited extent *diminution* of chromosome number can also occur is suggested in the Pteridophyta by the solitary evidence of *Hymenophyllum* (Chapter 16) and perhaps *Doodia* (Chapter 12). With fuller knowledge other and clearer examples would almost certainly be found. These would, however, in no way disturb the generality of the rule that in this group the aneuploid changes no less than the polyploid ones, though at a slower rate, tend on balance to *increase* chromosome numbers if a long enough period of time is considered. This observation could not have been made on the Cruciferae alone, and it is indeed possible that the Flowering Plants may respond to old age differently. If they do not, or if this phenomenon is at all widespread, we must recognize it as one at least of the possibly numerous factors which lead ultimately to the decay of once dominant groups.

To pursue this idea further, we may survey the Pteridophyta and ask ourselves where, if anywhere, could a new great group of the future arise; but we should be at a loss for an answer. If we have diagnosed the evidence aright, that all great groups must start from simple beginnings with low chromosome numbers, the choice is limited. We have *Selaginella*, *Isoetes hystrix*, *Hymenophyllum* and *Osmunda*. Only an irrepressible optimist would, however, expect big developments now from such peculiar and apparently stereotyped and specialized forms as the first three, and *Osmunda*, though probably still primitive, is known to have had its biggest burst of macroevolution in the very distant past, and all the fossil evidence we possess gives no instance of a recrudescence of effective evolutionary activity after so great a lapse of time except perhaps of the 'gerontic' sort, i.e. as a senile outburst preceding extinction. We seem forced therefore to the con-

clusion that the evolutionary potential of the Pteridophyta as a source of major innovations into the earth's flora is running down.

The reasons for this seem very varied even on the flimsy evidence we have. High specialization is known to check evolutionary potential in other groups, and this is perhaps the condition in *Isoetes* and *Hymenophyllum*. *Selaginella* appears to be stereotyped, perhaps from structural reasons, for among the several hundred species which differ in details, none is sufficiently distinct to tempt taxonomists to call it a genus. *Selaginella* now seems incapable of any form of macroevolution and has probably been in this condition for millions of years, even if it was not always so.

That cytological conditions in themselves can be factors which lead to a slowing down of evolutionary activity is, however, a conclusion which more particularly emerges from the present study and which, if true, is perhaps one of the more valuable parts of it. In the Osmundaceae we have already remarked upon the coincidence of a primitive morphology and extreme uniformity of nuclear structure among the modern genera. The cytological condition here seems to be primitive, and it is perhaps also relevant to recall the marked difficulty recorded in Chapter 3 of obtaining viable sporophytes with any considerable departure from the normal complement of chromosomes. Another observation, well known to gardeners, is the great scarcity of mutant forms of even much-used ornamental plants like *Osmunda regalis* in contrast to the profusion of monstrosities which most other British species have yielded. We seem here to be dealing with an ancient group in which the nuclear constitution has, for unknown reasons, become so stable that it is now almost incapable of change either genetically or cytologically. To this fact the long retention of primitive morphological characters may perhaps be due, and the success of such species as we have, over such very prolonged periods of time, may perhaps be attributed more to the physiological resilience which makes them so tenacious of life under very varied conditions of climate or culture, than to any specially adaptive features in their morphological characters as such.

That high chromosome number in itself may act as an internal factor tending to slow down evolution is strongly suggested by a comparison between the two almost equally ancient genera of *Selaginella* and *Equisetum*. In the one, *Selaginella*, we have 800 taxonomic species. In the other, *Equisetum*, a genus which in temperate latitudes is at least as successful if area of ground occupied is a valid guide, there are only some two dozen species on the whole earth's surface. Both have existed for millions of years, and should have had ample time to develop their full potentialities. Is the difference between them perhaps in part due to the fact that genetical innovations become effective more easily where the haploid chromosome number is 9 than when the nucleus has become loaded up as it were, by aneuploidy or by polyploidy, to a prevailing gametic number of 108? If this is so, we may include high chromosome number among the factors which lead to evolutionary stagnation, and see in it perhaps one of the commonest reasons why so many of these ancient groups have remained primitive in spite of a tenacity to life which has ensured their survival. We may also predict that the Filicales, that large and lively group from which so much of our evidence has been derived, are tending that way and are almost certain, in the future, to become engulfed in their own increasing complexity and probably to succumb, except for their hardiest or

luckiest descendants, as the inevitable consequence of the working of their internal evolutionary machinery.

This is a highly mechanistic conception which disregards the actual morphology of the plants themselves and also their degree of structural 'adaptation' to their mode of life, not from any prejudice against the Darwinian or other theories of evolution but by the force of logic in the facts before us. Adaptation there must clearly be or a plant cannot survive, and if an ecological niche suitable for such survival is not available at the right time a potential new species will not become established or an old one will die out. But to look here for the mainspring of Macroevolution seems to me personally a fruitless quest. That once established, most species have a very considerable power of physiological adjustment to their environment by Microevolution has been proved in the Flowering Plants by the work on experimental ecology initiated by Turesson (1922) and powerfully amplified among others by Clausen, Keck and Hiesey (1940–1948) in their admirable *Experimental Studies in the Nature of Species*. Such work has not yet been extended to the Pteridophyta and it is very desirable that it should be. It shows unmistakably that it is possible and indeed probable that the *pace* of evolution may be accelerated or retarded by environmental pressure, since the greater the opportunity the more frequently will new forms become established, and, conversely, the more extreme the changes in a given environment the greater will be the number of species to die out. Great evolutionary activity may therefore accompany or follow periods of violent climatic oscillations, such as an ice age. But that the major evolutionary trends have been primarily caused by such changes seems impossible to believe. We have been studying, in the Pteridophyta, an ancient group which has survived innumerable cosmic vicissitudes and which had already become subdivided almost at the dawn of the fossil record into the various main branches which still survive, together with others which have died out. In our study of species we have encountered several examples of parallel evolution and a great many examples of the production of new forms by mechanical processes such as hybridization, polyploidy and so on. At the same time, in studying the major groups as a whole, we have in the last few paragraphs had repeatedly to call attention to a variety of different mechanisms by means of which evolution seems to have been slowed down or stopped. At no point have we been constrained to look outside the organism for a directive influence.

This is perhaps the one point at which serious comment on generalized evolutionary theories may perhaps be offered, while observing the limitations on the scope of the argument explained in earlier pages. In most general theories, from the time of Lamarck almost to the present day, attention has primarily been directed to the search for some mechanism either outside or inside a living plant or animal by means of which, in the course of generations, its character, appearance and functions might become changed in an orderly manner, and without which these might be presumed to remain unchanged. We may now perhaps ask ourselves whether an avoidable difficulty may not have been introduced into the quest by this last assumption, unconsciously accepted as it usually is. The emphasis which we have repeatedly had to lay on the detection of mechanisms by means of which evolution appears to have been stopped, may suggest that a more helpful basic assumption will perhaps be found to lie in the truism that evolution, as such, is

a phenomenon for which no cause need be assigned other than the fundamental instability of living matter.

The apparent orderliness of evolutionary progressions, when viewed from a distance against the background of the geological time scale, may perhaps only express the fact that at any one period, in a given organism with a given structure and development, only a limited number of types of innovation are possible without prejudice to its efficiency as an individual, but that such changes as can occur most easily will do so repeatedly. The majority even of potentially successful innovations will be expected to disappear without trace, together with the vastly greater number of intrinsically unsuccessful ones, since the accident of opportunity must also coincide. Given long enough, however, an inherent instability in certain directions rather than in others will express itself taxonomically, as parallel evolution or an orthogenetic* trend. Both of these have been encountered in the Pteridophyta. We need only recall the soral characters of *Dryopteris* or *Athyrium* and the complex apparatus of obligate apogamy for examples to demonstrate the repeated origin of similar innovations in different places. Even the polyphyletic origin of a species is perfectly possible in certain cases, e.g. hexaploid *Polypodium*. On the other hand, the polyploid series itself which has been met with so abundantly could be thought of as an example of the stepwise increase of a character caused by the repeated introduction of similar innovations in time, which could easily have a morphological equivalent. If indeed, to make this suggestion more precise, we replace, in imagination, apogamy by heterospory and advancing polyploidy by progressive precocity of the gametophyte, we are almost within sight of macroevolution leading to the seed habit, without seriously overstraining credulity as to what might reasonably be supposed to have happened.

That the polyploid series is a valid analogy, indeed perhaps even a special case, of an orthogenetic trend, is further suggested by its apparent relevance to another observation, no less remarkable for being familiar, namely, the surprising tendency of biological systems to change in the direction of increasing complexity. We are told by physicists that one of the most fundamental statements of experience in the inorganic world is the second law of thermodynamics which says that entropy, by which is meant randomness or disorder, always tends to increase. Yet here before us in both plant and animal kingdoms we find the most elaborate kinds of order spontaneously generating themselves, not indeed out of nothing, but from simpler beginnings, and this, even at the price of ultimate extinction from over-elaboration. What is the reason for this, at first sight, flagrant contradiction? That the energy of respiration is sufficient answer is difficult to believe.

