AN EVOLUTIONARY STUDY OF THE FERN *ASPLENIUM TRICHOMANES*.

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Thesis submitted for the Degree of Doctor of Philosophy in the University of Leeds.

Department of Botany

1958.

VOLUME I : TEXT

CONTENTS.

ABSTRACT.

The aggregate species *Asplenium trichomanes* includes three different cytotypes:- a diploid with *n* = 36 chromosomes, a tetraploid with *n* = 72, and a hexaploid with *n* = 108. Although the diploid and tetraploid cytotypes are widely though discontinuously distributed in both Northern and Southern Hemispheres, the hexaploid is known only from New Zealand. All three plants are distinguishable on morphological characters, and possess distinctive ecological preferences. Cytogenetic analysis of a series of artificial hybrids between different representatives of the cytotypes obtained from various parts of the world has demonstrated the close genetical inter-relationship of the entire species complex, and given information concerning the nature of relationships existing between and within the three cytotypes.

FOREWORD.

The bulk of the work described in this thesis was carried out in the Department of Botany of the University of Leeds between July 1952 and June 1955, while holding a Nature Conservancy Research Studentship.

Towards the end of this period, it became apparent that a study of the forms of *Asplenium trichomanes* present in Australia and New Zealand was crucial for elucidation of the problem. Fortunately, an opportunity occurred for personal study in New Zealand, and in consequence twelve months were spent at Canterbury University College, Christchurch, New Zealand, from August 1955 to August 1956, while holding a New Zealand National Research Fellowship. It proved possible to make a brief visit to Victoria and New South Wales on the way out to New Zealand, and a longer stay of six weeks in Australia on the way back, in order to study the forms of *Asplenium trichomanes* present in Australia in their native habitat.

On return to the United Kingdom, the investigation was continued at Leeds, and is still in progress.

INTRODUCTION.

GENERAL INTRODUCTION.

Prior to the commencement of this investigation, it had already been discovered by Manton (22), that two cytological forms of Asplenium trichomanes L existed in Britain. One form or cytotype* is a diploid, with n = 36 chromosomes, while the other, evidently much the commoner plant, is a tetraploid with n = 72 chromosomes. The diploid had only been found at Aber Falls, in Caernarvonshire, and this one example was seen to be clearly morphologically very much alike the more common tetraploid cytotype, which had been determined from several localities scattered through the country. It was also known that both of these forms occurred on the continent of Europe, although again the diploid had only been found once, in the Auvergne in France.

*Footnote.
The term cytotype is used here and throughout this thesis in its general sense as a term for a member of a polyploid series, including the diploid level on which the series is based. The term is not used in its restricted sense according to the definition of Valentine (34). The diploid, tetraploid, and hexaploid chromosome races of Asplenium trichomanes s.l. are thus described here as cytotypes, although they have distinctive geographical and ecological distributions, and according to the categories of Valentine (34), would almost certainly correspond to ~~district~~ distinct coenospecies. (See also Clausen et al., 5). By its definition, use of the term coenospecies would imply the demonstration that no gene exchange can occur between the three chromosome races, although in actuality this fact has only been established for these plants with a certain degree of probability. The use of the term cytotype in its less restricted sense has therefore been preferred.

It was clearly very desirable that an effort should be made to find out more about the rarer diploid cytotype of Asplenium trichomanes, and especially about its relationship with the tetraploid, in view of their morphological similarity. Indeed the two cytotypes were evidently so much alike in appearance that it seemed possible that the tetraploid was an autotetraploid derived from the apparently rarer diploid. Since no example of autopolyploidy had been demonstrated for any fern in a state of nature, it was quite clear that a cytogenetical investigation of these two cytotypes ought to be undertaken, in order either to confirm or disprove the hypothesis of autopolyploidy in this case.

Early in the investigation it was realised that the study would have limited value and interest if confined to European material, especially since Asplenium trichomanes has an extremely wide distribution throughout the world. Indeed, there is no other fern, unless it be Cystopteris fragilis s.l., which has such a comprehensive range in the temperate regions of the world as has Asplenium trichomanes. The possibility existed that by extending the investigation to extra-European material, results of considerable evolutionary and phytogeographic importance might emerge from the work. Accordingly, efforts were made to obtain material from various parts of the world, mainly from

correspondents overseas. Fortunately, these efforts were largely successful, and it eventually became possible to consider the study as an investigation into the structure of the Asplenium trichomanes species complex over its entire distributional range.