This may perhaps suggest that to understand evolution in general terms we need to

* Although the sense in which this word is used here will probably be clear from the context and from what follows above, it should perhaps be emphasized that it does *not* imply the existence of any 'inscrutable creative force' as is sometimes assumed (e.g. Sewall Wright, 1949). A useful definition may be quoted from the glossary to the symposium on *Genetics, Palaeontology and Evolution* (Jepsen, Simpson & Mayr, 1949) which has reached me at the last moment before this manuscript goes to press and which cannot unfortunately be discussed as a book. '*...orthogenesis. Evolution continuously in a single direction over a considerable length of time. Usage differs considerably, but the term usually carries the implication that the direction is determined by some factor internal to the organism....*'

look not outside but inside the organism and in particular to study, with all the new tools foreshadowed in Chapter 1, and no doubt many others besides, not merely the external attributes of chromosomes (their numbers, shapes and homologies) as in this book, but rather their intimate molecular structure. We have here the usual atomic components of the inorganic world harnessed together in a manner which strikes a physicist as unfamiliar. This has been eloquently expressed by Schroedinger in a little book called *What is Life?* with which all biologists should be acquainted. We know enough about chromosome structure to be certain that their remarkable power of initiating and controlling development is due not to the statistically determined behaviour of bulk matter but to the delicate adjustment of the spacial pattern of a relatively small number of individual atoms in a molecular fabric. It is this fabric which now needs to be studied to determine its properties and laws of behaviour. Moreover, once again this must be done objectively, without preconceptions derived from the inorganic world. For though we may be certain that matter has not ceased to be matter by becoming harnessed into a novel configuration, the consequences of such a configuration must also be expected to be novel and must first be explored for their own sake before they can be used to explain other phenomena.

The problem of evolution is thus only one aspect of a larger problem of life, of growth and of reproduction. And if we could really know how an organism contrives both to develop and to transmit its likeness with such surprising fidelity from generation to generation we might after all have unravelled the greater mystery.

APPENDIX 1

NOTES ON THE CYTOLOGICAL TECHNIQUE

FIXATION

The best fixatives for sections of most members of the Pteridophyta are half-strength chromacetic-formalin for roots and 2BD for sporangia. Full-strength chromacetic-formalin can also be used for sporangia and for these is better than the half strength. The formulae of these fixatives are given below. Sporangia should always be *momentarily* dipped in alcohol (70–90%) or acetic-alcohol as a preliminary to fixation, though care should be taken not to carry over excess of alcohol into the aqueous fixative; a convenient method is to dip a sorus or sporangium into spirit for just long enough to wet it, then to put it momentarily in contact with a piece of fabric to absorb the surplus alcohol before transferring quickly to the proper fixative. Very large sporangia such as those of the Psilotales should be punctured before fixing.

Bleaching before staining is of course necessary after the osmic fixative. This can be done in the usual way by leaving wax-free sections in diluted hydrogen peroxide overnight.

For squash preparations of every kind acetic-alcohol fixation is generally satisfactory. The precise concentration is not critical, 1 : 3 glacial acetic acid : absolute alcohol is correct for some genera, e.g. *Polypodium*, though for others 1 : 2 is sometimes better. In cold weather, fixation of several days (3 days to a week) is required for successful carmine mounts, though in the height of summer overnight is sufficient. For Feulgen squashes 10 min. to half an hour's fixation is often satisfactory.

FORMULAE OF AQUEOUS FIXATIVES

I. *Chromacetic-formalin:*

Solution A:	Chromic acid	1 gm.
	Water	65 c.c.
	Glacial acetic acid	10 c.c.
Solution B:	Commercial formalin	40 c.c.
	Water	35 c.c.

Mix in equal parts immediately before use. For the half-strength fixative dilute with an equal volume of water after mixing.

II. 2BD (La Cour, 1931):

Chromic acid 1%	100 c.c.
Potassium bichromate 1%	100 c.c.
Saponin	0·1 g.
Osmic acid 2%	30 c.c.
Acetic acid 5%	30 c.c.

Since osmic acid in solution is unstable a stock solution without this component was generally made up. When required for use, 1 c.c. of 2% osmic added to 7·3 c.c. of stock solution makes the complete fixative.

STAINING

For most sections of meiosis Heidenhain's haematoxylin used as described below was successful, and with good fixation a counterstain with Bismark brown to show up cytoplasm and cell walls is a great improvement. For roots a more transparent stain is needed wherever chromosome numbers are high, and for this purpose gentian violet was satisfactory. With both these stains a difficulty very frequently met with in roots is the presence of large amounts of some substance in the cytoplasm (perhaps tannin) which holds the stain tenaciously and is liable to disfigure preparations, although, if this can be tolerated, clear staining of the chromosomes even in heavily affected cells can be obtained (see, for example, Fig. 187). This difficulty is less troublesome after osmic fixation, but the chromosomes are as a rule easier to count after chromic fixation (in roots) so that the disfigurement has usually to be accepted. The innermost layers of roots are free from this difficulty but are generally less suitable for chromosome counts. Feulgen staining was used very rarely with sectioned material (see Fig. 71*d*), though it appeared to present no difficulty. With squash material this stain was sometimes brilliantly successful after acetic-alcohol fixation, notably in *Equisetum* and *Ophioglossum*. In some other cases, notably meiosis in the Osmundaceae and Polypodiaceae, it failed completely after this fixative and was not further investigated, since in most of these species acetocarmine was found to be adequate. The schedule for acetocarmine and Feulgen staining used will be described below under Squashes.

STAINING PROCEDURE WITH HAEMATOXYLIN

Mordant half an hour. Wash in running water for three-quarters of an hour. Stain overnight. Rinse in running water (half-hour). Differentiate in alum. Wash for 4 hr. Dehydrate, clear and mount. For details of the solutions and for the insertion of a counter stain see next section.

Note. In places with hard water, preparations should probably be dipped in distilled water before entering the stain. This, however, was not necessary in either Manchester or Leeds, where the domestic tap water is exceedingly soft.

FORMULAE OF STAINS

Haematoxylin was prepared and used as advised by Dame Helen Gwynne-Vaughan. The dry stain, supplied by Messrs Gurr of London, was dissolved in absolute alcohol in a 10% concentration and left for at least 3 months. When required for use, 5 c.c. of this solution is made up to 100 c.c. with distilled water; it is then stirred with a glass rod previously dipped in mordanting alum and then left for 4 days. After this, staining is at first somewhat faint, though preparations become brighter with keeping. The pot improves considerably after a month of constant use, and it will then remain in good condition for at least a year. The stock solution will remain in good condition for several years but

probably not indefinitely. After 10 years I have sometimes had to discard an old stock owing to the formation of a chocolate-coloured precipitate when it was mixed with water. In old stocks it is also sometimes better to dilute down to $\frac{1}{4}\%$ instead of $\frac{1}{2}\%$ for the working strength, though at this dilution the stain becomes exhausted more rapidly; it may, however, give very bright and clear preparations while it lasts.

Alum. The correct strength of iron alum varies somewhat with the material, but usually the Pteridophyta require much weaker alum than the Flowering Plants. A 2% solution for mordanting and an 8% solution for differentiating is often satisfactory. (In Flowering Plants I have generally used the strengths in the reverse order.)

Bismark brown. A 2% solution in 90–95% alcohol is convenient. The counterstain can then be introduced, after haematoxylin, last thing before finishing the dehydration. Alternatively, the stain may be dissolved in a weaker alcohol (50 or 70%) and introduced during the dehydration, a procedure which is sometimes preferred as leading to better washing off of surplus stain. The length of time required in the stain varies with the age of the solution but should be of the order of 2 min.

Acetocarmine. The only secret in this stain is to have one's solution strong enough. Heat a 45% aqueous solution of glacial acetic acid to boiling-point with excess of carmine. Cool and filter. Use distilled water and keep the vessel covered while heating.

Schiff's reagent (Leuco-basic fuchsin) for Feulgen technique. Pour 100 c.c. boiling, distilled water on to 0·5 g. of Gurr's basic fuchsin. Agitate thoroughly and cool to 50° C. Filter and add 10 c.c. N/1-hydrochloric acid and 0·5 g. potassium metabisulphite. Shake well and keep in the dark in a glass-stoppered bottle for 12–18 hr. During this time the solution bleaches to a pale straw colour and is then ready for use. Stored as above, the reagent will keep in good condition for up to 3 months.

SQUASH METHODS

I. *Simple acetocarmine.* With large sporangia the very simplest of the squash methods may be satisfactory. Thus in all the Osmundaceae 12 hr. fixing in acetic-alcohol followed by breaking of a group of sporangia with a flat-ended needle into a drop of acetocarmine will provide ideal material for squash preparations, without the need for any manual pressure, *provided that* all the empty sporangia and other solid bodies are removed. It is then only necessary to put on a cover-slip, conveniently a no. 1 thickness, $\frac{7}{8}$ in. square, and boil the preparation violently under one corner for a second or two, during which process the cover-slip itself provides all the pressure which is needed. The initial size of the drop of liquid should be such that after boiling the cover-slip clings closely to the slide without air bubbles. In this condition it may be ringed with wax for further study in the fresh condition or it may at once be made permanent. If this is to be done it should be left for as long as possible, i.e. half an hour or until air begins to enter, before lifting the cover-slip, to ensure the maximum adhesion of the squashed cells, and it may be beneficial to the intensity of colour to heat gently several times, care being taken not to reach boiling-point again or the cover-slip will merely blow off explosively and the preparation be lost.

II. *Making the preparation permanent.* The method used has been almost exactly that originally devised by McClintock, though for ease of reference the details may be given here as follows:

(i) Dissolve off the cover-slip by immersing the slide face upwards in 45% acetic acid. It is essential not to attempt to hurry this process by poking the cover-slip with a needle or the more tightly attached cells will wash off.

(ii) Dehydrate by passing slide and cover-slip through graded mixtures of acetic acid and absolute alcohol of the following strengths 1 : 1, 1 : 3, 1 : 9, absolute.

(iii) Partially clear in 1 : 1 absolute and xylol from which it is safe to mount in balsam. It is essential in mounting that the cover-slip should be replaced exactly over the area from which it came. This may be clearly marked by the lines of dried carmine formed round the edges of the cover-slip after the original boiling and which should on no account be cleaned off until the preparation is finished, but if this is not sufficient it may be convenient to mark the position of two corners of the cover-slip with a diamond on the back of the slide before immersing it in the acetic acid.

III. *Acetocarmine squash with manual pressure.* With small sporangia in which it is impossible to remove the empty cases entirely additional pressure is needed. This applies particularly to the Polypodiaceae with mixed sori, in which ripe spores which resist pressure are always liable to be present on the same slide as the softer mother cells. The spore mother cells are squashed out of their sporangia as thoroughly as possible with a flat-ended needle into the stain and the larger lumps of tissue such as the indusia removed. Not too many sori should be dealt with at one time, six or so on a slide are generally enough. A cover-slip, which may be large or small according to taste, is then put on and the preparation heated gently. Whether it should be boiled or not depends on the intensity of colour and the softness of the material. If staining is faint, boiling enhances it; on the other hand, if the cells are soft they may blow to pieces on boiling. In either case the pressure which produces flattening of the cells is applied by hand. The warm preparation is put down on the bench, momentarily covered with a piece of blotting paper and a finger passed rapidly over it with the precise degree of pressure which can only be learned by experience. The field should then be searched at once, preferably without ringing, and ruthlessly discarded if it does not show any specially favourable cells. This is essential, because in the Polypodiaceae, where only 16 or sometimes 8 mother cells may be the entire contents of a sporangium, successfully squashed and usable cells may occur singly on a slide and if not found at once be looked for in vain afterwards.

As a routine procedure in dealing with this type of material every instance of an exceptionally perfect cell was photographed at once and perhaps also drawn while the preparation was in the first wet condition, as a precaution against its loss on transfer to balsam. A few such photographs have been included in this book (e.g. Figs. 183*b*, 218*b*). In every case, however, the transfer to balsam by the method listed in paragraph II above was attempted, the only additional precaution needed being slow dehydration, a delay of a quarter of an hour or longer in each of the alcohols being not too much. In most cases a cell could be rephotographed in balsam and the greater number of the figures in this book are of these; occasionally the critical cell became detached and lost

or spoiled when the slide and cover-slip were first separated, and the benefit was then reaped of the preliminary photographs.

IV. *Modification of the acetocarmine technique.* Orcine and Lacmoid were both tried as a substitute for carmine (La Cour and Darlington), though no benefit was felt. For the Pteridophyta, carmine when properly used is very satisfactory, and the reagent is also stable enough to remain in good condition for a year with only occasional filtering. Lacmoid, under certain circumstances, had the disadvantage of rendering the chromosome brittle and was discarded for this reason.

Experiments were also made with alternative mountants such as 'Euparal', and though satisfactory these showed no advantage over balsam and were therefore not extensively employed. Euparal, indeed, has a disadvantage over balsam in that it is soluble in the immersion fluid that was used (Leitz 'Objektol'), which precludes the making of high-power observations near the edge of a slide. For this reason alone it would have been discarded.

V. *Feulgen squashes.* These were only used sparingly as a check on certain troublesome genera such as *Equisetum*, which had proved difficult to complete by other means. Both in *Equisetum* and *Ophioglossum* very beautiful preparations were obtained by this means, though the much smaller size of the individual chromosomes after this treatment than when swollen by acetocarmine meant that little if any additional facts were learnt. The chromosomes also became somewhat brittle and liable to separate at secondary constrictions.

The procedure adopted was as follows:

(i) Fix in acetic-alcohol for 10 min. to half an hour.

(ii) Transfer to water.

(iii) Hydrolyse in normal HCl at 60° C. for 10–20 min. according to the material.

(iv) Transfer to Schiff's reagent (see p. 295) in the dark for half to 2 hr.

(v) Transfer to 45% acetic acid on a slide, tease out, cover, warm gently and squash *gently* under a piece of blotting paper.

(vi) Examine at once, and if successful transfer to balsam by McClintock's method (p. 296 above).

Examples of this technique are reproduced in Figs. 218*a* and 220.

OBSERVING

There was nothing worthy of special note about the methods of observing except perhaps the very close use of photography as an integral part of observation at every stage (for further details see Appendix 2), and the liquid used as an immersion fluid. The frequent necessity of making detailed observations on unringed liquid mounts made the change from cedar oil as the immersion fluid to 'Objektol' very advantageous. Objektol (which is obtainable commercially from Messrs. Leitz of London) does not stiffen on exposure to air and can be washed off with water. This means that its presence is not a difficulty when slides have to be processed further, since it merely disperses in the acetic acid, and being very liquid throughout the examination reduces considerably the risk of damage to the slide by accidental movement of the cover-slip which can occur when cedar oil has become stiff.

APPENDIX 2

NOTES ON THE PHOTOGRAPHIC TECHNIQUE

Though photography has been very extensively employed as an integral part of observation at every stage, there is nothing specially remarkable about the photographic technique for straightforward observations such as those reproduced in half-tone throughout the book. For photomicrographs of haematoxylin and gentian violet preparations and also for natural-size photographs, Ilford Special Rapid Panchromatic plates with appropriate colour filters were used. For carmine mounts some of the later photographs were taken on Thin Film Half-Tone plates, since these give greater contrast especially with a red object.

The methods used for the making of text-figures are perhaps less well known and the following processes may be of interest:

(a) *Photographic basis for drawings.* A camera lucida was not used for any of the black and white diagrams, but all were drawn on the basis of a photograph which in many cases is the photograph reproduced. The original photographs were all taken at standard magnifications of 500, 1000 or 2000 diameters, according to circumstances and from them drawings were made according to the following procedure. An enlarged print at a higher magnification (2000, 3000 or 4000) is made on to matt-surface bromide paper. This is then inked over in the usual way, after which the photographic image is bleached (see below) and the drawing alone remains. A very suitable paper for this purpose was found in Kodak WSM. 3.S which has a very convenient texture both for drawing and for subsequent treatment, but any ordinary matt paper can be used. The advantage of this method of obtaining diagrams is that far greater accuracy is achieved than with a camera lucida and with much less effort to the observer; a thing of importance where nuclei of the complexity of those shown here are to be analysed.

(b) *Method of bleaching.* Any normal photographic method can be used, but to avoid the handling of KCN, which is a dangerous reagent outside a chemical laboratory, the following procedure may be recommended as having proved satisfactory.

A bleaching solution is prepared immediately before use by adding sufficient of a stock solution of 10% potassium ferricyanide to the normal stock solution of 20% hypo to produce a deep yellow colour. (The exact strength is not critical, but it is considerably stronger than that used for normal reduction.) The print to be bleached is then soaked in water until uniformly limp and is then immersed in the bleaching solution until the image has disappeared. The diagram which remains is then rinsed in water, care being taken not to rub the ink, which is otherwise liable to come off or run, and is then given a brief bath in acid hypo to prevent or to remove yellow stains. It is then washed in running water for half an hour. To assist drying it may be gently blotted. When dry it may be necessary to touch up the ink here and there and the diagram is then ready.

(c) *Duplication of drawings by means of a paper negative.* If it is undesirable to use the original drawing for subsequent purposes it is a very simple matter to obtain facsimiles

which are virtually indistinguishable from originals. One method is to make a paper negative by treating the drawing as a transparency and making a contact print of it on to any slow contrasty printing paper. The two pieces of paper should be kept closely in contact with the unexposed sensitive surface against the drawing either in a printing frame or by other means. Illumination is through the back of the drawing, and a negative print results, from which positives may be obtained by a repetition of the process. Any ordinary gaslight paper can be used, though Ilford Reflex Document paper no. 50 was found specially suitable and cheaper than gaslight paper for large diagrams.

(d) *Duplication of drawings by reflex copying.* If a drawing is much touched up, or has writing on the back, or is on rather opaque paper or board, it may be inconvenient to illuminate through it, and in that case reflex copying is to be preferred. The drawing is placed face upwards and is covered by a piece of Ilford Reflex Document paper no. 50 with the sensitized side downwards. Close contact is essential, and may be obtained either in a printing frame or by covering with a piece of glass held down by weights. Illumination is through the back of the Reflex Document paper. The length of exposure can easily be ascertained by trial, but an average duration is 20 sec. with a 60 W. bulb at 2 ft. After development a paper negative is again obtained, from which positives may be printed off by normal contact methods.