The investigation had been under way for some eighteen months when news was received from Mr. G. Brownlie, of Christchurch, New Zealand, of his discovery of the existence of a hexaploid cytotype of Asplenium trichomanes in New Zealand. Owing to his kind co-operation, material of this plant was subsequently included in this study.

Comparative autecological studies on the diploid and tetraploid cytotypes were carried out in Britain. At a later stage, it was possible to study localities of these two plants in Australia, and also to make an intensive study in New Zealand of the autecology of the hexaploid cytotype.

The morphology of all three cytotypes was examined comparatively both at the visual and microscopic levels, in order to ascertain what characters could be used for the determination of herbarium specimens, and eventually be recommended for general taxonomic use.

The cytogenetic investigation included a cross-breeding programme between the three cytotypes using as parents various strains from different parts of the world. Also

included in this hybridisation programme was Asplenium adulterinum Milde, a known allotetraploid, of which the diploid cytotype of Asplenium trichomanes is apparently one parent.

Thus this thesis consists of an account of the three known cytotypes of Asplenium trichomanes, describing their geographical distributions, their autecology in Britain, Australia, and New Zealand and their comparative morphology, together with the results of a hybridisation programme involving stocks of the three cytotypes from localities in various parts of the world.

Rather than devoting a separate section of the thesis to description of the numerous unrelated methods used in the course of the investigation, it has been considered more suitable to describe the various techniques independently under the sections to which each is appropriate.

Since the part-parental relationship of the diploid cytotype of Asplenium trichomanes to Asplenium adulterinum has been assumed in the design of the cytogenetical investigation, an account is given of the evidence demonstrating that Asplenium adulterinum is in fact an allotetraploid with Asplenium viride Hudson, and the diploid cytotype of Asplenium trichomanes as its apparent parents in the form of a supplement to the main body of the thesis.

SOURCES OF LIVE MATERIAL.

Although the taxonomist working in this country on a group of plants of global distribution is admirably served by the immense Herbarium collections built up over the years at South Kensington and at Kew, the cytologist must work harder for the material that is to form the basis of his research, since he requires live plants for his work.

The author has been personally fortunate in having the opportunity to collect his own material not only in Britain and Norway, but also in Australia and New Zealand. Nevertheless, the bulk of the overseas material used in the investigation has been sent by well-disposed foreign correspondents. In this way, living material has been acquired from the eastern United States, from Canada, Japan, Hawaii, Basutoland, Australia, and New Zealand. A third source of material has been spores from recent collections received by the British Museum Herbarium, thanks to the kindness of the Keeper. Plants have thus been raised from several localities in the Himalayas, and from one station in the Yemen, from which two regions no live material would otherwise have been available.

THE TAXONOMIC LIMITS OF THE SPECIES AGGREGATE.

At an early stage in the investigation it was realised that although Asplenium trichomanes Linnaeus is a very well known and unequivocal species to European botanists, as soon as one's attention is directed outside Europe, it becomes evident that the interpretation of the name by different authors has varied considerably. Certain authorities have included within Asplenium trichomanes what are distinct plants, while on the other hand there has also been some evident creation of synonymy. This was perhaps unavoidable, especially since early authors working at some distance from Europe had only descriptions, often quite inadequate, of other workers' species to guide them. However, since it is clearly necessary to formulate a precise idea of the inclusive taxonomic limits of a particular species complex before undertaking a study of the group, this taxonomic confusion does mean that before the work on the Asplenium trichomanes species aggregate can be described, it is first necessary to indicate the author's* own interpretation of the taxonomic limits of Asplenium trichomanes s.l., thus defining the scope of the present investigation. This will now be done.

I include within Asplenium trichomanes s.l. such taxa as A.anceps Buch (described from the Canary Islands, but

found elsewhere), A.melanocaulon Willdenow (North America), A.densum Brackenridge (Hawaii), and A.melanolepis Colenso (New Zealand). I also include, though with less certain conviction, the A.anceps var. proliferum of Nakai, and A.tripteropus Nakai, both from Japan.