(e) *Silhouettes of fern leaves.* A paper negative is made by putting the leaf in contact with slow contrasty sensitized paper and exposing it as if for a photographic print. The use of a printing frame is convenient if the specimen is small and ordinary contrasty gaslight paper to be used. If the specimen is large or a part of a herbarium sheet which it is inconvenient to disturb, Reflex Document paper may be preferred, placed either above or below the specimen (cf. (c) and (d) above). The negative so obtained may be used for printing off positives in the usual way. This method is very valuable, since complete accuracy and great speed are obtained at very small cost.

APPENDIX 3

GENERAL SUMMARY OF THE PRINCIPAL NEW FACTS RECORDED

(1) Chromosome counts have been given of all known British members of the Pteridophyta and of some non-British species and hybrids together with almost all known examples of ferns with apogamous life histories.

(2) Allopolyploidy has been demonstrated or strongly suggested in the following normal taxonomic species:

Dryopteris Filix-mas (L.) Schott sens.strict.emend. (Chapter 4).
Cystopteris fragilis (L.) Bernh. in part (Chapter 7).
Polypodium vulgare L. in part (Chapter 8).
Polystichum aculeatum (L.) Roth (Chapter 9).
Scolopendrium hybridum Milde (Chapter 9).
Woodsia alpina (Bolton) Gray (Chapter 9).
Doodia caudata (Cav.) R.Br. (Chapter 12).

(3) Allopolyploidy has been demonstrated or suspected in all sufficiently investigated apogamous species (Chapter 11), namely:

Cyrtomium falcatum auct. (= *C. falcatum* Presl, *Fortunei* J.Sm. and *caryotideum* Presl)
Dryopteris atrata (Wallich) Ching
D. Borreri Newman
D. remota A.Br.
Pteris cretica L.
Pellaea atropurpurea (L.) Link
Asplenium monanthes L.

(4) Further work with a view to taxonomic revision is required to establish the nature of the following new forms:

Diploid *Dryopteris dilatata* (Chapter 5).
Diploid *D. Villarsii* (Chapter 5).
Diploid *Asplenium Trichomanes* (Chapter 6).
Asplenium Adiantum-nigrum var. *acutum* (Chapter 6).
Various segregants from *Polypodium vulgare* (Chapter 8).
Various segregants from *Cystopteris fragilis* (Chapter 7).

(5) The following naturally occurring sterile hybrids have been examined and their nature discussed:

Dryopteris uliginosa (Newman) Druce (Chapter 5).
D. dilatata (Hoffm.) A. Gray × *D. spinulosa* (Müll.) Watt (Chapter 5).
'*D. remota* Moore', from Windermere (Chapter 5).

Asplenium germanicum auct.non Weiss (Chapter 6).
Polystichum illyricum Hahne (Chapter 9).
Woodsia ilvensis (L.) R.Br. × W. *alpina* (Bolton) Gray (Chapter 9).
Equisetum trachyodon A.Br. (Chapter 13).
E. Moorei Newman (Chapter 13).
E. litorale Kuhlw. (Chapter 13).

(6) The following hybrids have been synthesized and their cytology and structure described:

Dryopteris Filix-mas (L.) Schott s.str. × *D. abbreviata* (Lam. & DC.) Newm. (Chapter 4).
Polystichum aculeatum (L.) Roth × *P. angulare* Presl (Chapter 9).

(7) *Lycopodium Selago* L. is shown to be problematical and to require further work to determine its chromosome number and to reconcile the evidence of extensive failure of pairing at meiosis, suggestive of a hybrid origin, with the known fact of the occasional occurrence of gametophytes. (Chapter 15.)

(8) The highest chromosome number yet recorded in the plant kingdom has been encountered in *Ophioglossum vulgatum* L., where $n = 250–260$. (Chapter 16.)

(9) A haploid sporophyte has been obtained by induced apogamy in *Scolopendrium vulgare* Sm. (Chapter 12). Other suspected cases of haploids have been shown to admit of alternative explanations. (Chapters 11 and 12.)

(10) A photographic demonstration of the cytology of sporangial development in apogamous ferns has been given. (Chapters 10 and 11.)

(11) Multivalent chromosome pairing has been demonstrated for the first time in the Pteridophyta in the autopolyploid series of *Osmunda* which is described. (Chapter 3.)

(12) Spiral structure of chromosomes has been demonstrated in *Equisetum*, *Psilotum* (rather imperfectly), *Hymenophyllum*, *Todea* and *Leptopteris*, in addition to *Osmunda* in which it was already known.

(13) Some new or little-known biological observations are quoted on the cultural needs of *Ophioglossum lusitanicum* L. and *Isoetes hystrix* Durieu (Chapters 15 and 16); on the prothallial structure of *Isoetes hystrix*, *Ophioglossum vulgatum* and *Psilotum* (Chapters 14, 15 and 16), and on the coning habits of the European species of *Equisetum* (Chapter 13).

(14) Statistical comparisons are made between the frequencies of polyploidy in the fern floras of Britain and Madeira and of both with the Flowering Plant floras of N.W. Europe. From this, conclusions are drawn as to the cause and meaning of polyploidy (Chapter 17).

(15) Other evolutionary conclusions are discussed in Chapter 17.

APPENDIX 4

CHROMOSOME NUMBERS

Complete list of chromosome numbers in the Pteridophyta (new, emended and verified) introduced throughout the book. The entries under country of origin refer in each case to the source of the cytological material actually used, though greater detail will in many cases be found in the partial lists at the ends of the appropriate chapters and in the text. The treatment of the specific names differs slightly from that of the text in that preference is in this case given to the most technically correct name known to me, although where a familiar name was retained in the body of the book but will now be displaced the synonym is given in brackets to avoid confusion. The use of inverted commas denotes either an invalid name according to the principles of nomenclature, which has been retained from lack of a suitable synonym, or the application of a valid name to more than one thing, one of which if not both will need a new specific epithet when further work has been done. The order of arrangement of the genera of ferns follows Christensen's *Index Filicum*, supplement 1934, except where a genus has had to be split to conform to the cytological evidence.

FILICALES

Name	Country of origin	$2n$	n	Reproduction	Chapter(s)
HYMENOPHYLLACEAE					
Trichomanes:					
T. radicans Sw.	Ireland	—	72	Normal	17
Hymenophyllum:					
H. unilaterale Bory	England	—	18	Normal	17
H. tunbridgense (L.) Sm.	Scotland	—	13	Normal	17
POLYPODIACEAE					
Woodsia:					
W. ilvensis (L.) R.Br.	Wales	—	*c.* 41 (i.e. 41–42)	Normal	7 and 9
W. alpina (Bolton) S. F. Gray	Scotland	—	*c.* 82 (i.e. 82–84)	Normal	7 and 9
Cystopteris:					
C. montana (Lam.) Desv.	Switzerland	—	84	Normal	7
C. Dickieana Sim	Hort. (Scotland)	—	84	Normal	7
'*C. Baenitzii* Dörfl.'	Norway Greenland	—	84	Normal	7
C. fragilis (L.) Bernh.	Britain Switzerland Finland Sweden Norway Iceland Canada	—	84	Normal	7
—	Britain Switzerland	—	126	Normal	7
'*C. alpina* Desv.'	Switzerland	—	126	Normal	7

Name	Country of origin	2n	n	Reproduction	Chapter(s)
Dryopteris:					
D. abbreviata (Lam. et DC) Newm.	British Isles	82	41	Normal	4
D. aemula (Ait.) O. Kuntze	British Isles	82	41	Normal	5
D. Borreri Newm.	British Isles Switzerland	82	82	Apogamous	5 and 11
—	British Isles Switzerland Norway	123	123	Apogamous	5 and 11
D. remota (A.Br.) Hayek	Ireland	123	123	Apogamous	5 and 11
'*D. remota* var. *Boydii*'	Scotland	*c.* 123	*c.* 123	Apogamous	5 and 11
D. remota var. *subalpina* Borbas	Hort. (Switzerland)	*c.* 123	*c.* 123	Apogamous	5
'*D. remota* Moore'	England	*c.* 164	Irregular	Sterile	5
D. atrata (Wall.) Ching	Botanic Gardens	123	123	Apogamous	11
D. Filix-mas (L.) Schott s.str.	Britain Switzerland Sweden	164	82	Normal	5
D. spinulosa (Müll.) Watt	Britain Sweden	164	82	Normal	5
D. cristata (L.) A. Gray	England Switzerland	164	82	Normal	5
D. dilatata (Hoffm.) A. Gray	British Isles	164	82	Normal	5
D. dilatata (form of)	Switzerland Norway Sweden	82	41	Normal	5
D. Villarsii Woynar (= *D. rigida* (Hoffm.) Underw.)	England	164	82	Normal	5
	Switzerland	82	41	Normal	5
D. uliginosa (Newm.) Druce	Switzerland	164	Irregular	Sterile	5
D. spinulosa × *D. dilatata*	England	164	Irregular	Sterile	5
D. Filix-mas × *D. Borreri*	England	164	164	Apogamous	5 and 11
	Ireland	205	205	Apogamous	5 and 11
D. Filix-mas × *D. abbreviata*	Synthesized	123	Irregular	Sterile	4
Thelypteris:					
T. palustris Schott	England	70	35	Normal	5
T. Oreopteris (Ehrh.) C.Chr.	England	68	34	Normal	5
Gymnocarpium:					
G. Dryopteris (L.) Newm.	Britain Sweden	—	80	Normal	5
G. Robertianum (Hoffm.) Newm.	England	—	*c.* 80 (i.e. 80–84)	Normal	5
Phegopteris:					
P. polypodioides Fée	British Isles Sweden	90	90	Apogamous	5
Polystichum:					
P. Lonchitis (L.) Roth	British Isles Switzerland	82	41	Normal	6 and 9
P. setiferum (Forsk.) Woynar (= *P. angulare* (Kitaib.) Presl)	Britain Switzerland	82	41	Normal	6 and 9
P. aculeatum (L.) Roth	Britain Switzerland	164	82	Normal	6 and 9
P. falcinellum (Sw.) Pr.	Madeira	—	164	Normal	17
P. illyricum Hahne (= *P. Lonchitis* × *P. aculeatum*)	Switzerland	123	Irregular	Sterile	9
P. aculeatum × *P. angulare*	Synthesized	123	Irregular	Sterile	9
Cyrtomium:					
C. falcatum (L.f.) Presl	Hort.	123	123	Apogamous	10 and 11
C. Fortunei J.Sm.	China	123	123	Apogamous	10 and 11
C. caryotideum (Wall.) Presl	Uganda	123	123	Apogamous	10 and 11
Athyrium:					
A. Filix-femina (L.) Roth	Britain Sweden	—	40	Normal	6
A. alpestre (Hoppe) Rylands	Scotland	80	—	Unknown	6
A. flexile (Newm.) Syme	Scotland	80	40	Normal	6
Phyllitis (= *Scolopendrium*):					
P. Scolopendrium (L.) Newm. (= *Scolopendrium vulgare* Sm.)	Britain	72	36	Normal	7, 9 and 12
P. hemionitis (Lag.) O. Kuntze	France	72	36	Normal	9
P. hybridum (Milde) Christensen	Lussinpiccolo	*c.* 144	*c.* 72	Normal	9

Name	Country of origin	2n	n	Reproduction	Chapter(s)
Asplenium:					
A. fontanum (L.) Bernh.	Switzerland	72	36	Normal	6
A. viride Huds.	Britain	72	36	Normal	6
A. marinum L.	Britain	72	36	Normal	6
A. Adiantum-nigrum L. var. *acutum* (Bory) Pollini	Madeira	72	—	Normal	6
A. Petrarchae DC.	France	—	72	Normal	6
A. ruta-muraria L.	Britain	—	72	Normal	6
A. septentrionale (L.) Hoffm.	Britain Switzerland Finland	144	72	Normal	6
A. lanceolatum Huds.	England	—	72	Normal	6
A. Adiantum-nigrum L.	England	—	72	Normal	6
'*A. Trichomanes* L.'	England Switzerland France	144	72	Normal	6
	Wales	—	36	Normal	6
A. aethiopicum (Burm.) Bech.	Madeira	—	144	Normal	17
A. germanicum auct.non Weiss (=*A. Breynii* Retz.)	Wales Italy Switzerland Sweden	108	Irregular	Sterile	6
A. monanthes L.	Madeira	108	108	Apogamous	11
Ceterach:					
C. officinarum Lam. & DC.	England France	—	72	Normal	6
C. aureum (Cav.) v. Buch	Teneriffe	—	72	Normal	6
Blechnum:					
B. spicant (L.) With.	Britain	68	34	Normal	7
Doodia:					
D. caudata (Cav.) R.Br.	Botanic Garden	—	65–70	Normal	12
	Induced	*c.* 70	Irregular	Sterile	12
	Induced	Over 200	Unknown	Unknown	12
Pellaea:					
P. atropurpurea (L.) Link	California	87	87	Apogamous	11
Cryptogramma:					
C. crispa (L.) R.Br.	England	—	60	Normal	7
Adiantum:					
A. capillus-veneris L.	Ireland Italy Spain	60	30	Normal	7
A. reniforme L.	Madeira and Teneriffe	—	150	Normal	17
Pteris:					
P. cretica L.	Italy	58	58	Apogamous	10 and 11
var. *albolineata* Hook.	Hort.	*c.* 90	*c.* 90	Apogamous	11
P. cretica L.	Uganda	*c.* 120	*c.* 120	Apogamous	11
Pteridium:					
P. aquilinum (L.) Kuhn	Britain Malay	—	52	Normal	7
Polypodium:					
'*P. vulgare* L. var. *serratum* (Willd.) Milde'	France Switzerland Italy England and Ireland	74	37	Normal	8
P. vulgare L.	British Isles Scandinavia Switzerland France	—	74	Normal	8
'*P. vulgare* L.'	British Isles Western Europe	—	111	Normal	8
P. virginianum L.	Nova Scotia	—	37	Normal	8
'*P. vulgare* L. var. *occidentale* Hook.'	Western N. America	—	37	Normal	8
OSMUNDACEAE					
Todea:					
T. barbara (L.) Moore	Botanic Gardens	—	22	Normal	16
Leptopteris:					
L. Frazeri (Hk. & Grev.) Presl	Botanic Gardens	—	22	Normal	16
L. hymenophylloides (A. Rich.) Presl	Botanic Gardens	—	22	Normal	16

Name	Country of origin	2n	n	Reproduction	Chapter(s)
Leptopteris (cont.)					
L. superba (Col.) Presl	Botanic Gardens	—	22	Normal	16
Osmunda:					
O. regalis L.	British Isles	44	22	Normal	3
	Synthesized	66	Irregular	Sterile	3
	Synthesized	88	44	Normal	3
O. cinnamomea L.	Botanic Gardens	—	22	Normal	16
O. Claytoniana L.	Botanic Gardens	—	22	Normal	16
O. gracilis Hort.	Botanic Gardens	—	22	Normal	16
O. palustris Hort.	Botanic Gardens	—	22	Normal	16
O. javanica Bl.	Malay	—	22	Normal	16
OPHIOGLOSSACEAE					
Ophioglossum:					
O. vulgatum L.	England	—	250–60	Normal	16
O. lusitanicum L.	Guernsey	—	125–30	Normal	16
Botrychium:					
B. lunaria (L.) Sw.	England	—	45	Normal	16
EQUISETALES					
Equisetum subgenus *Eu-equisetum:*					
E. arvense L.	England	—	Prob. 108	Normal	13
E. sylvaticum L.	England	—	Prob. 108	Normal	13
E. maximum Lam.	England	—	Prob. 108	Normal	13
E. pratense Ehrh.	Hort.	—	Prob. 108	Normal	13
E. palustre L.	England	—	Prob. 108	Normal	13
E. limosum L.	England	—	Prob. 108	Normal	13
E. litorale Kuhlw.	Ireland	—	Irregular	Sterile	13
Equisetum subgenus *Hippochaete:*					
E. ramosissimum Desf.	Italy	—	Prob. 108	Normal	13
E. hiemale L.	England	—	Prob. 108	Normal	13
E. robustum A.Br.	Botanic Gardens	—	Prob. 108	Normal	13
E. scirpoides Michx.	Norway	—	Prob. 108	Normal	13
E. variegatum Schleich.	British Isles	—	Prob. 108	Normal	13
E. trachyodon A.Br.	Ireland	Prob. 216	Irregular	Sterile	13
E. Moorei Newm.	Ireland	—	Irregular	Sterile	13
PSILOTALES					
Psilotum:					
P. flaccidum Wall.	Botanic Gardens	—	52–54	Unknown	14
P. nudum (L.) Beauv.	Ceylon	*c.* 100	—	Unknown	14
	Malay	*c.* 200	*c.* 100	Normal	14
	Australia				
	New Zealand				
Tmesipteris:					
T. tannensis (Spreng.) Bernh.	Botanic Gardens	Over 400	Over 200	Unknown	14
LYCOPODIALES					
Lycopodium:					
L. inundatum L.	Scotland	—	78	Normal	15
L. clavatum L.	England	68	34	Normal	15
L. annotinum L.	England	*c.* 68	*c.* 34	Normal	15
	Switzerland				
	Sweden				
L. alpinum L.	Britain	*c.* 48	24–25	Normal	15
L. Selago L.	Britain	At least	Irregular	Problematical	15
	Sweden	260			
Isoetes:					
I. lacustris L.	Britain	Not less than 100	54–56	Normal	15
I. echinospora Durieu	Ireland	Not less than 100	—	Normal	15
I. hystrix Durieu	Britain	20	10	Normal	15
	Morocco				
Selaginella:					
S. spinulosa A.Br.	England	18	9	Normal	15
S. helvetica (L.) Link	Switzerland	18	—	Unknown	15
S. denticulata (L.) Link	Italy	18	9	Normal	15

BIBLIOGRAPHY

Allen, R. F. (1914). Studies in spermatogenesis and apogamy in ferns. *Trans. Wis. Acad. Sci. Arts Lett.* **17**, 1–56.

Andersson-Kottö, I. (1930). Variegation in three species of ferns (*Polystichum angulare*, *Lastrea atrata* and *Scolopendrium vulgare*). *Z. indukt. Abstamm.- u. VererbLehre*, **56**, 115–201; see especially pp. 184–6.

Andersson-Kottö, I. (1931). The genetics of ferns. *Bibliogr. genet.* **8**, 269–94.