So far, these conclusions agree with those of Christensen (4), and it is necessary to accept the judgement of Christensen concerning certain other taxa which I have not seen. Thus he also reduces to synonymy within Asplenium trichomanes the following; A.newmanii Bolle (Canary Isles), A.caput-serpentis Henriques (Portugal), A.pechnellii Kuntze (Germany), and A.alatum Dulac (Pyrenees)

Certain other taxa, although at some time confused with Asplenium trichomanes, are nevertheless quite distinct, and therefore do not come within the scope of this thesis. An excellent example is A.castaneum Schlechtendal and Chamisso (from Mexico and the Andes), which though included by Hooker and Baker (17), within Asplenium trichomanes, is distinguished clearly from Asplenium trichomanes by major characteristics of the frond, indusium, rhizome scale and spore. Other taxa, which though evidently related to Asplenium trichomanes, are certainly specifically distinct, include A.resiliens Kunze (North and Central America), A.lealii Alston (South America), A.palmeri Maxon (Mexico),

A.vespertinum Maxon (San Diego., California), A.heterochroum Kunze (Florida, Cuba, and Bermuda), A.nesioticum Maxon (Jamaica), A.extensum Fee (Columbia and Peru), A.microtum Maxon (Yunnan), A.rectangulare Bonaparte (Madagascar), and A.trialatum Christensen (Szechuan and Formosa). None of these plants will be considered further within this thesis.

The status of Asplenium underwoodii Maxon, known only from one almost inaccessible peak in Jamaica, is less certain. The only herbarium specimen I have seen lacks any one diagnostic character to separate it from Asplenium trichomanes, but in the absence of fuller information it is expedient to accept for the time being Maxon's opinion (24), that this plant is distinct from any form of Asplenium trichomanes. For this reason, this plant also will not be considered further in this thesis.

GEOGRAPHICAL DISTRIBUTION. (Figs. 1-7).

This section of the thesis commences with an account of the methods used in investigation of distributions.

The geographical distributions of the aggregate species and its diploid, tetraploid, and hexaploid cytotypes will then be described, in that order. For each cytotype concise lists of chromosome counts and herbarium records are first given, followed by a general description of the distribution of that cytotype.

METHODS OF INVESTIGATION.

The descriptions of the geographical distributions of the aggregate species and of the three cytotypes have been drawn up solely on the basis of living and dried material examined by the author.

Descriptions of the distributions of the cytotypes must be based on cytological results, since actual chromosome counts afford the only definitive means of identification of plants which are primarily characterised by chromosome number. Chromosome counts have mostly been made on meiotic material, but some determinations are based on examination of root-tip mitoses. Details of the cytological techniques are given later (pp. 85 - 86).

As is described in the section on the comparative

morphology of the cytotypes (pp.58 - 78), certain differences exist between the spores and rhizome scales of these three plants. These characters have been used as a basis for the identification of herbarium specimens, thus gaining additional knowledge of the distribution of the cytotypes.

In this account of the distributions of the cytotypes the lists of chromosome counts have been shortened for the sake of conciseness. For Britain, only vice-counties are given, with the number of records for each. For records outside Britain, only the country, state, or general district of origin is stated. Fuller details of locality and other data concerning each record are given in an Appendix at the back of the thesis.

The lists of records based on determinations of herbarium specimens are confined to those from parts of the world from which there is no record based on a chromosome count. These are also abbreviated in the text to a statement of general location, with fuller details being given in an Appendix.

GEOGRAPHICAL DISTRIBUTION (contd).

DISTRIBUTION OF THE SPECIES AGGREGATE (Fig.3).

Although the limits of the species aggregate are restricted according to its definition in the Introduction (p.8), Asplenium trichomanes s.l. still has an extremely wide range. It occurs throughout Europe, temperate Asia and North America, excepting the Arctic regions of these continents. It occurs in the Azores, Madeira, and the mountains of North Africa, and is scattered throughout the main African mountain chain from British Somaliland to Cape Province. It is also found in Japan and Hawaii, on some of the highest mountains of Indonesia and New Guinea, and in Australia and New Zealand.