Andersson-Kottö, I. & Gairdner, A. E. (1930). In Andersson-Kottö (1930).

Andersson-Kottö, I. & Gairdner, A. E. (1936). Inheritance of apospory in *Scolopendrium vulgare*. *J. Genet.* **32**, 189–228.

Andersson-Kottö, I. & Gairdner, A. E. (1938). Note on the chromosome numbers obtained from crosses with the 'peculiar' aposporous type of *Scolopendrium vulgare*. *J. Genet.* **36**, 509–14.

Ascherson, P. & Graebner, P. (1896). *Synopsis der Mitteleuropäischen Flora*. Jena, **1**, 52.

Babcock, E. B. (1947). The genus *Crepis*. *Univ. Calif. Publ. Bot.* no. 21.

Babcock, E. B. (1947*a*). Cytogenetics and speciation in *Crepis*. *Advances in Genetics*, **1**, 69–93. New York: Demerec.

Babington, C. C. (1922). *Manual of British Botany*, 10th ed.

Backhouse, J. (1853). *Pseudathyrium flexile*. *Phytol.* **4**, 974.

Backhouse, J. (*see* Newman, 1853).

Baenitz, C. (1891). *C. Baenitzii*. *Prospect. Herb. Europ.* **4**.

Balfour, J. H. (1867). Botanical excursions made by Professor J. H. Balfour in the years 1846–1878 inclusively. *Notes R. Bot. Gdn. Edinb.* (1902), **11**, 418–19.

de Bary, A. (1858). Über die Keimung der Lycopodien. *Bericht. d. naturf. Ges. zu Freiburg*, pp. 467–72.

de Bary, A. (1878). Ueber apogame Farne u. die Erscheinung der Apogamie im Allgemeinen. *Bot. Ztg.* **36**, 450–95.

Bateson, W. (1894). *Materials for the Study of Variation*. London.

Bateson, W. (1913). *Problems of Genetics*. Silliman Lectures. Yale University Press.

de Beer, R. (1913). Studies in spore development. III. The premeiotic and meiotic nucleus division of *Equisetum arvense*. *Ann. Bot., Lond.*, **27**, 643–59.

Belling, J. & Blakeslee, A. F. (1923). The reduction division in haploid, diploid, triploid and tetraploid Daturas. *Proc. Nat. Acad. Sci., Wash.*, **9**, 106–11.

Bisby, G. R. (1945). *An Introduction to the taxonomy and nomenclature of Fungi*. Kew.

Black, C. A. (1909). The development of the embedded antheridium in *Dryopteris stipularis* (Willd.) Maxon and '*Nephrodium molle*'. *Bull. Torrey Bot. Cl.* **36**, 557–72.

Blackburn, K. B. & Harrison, J. W. H. (1921). The status of the British rose forms as determined by their cytological behaviour. *Ann. Bot., Lond.*, **35**, 159–88.

Bower, F. O. (1923). *The Ferns*, **1**. Cambridge.

Bower, F. O. (1926). *The Ferns*, **2**. Cambridge.

Bower, F. O. (1928). *The Ferns*, **3**. Cambridge.

Bower, F. O. (1929). The evolutionary relations of the British ferns. *Naturalist, Lond.*, pp. 15–29.

Bower, F. O. (1935). *Primitive Land Plants*. London.

Braun-Blanquet, J. (1936). Un joyau floristique et phytosociologique 'L'Isoetion' méditerranéen. *Comm. Stat. Internat. Géobot. Méditerr. et Alpine*, **42**, 1–23. Also in *Bull. Soc. Etud. Sci. Nat. Nimes*, **47**, 1930–5.

Bruchmann, H. (1885). Das Prothallium von *Lycopodium*. *Bot. Centralbl.* **21**, 23–7 and 309–13.

Bruchmann, H. (1898). *Die Prothallien u. die Keimpflanzen mehrerer europäischer Lycopodien*. Gotha.

Bruchmann, H. (1904). Ueber das Prothallium und die Keimpflanze von *Ophioglossum vulgatum* L. *Bot. Zeit.* **62**, 227–47.

Bruchmann, H. (1910). Die Keimung der Sporen und die Entwicklung der Prothallien von *Lycopodium clavatum* L., *L. annotinum* L. und *L. Selago* L. *Flora, Jena*, **101**, 220–67.

Ching, R. C. (1933). Pteridophyta of Kiangsu Province. *D. atrata* (Wallich) Ching. *Sinensia*, **3**, 326.

Ching, C. (1940). Natural classification of the family Polypodiaceae. *Sunyatsenia*, **5**, 201–67.

Christensen, C. (1905–6). *Index Filicum*. 1913, 1917, 1934, Supplementa I–III. Copenhagen.

Christensen, C. (1920). A monograph of the genus *Dryopteris*. Pt. II. *Kl. danske vidensk. Selsk. Skr.*, Naturv. og Mathematisk, Afd. 8, Raekke 6.
Christensen, C. (1928). On the systematic position of *Polypodium vulgare*. *Dansk. bot. Ark.* **5**, 1–10.
Christensen, C. (1930). The genus *Cyrtomium*. *Amer. Fern J.* **20**, 41–52.
Christensen, C. (1938). Filicinae in Verdoorn's *Manual of Pteridology*, p. 522. The Hague.
Clapham, A. R. (1946). Check list of British vascular plants. *J. Ecol.* **33**, 308–37.
Clausen, J., Keck, D. D. & Hiesey, W. M. (1940, 1945, 1948). Experimental studies in the nature of species. I, II, III. *Publ. Carneg. Instn*, nos. 520, 564, 581.
Copeland, E. B. (1947). *Genera Filicum*. Waltham, Mass.
Creighton, H. & McClintock, B. (1931). A correlation of cytological and genetical crossing-over in *Zea Mays*. *Proc. Nat. Acad. Sci., Wash.*, **17**, 492–7.
Darlington, C. D. & Janaki Ammal, E. K. (1945). *Chromosome Atlas of Cultivated Plants*. London.
Darnell-Smith, G. P. (1917). The gametophyte of *Psilotum*. *Trans. Roy. Soc. Edinb.* **52**, 79.
Dobzhansky, T. (1937). *Genetics and the Origin of Species*. New York.
Döpp, W. (1932). Die Apogamie bei *Aspidium remotum* A.Br. *Planta*, **17**, 86–152.
Döpp, W. (1939). Cytologische u. genetische Untersuchungen innerhalb der Gattung *Dryopteris*. *Planta*, **29**, 481–533.
Dörfler, J. (1891). *Baenitz Herb. Eur.* p. 6510, Prospect 4.
Druce, G. C. (1919). *Cystopteris regia* Presl var. *Dickieana* (Sim) Druce. *Rep. Bot. Soc. Exch. Cl.* **5**, 317–18.
Druce, G. C. (1932). *Comital Flora of the British Isles*. Arbroath.
Druery, C. T. (1912). *British Ferns and their Varieties*. London.
Duncan, R. E. (1941). Apogamy in *Doodia caudata*. *Amer. J. Bot.* **28**, 921–31.
Duval-Jouve, J. (1864). *Histoire Naturelle des* Equisetum *de France*. Paris.
Fankhauser, J. (1873). Über den Vorkeim von *Lycopodium*. *Bot. Zeit.* p. 1.
Farmer, J. B. & Digby, L. (1907). Studies in apospory and apogamy in ferns. *Ann. Bot., Lond.*, **21**, 161–99.
Farquet, P. (1933). Matériaux pour servir à la connaissance du *Polypodium vulgare* et de ses sous-espèces, *Ber. schweiz. bot. Ges.* **42**, 484–506.
Foucaud. (1894). Un *Spartina* nouveau. *Soc. Sci. Nat. de la Charente Inférieure* (*Acad. de la Rochelle*), p. 8.
Gairdner, A. E. *See* Andersson-Kötto (1930).
Gaiser, L. D. (1926). A list of chromosome numbers in angiosperms. *Genetica*, **8**, 401–84.
Gaiser, L. D. (1930). Chromosome numbers in angiosperms. II. *Bibliogr. genet.* **6**, 171–466.
Gaiser, L. D. (1930). Chromosome numbers in angiosperms. III. *Genetica*, **12**, 161–249.
Goebel, K. (1887). Ueber Prothallien und Keimpflanzen von *Lycopodium inundatum*. *Bot. Zeit.* p. 160.
Goldschmidt, R. (1940). *The Material Basis of Evolution*, Silliman Lectures. Yale University Press.
Grégoire, V. (1910). Les cinèses de maturation dans les deux règnes. *Cellule*, **26**, 223–422.
Haračić, A. (1893). Über das Vorkommen einiger Farne auf der Insel Lussin. *Verh. zool.-bot. Ges. Wien*, **43**, 207–12.
Hegi, G. (1906). *Illustrierte Flora von Mittel-Europa*, **1**. Munich.
Heilbronn, A. (1910). Apogamie, Bastardierung und Erblichkeitsverhältnisse bei einigen Farnen. *Flora, Jena*, **101**, 1–42.
Heim, C. (1896). Untersuchungen über Farnprothallien. *Flora, Jena*, **82**, 329–42.
Heinz, A. (1892). Ueber *Scolopendrium hybridum* Milde. *Ber. dtsch. bot. Ges.* **10**, 412–22.
Hoffman, C. (1899). Abstract in Morton (1914*a*).
Holloway, J. E. (1917). The prothallus and young plant of *Tmesipteris*. *Trans. N.Z. Inst.* **50**, 1–44.
Holloway, J. E. (1921). Further studies on the prothallus, embryo and young sporophyte of *Tmesipteris*. *Trans. N.Z. Inst.* **53**, 386–422.
Holloway, J. E. (1938). The embryo and gametophyte of *Psilotum*. A preliminary note. *Ann. Bot., Lond.*, N.S., **2**, 807.
Holloway, J. E. (1939). The gametophyte, embryo and young rhizome of *Psilotum triquetrum* Swartz. *Ann. Bot., Lond.*, N.S., **3**, 313–36.
Holttum, R. E. (1947). A revised classification of leptosporangiate ferns. *J. Linn. Soc.* (*Bot.*), **53**, 123–58.
Hooker, J. (1884). *Student's Flora*, 3rd ed. p. 471. London.
Howard, W. H. & Manton, I. (1946). Autopolyploid and allopolyploid watercress with the description of a new species. *Ann. Bot., Lond.*, N.S., **10**, 1–13.

HOWAT, I. (1922). Abstract in Morton (1925).

HULTÉN, E. (1941). Flora of Alaska and Yukon, Part I, p. 13. In *Lunds Univ. Årsskr.*, N.F., Avd. 2, **37**. (*Woodsia* hybrid in Alaska.)

HUSKINS, C. L. (1931). The origin of *Spartina Townsendii*. *Genetica*, **12**, 531–8.

HUXLEY, J. (1942). *Evolution, the Modern Synthesis*, p. 33. London.

IVANCICH, A. (1923). Su alcune varietá dello *Scolopendrium hybridum* Milde di Lussino. *Boll. Soc. adriat. Sci. nat.* **28**, 1–10.

JANSSENS, F. A. (1909). La théorie de la chiasmatypie. *Cellule*, **25**, 389–411.

JANSSENS, F. A. (1924). La chiasmatypie dans les insectes. Spermatogenèse dans *Stethophyma grossum*, *Chortippus parallelus*. *Cellule*, **34**, 135–359.

JARETZKY, R. (1932). Beziehungen zwischen Chromosomenzahl und Systematik bei den Cruciferen. *Jb. wiss. Bot.* **76**, 485–527.

JEPSEN, G. L., SIMPSON, G. G. & MAYR, E. (1949). *Genetics, Palaeontology and Evolution*. Princeton.

JORDAN, A. (1852). *Pugillus plantarum novarum*. Paris.

JORDAN, A. (1864). *Diagnoses d'espèces nouvelles ou méconnues*. Paris.

KARPECHENKO, G. D. (1928). Polyploid hybrids of *Raphanus sativus* × *Brassica oleracea*. *Z. indukt. Abstamm.- u. VererbLehre*, **48**, 1–85.

KESTNER, P. (1935). A letter on hybrid ferns. *Brit. Fern. Gaz.* **7**, 22.

KIDSTON, R. (1892). On the occurrence of *Equisetum* in the Yorkshire coal-measures. *Ann. Mag. Nat. Hist.* **9**, 138.

KIDSTON, R. & GWYNNE-VAUGHAN, D. T. (1907–14). On the fossil Osmundaceae. I–V. *Phil. Trans. Roy. Soc. Edin.* **45**, 759–80; **46**, 213–32, 651–67; **47**, 455–77; **50**. 469–80.

KIHARA, H. & ONO, T. (1926). Chromosomenzahlen u. systematische Gruppierung der *Rumex* Arten. *Z. Zellforsch.* **4**, 475–81.

KOMAROV, V. L. (1934). *Flora U.R.S.S.* **1**. Leningrad.

KUPFER, R. (1929). *Equisetum trachyodon* A.Br. in Ostbaltikum. *Acta Hort. bot. Univ. latv.* **4**, 247.

LAMARCK, J. & DE CANDOLLE, A. P. (1815). *Flore Française*, **2**, 560.

LANG, W. H. (1898). Apogamy and the development of sporangia on prothalli. *Philos. Trans.* B, **190**, 187–238.

LANG, W. H. (1899). The prothallus of *Lycopodium clavatum* L. *Ann. Bot., Lond.*, **13**, 279–317.

LANG, W. H. (1923). On the genetic analysis of a heterozygotic plant of *Scolopendrium vulgare*. *J. Genet.* **13**, 167–75.

LANG, W. H. (1924). On some deviations from the normal morphology of the shoot in *Osmunda regalis*. *Mem. Manchr. Lit. Phil. Soc.* **68**, 53–67.

LANG, W. H. (1929). On a variety of *Scolopendrium* that bears sporangia on the prothallus. *Ann. Bot., Lond.*, **43**, 355–74.

LAWSON, A. A. (1917). The prothallus of *Tmesipteris tannensis*. *Trans. Roy. Soc. Edinb.* **51**, pt. 3, pp. 785–94.

LAWSON, A. A. (1917). The gametophyte generation of the Psilotaceae. *Trans. Roy. Soc. Edinb.* **52**, 93–113.

LENOIR, M. (1926). Évolution des chromatines. I. Les phénomènes de la cinèse dans la tige aérienne de l'*Equisetum arvense* L. *Arch. morph. gén. éxp.* **26**, 1–53.

LENOIR, M. (1932). Évolution des chromosomes hétérotypiques pendant la diacinèse chez l'*Equisetum palustre* L. *C.R. Soc. Biol., Paris*, **110**, 77–8.

DE LITARDIÈRE, R. (1920). Recherches sur l'élément chromosomique dans la caryocinèse somatique dans Filicinées. *Cellule*, **31**, 255–473.

LÖVE, A. & LÖVE, D. (1943). The significance of differences in the distribution of diploids and polyploids. *Hereditas, Lund*, **29**, 145–63.

LÖVE, A. & LÖVE, D. (1948). Chromosome numbers of northern plant species. *Dep. Agric. Rep.*, Series B, no. 3. Rejkjavik.

LUERSSEN, C. (1889). Die Farnpflanzen. In Rabenhorst's *Kryptogamenflora*, **3**, 402.

MANTON, I. (1932). Contributions to the cytology of apospory in ferns. I. A case of induced apospory in *Osmunda regalis* L. *J. Genet.* **25**, 423–30.

MANTON, I. (1932*a*). Introduction to the general cytology of the Cruciferae. *Ann. Bot., Lond.*, **46**, 509–56.

MANTON, I. (1934). The problem of *Biscutella laevigata*. *Z. indukt. Abstamm.- u. VererbLehre*, **67**, 41–57.

MANTON, I. (1935). The cytological history of watercress. *Z. indukt. Abstamm.- u. VererbLehre*, **69**, 132–57.

MANTON, I. (1935*a*). Some new evidence on the physical nature of plant nuclei from intra-specific polyploids. *Proc. Roy. Soc.* B, **118**, 522–47.

MANTON, I. (1937). The problem of *Biscutella laevigata*. II. *Ann. Bot., Lond.*, N.S., **1**, 439-62.
MANTON, I. (1939). Cytology of the common male fern in Britain. *Nature, Lond.*, **144**, 291.
MANTON, I. (1939). Evidence on spiral structure and chromosome pairing in *Osmunda regalis* L. *Philos. Trans.* B, **230**, 179-215.
MANTON, I. (1942). A note on the cytology of *Psilotum* with special reference to vascular prothalli from Rangitoto Island. *Ann. Bot., Lond.*, N.S., **6**, 283-92.
MANTON, I. (1945). Chromosome length of the early meiotic prophases in *Osmunda*. *Ann. Bot., Lond.*, N.S. **9**, 155–78.
MANTON, I. (1947). Polyploidy in *Polypodium vulgare*. *Nature, Lond.*, **159**, 136.
MANTON, I. (1950). Demonstration of Compound Cilia in a fern Spermatozoid by means of the ultra-violet microscope. *J. Exp. Bot.* **1** (in the Press).
MARCHAL, E. (1920). Recherches sur les variations numériques des chromosomes dans la série végétale. *Mém. Acad. R. Belg. Cl. Sci.* 2me série 8°, **4**, fasc. 1, pp. 108.
MARCHAL, ÉL. & MARCHAL, ÉM. (1907, 1909, 1911). Aposporie et sexualité chez les mousses, I, II, III. *Bull. Acad. Belg. Cl. Sci.* (1907) pp. 765–89; (1909) pp. 1249–88; (1911) pp. 750–78.
MARQUAND, E. D. (1901). *Flora of Guernsey*. London.
MARTENS, P. (1943). Les organes glanduleux de *Polypodium virginianum*. *Bull. jard. bot. Brux.* **17**, 1–13.
MAUDE, P. (1939). The Merton Catalogue. *New Phytol.* **38**, 1–31.
MAUDE, P. (1940). Chromosome numbers in some British plants. *New Phytol.* **39**, 17–32.
MELVILLE, R. (1938). *Isoetes hystrix* at the Lizard. *J. Bot.* **76**, 17–19.
MENDEL, G. (1865). Versuche über Pflanzen-Hybriden. *Verh. naturf. Ver. Brünn*, **10**, 1. Reprinted *Flora, Jena*, 1901. English translation in *J.R. Hort. Soc.* **26**, 1901. Also in Bateson's *Mendel's Principles of Heredity*, 1909, pp. 317–61, Cambridge.
MILDE, J. (1864). *Scolopendrium hybridum* Milde. *Verh. zool.-bot. Ges. Wien*, **14**, 235–8.
MILDE, J. (1867). *Monographia Equisetorum*. Dresden.
MILDE, J. (1867*a*). *Fil. Eur.* p. 148. Leipzig.
MOOR, M. (1936). Zur Soziologie der Isoëtetalia. *Beitr. z. geobot. Landesaufnahme der Schweiz.* **20**, 1–148.
MOORE, T. (1848). *Handbook of British Ferns*, 1st ed. p. 43. London.
MORTON, F. (1914). Beiträge zur Kenntnis der Pteridophytengattung *Phyllitis*. Über die Auffindung von *Phyllitis hemionitis* (Lag.) O. Kuntze im Quarnerogebiete. *Öst. bot. Z.* **64**, 19–25.
MORTON, F. (1914*a*). Systematische Stellung, Verbreitung und Oekologie von *Phyllitis hybrida* (Milde) Christensen. *Öst. bot. Z.* **64**, 25–36.
MORTON, F. (1925) Nachträge zur Kenntnis von *Phyllitis hybrida*. *Öst. bot. Z.* **74**, 244–7.
NEWMAN, E. (1844). *History of British Ferns*, 2nd ed. p. 202. London.
NEWMAN, E. (1851). Genus *Gymnocarpium*. *Phytologist*, **4**, 371.
NEWMAN, E. (1853). *Pseudathyrium flexile*. *Phytologist*, **4**, 974.
NEWMAN, E. (1854). *History of British Ferns*, 3rd ed. London.
NEWTON, W. C. F. & DARLINGTON, C. D. (1929). Meiosis in polyploids. *J. Genet.* **21**, 1–56.
NORDHAGEN, R. (1947). *Dryopteris paleacea* (Sw.) C.Chr. og dens utbredelse i Norge. *Blyttia*, **5**, 89–118.
OKABE, S. (1929). Ueber eine tetraploide Gartenrasse von *Psilotum nudum* Palisot de Beauvois (= *P. triquetrum* Swartz). *Sci. Rep. Tôhoku Univ.* ser. 4, **4**, 373.
ORTUS SANITATIS (1491). Mainz. From a copy in the Rylands Library, Manchester.
PAYNE, L. G. (1939). The Crested Buckler Fern. *Lond. Nat.* (1938), pp. 29–31.
PRAEGER, R. L. (1909). *Lastrea remota* in Ireland. *Irish Nat.* **18**, 151–3.
PRAEGER, R. L. (1934). *The Botanist in Ireland*. Dublin.
PRESL, C. B. (1849). *Epimeliae Botanicae*, p. 38. Prague.
RABENHORST, L. (1889). *Kryptogamenflora*, **3**. Die Farnpflanzen. Leipzig.
REICHARDT, H. W. (1863). Bericht über die auf einer Reise nach den quarnerischen Inseln gesammelten Sporenpflanzen. *Verh. zool.-bot. Ges. Wien*, **13**, 461–78.
ROSENBERG, O. (1909). Cytologische und morphologische Studien an *Drosera longifolia* × *rotundifolia*. *K. svenska VetenskAkad. Handl.* **43**, 1–64.
ROSENBERG, O. (1917). Die Reduktionsteilung u. ihre Degeneration in *Hieracium*. *Svensk bot. Tidskr.* **11**, Heft 2, pp. 145–206.
ROSENDAHL, H. V. (1909). *C. fragilis* var. *cynapiifolia*, *C. fragilis* var. *acutidentata*. *Svensk bot. Tidskr.* **3**, 382.

ROSENDAHL, H. V. (1915). Om *Woodsia alpina* och den sydlig inlandsform af denna samt *Woodsia alpina* × *ilvensis* nov. hybr. *Svensk bot. Tidskr.* **9**, 414–20.

ROWLANDS, S. P. (1929). *Cystopteris fragilis* Bernh. var. *Dickieana*. *Brit. Fern Gaz.* **6**, 18–19.

SAMUELSSON, G. (1921). Svenska fyndorter för *Cystopteris Baenitzii*. *Svensk bot. Tidskr.* **15**, 98.

SCHRÖDINGER, E. (1944). *What is life? The physical aspect of the living cell.* Cambridge.

SHARP, L. W. (1934). *Introduction to Cytology*, 3rd ed. New York.

SHAW, H. K. A. (1947). The botanical name of the wild tetraploid watercress. *Kew Bull.* pp. 39–46.

SMITH, J. (1841). Ennumeratio Filicum Phillipinarum. *Hooker's J. Bot.* **3**, 412.

STANGE, F. (1887). Mittheilungen über seine Farnkulturen und die bei denselben beobachtete Apogamie. *Just's Jber.* Abt. 1, 598.

STANSFIELD, F. W. (1934). '*Lastrea dilatata Boydii*' (F.W.S.) and *Nephrodium subalpinum* Borbasio. *Brit. Fern Gaz.* **6**, 281–3.

STANSFIELD, F. W. (1936). *Brit. Fern Gaz.* **7**, 91.

STAPF, O. (1927). *Spartina Townsendii*. *Bot. Mag.* **152**, no. 9125.

STEIL, W. N. (1911). Apogamy in *Pellaea atropurpurea*. *Bot. Gaz.* **52**, 400–1.

STEIL, W. N. (1915). Apogamy in *Nephrodium hertipes*. Preliminary note. *Bot. Gaz.* **59**, 254–5.

STEIL, W. N. (1919). A study of apogamy in *Nephrodium hirtipes* Hk. *Ann. Bot., Lond.*, **33**, 109.

STEINECKE. (1932). *See* Tischler (1935*a*).

STEP, E. (1908). *Wayside and Woodland Ferns*. London.

STERN, C. (1931). Zytologisch-genetische Untersuchungen als Beweise für die Morgansche Theorie des Faktorenaustauschs. *Biol. Zbl.* **51**, 547–87.

STRASBURGER, E. (1875). *Zellbildung und Zelltheilung*. Jena.

STRASBURGER, E. (1894). The periodic reduction in the number of the chromosomes in the life history of living organisms. *Ann. Bot., Lond.*, **8**, 281–316.

TÄCKHOLM, G. (1922). Zytologische Studien über die Gattung *Rosa*. *Acta Hort. berg.* **7**, 97–381.

VON TAVEL, F. (1934). Letter re hybrid *Lastrea*. *Brit. Fern Gaz.* **6**, 308–11.

VON TAVEL, F. (1937). *Dryopteris Borreri* Newm. und ihr Formenkreis. *Verh. schweiz. naturf. Ges.* pp. 153–4.

TISCHLER, G. (1927–38). Pflanzliche Chromosomenzahlen. I–IV. *Tabul. biol., Berl.*, **4**, 1–83; **7**, 109–226; **11**, 281–304; **12**, 57–115.

TISCHLER, G. (1935). Die Bedeutung der Polyploidie für die Verbreitung der Angiospermen. *Bot. Jahrb.* **47**, 1–36.

TISCHLER, G. (1935*a*). Pflanzliche Chromosomen-Zahlen. *Tabul. biol., Berl.*, **11**, 304.

TURESSON. *See* Clausen, Keck & Hiesey (1945).

TURESSON, G. (1922). The genotypic response of the plant species to the habitat. *Hereditas, Lund*, **3**, 211–50.

VERDOORN, F. (1938). *Manual of Pteridology*. The Hague.

WALLACE, A. R. (1892). *Island Life*, 2nd ed. p. 363. London.

VON WETTSTEIN, R. (1932). Genetik. In Verdoorn's *Manual of Bryology*, p. 251.

WHITE, M. J. D. (1945). *Animal Cytology and Evolution*. Cambridge.

WILLIS, J. C. (1925). *Dictionary of the Flowering Plants and Ferns*, 5th ed. Cambridge.

WILSON, A. (1938). *The Flora of Westmorland*. Arbroath.

WINGE, Ö. (1940). Taxonomic and evolutionary studies in *Erophila* based on cytogenetic investigations. *C.R. Lab. Carlsberg*, série physiologique, **25**, 41–73.

WINKLER, H. (1916). Über die experimentelle Erzeugung von Pflanzen mit abweichenden Chromosomenzahlen. *Z. Bot.* **8**, 417–531.

WOLLASTON, G. B. (1855–6). *Lastrea Filix-mas*. *Phytologist*, N.S., **1**, 172.

WOLLASTON, G. B. (1875). Three species of *Lastrea Filix-mas*. *Brit. Fern Gaz.* **3**, 20–4.

WRIGHT, S. (1949). *See* Jepsen, Simpson & Mayr (1949).

YAMANOUCHI, S. (1908). Apogamy in *Nephrodium*. *Bot. Gaz.* **45**, 289–319.

YEATES, J. S. (1925). The nucleolus of *Tmesipteris tannensis* Bernh. *Proc. Roy. Soc.* B, **98**, 227–44.

ZIMMERMANN, W. (1938). Phylogenie. In Verdoorn's *Manual of Pteridology*, p. 615, fig. 19. The Hague.

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