In fact, it seems that this plant occurs in all the major mountain masses of the world, excepting only the Andes. Material from the Andean region attributed to Asplenium trichomanes is in my experience always either A.castaneum or A.lealii. It has been found in the district of Santa Catharina, in south-east Brazil, but this is the only authentic record of Asplenium trichomanes from South America.

In tropical latitudes, Asplenium trichomanes is only found at considerable altitudes, and in consequence it has an extremely discontinuous distribution in these regions.

GEOGRAPHICAL DISTRIBUTION (contd)

DISTRIBUTION OF DIPLOID CYTOTYPE (Figs 1,3,4 & 6).

CHROMOSOME COUNTS : n = 36

Great Britain.

Merioneth	v.c. 48	3	localities
Caernarvon	v.c. 49	4	"
Cumberland	v.c. 70	2	"
Stirling	v.c. 86	1	"
N. Perth	v.c. 89	2	"
N. Aberdeen	v.c. 93	2	"

Europe

France	1	locality
Switzerland	2	"
Norway	5	"
Germany	1	" , confirming Meyer (26,27)

Asia.

Assam	1	locality
Punjab	1	"
S.E.Tibet	2	"
Nepal	1	"

North America.

Ontario 1 locality, confirming Britton (2)

Australia

Victoria 1 locality

IMPORTANT DETERMINATIONS OF HERBARIUM MATERIAL.

North America

Oklahoma

Colorado

Arizona

Georgia

North Carolina

Asia.

China : Hupeh

" : Shensi

" : Yunnan

Pacific region

Indonesia : Lombok

Portugese Timor

New Guinea

(New Zealand ?) - see Appendix II, p. 176.

DESCRIPTION OF THE DISTRIBUTION OF THE DIPLOID CYTOTYPE.

The diploid cytotype is widely distributed throughout the Northern Hemisphere. In North America, it is known from several localities in the Rocky Mountains, and also in the east, where it has been found in the Appalachian Mountains, and at lower levels in Canada.

In Europe, it appears to be confined to mountainous regions and the lowland areas immediately adjacent to them. In Britain it is at present known from North Wales, the Lake District, and three widely separate localities in Scotland. It is found in Scandinavia, and in many parts of Central Europe.

There is a very large gap between the easternmost European record of this plant, in Macedonia, and its westernmost locality in Asia, in the Kangra Himalaya of the Punjab. Whether or not this discontinuity is real, or instead merely spurious and occasioned by the paucity of available material of Asplenium trichomanes s.l. from this part of the world, cannot at present be known. There are numerous records of the diploid from the Himalayas and also from Western China.

Though apparently completely absent from South America and Africa, there is a most interesting group of records from the S. W. Pacific region. In Australia, there are some few isolated localities in Victoria and New South Wales. Between these localities, and those in the far-distant Himalayas, there are four records, all at great elevations in tropical latitudes. Two come from the Owen Stanley Ranges of New Guinea, between 9,000 and 12,000 feet. Fifteen hundred miles to the west there is a record from Mt. Tatamailau, in Portugese Timor, at 9,000 feet. Another thousand miles further west is a record from Mr. Rindjani, on Lombok, an Indonesian island, at 6,500 feet. Since the oldest of these four records only dates back to 1936, it is very probable that further localities of this plant exist elsewhere in this region, perhaps on the slopes of the highest mountains of Celebes, Java and Sumatra.

GEOGRAPHICAL DISTRIBUTION (contd)

DISTRIBUTION OF THE TETRAPLOID CYTOTYPE (Figs 2, 3, & 5-7).

CHROMOSOME COUNTS : n = 72

Great Britain.

W. Cornwall	v.c. 1	2	localities
E. Cornwall	v.c. 2	2	"
S. Somerset	v.c. 5	1	"
S. Sussex	v.c. 13	1	"
W. Kent	v.c. 16	2	"
Surrey	v.c. 17	2	"
Northampton	v.c. 32	1	"
W. Gloucester	v.c. 34	1	"
Brecon	v.c. 42	2	"
Merioneth	v.c. 48	2	"
Caernarvon	v.c. 49	4	"
Anglesey	v.c. 52	1	"
Derby	v.c. 57	1	"
Mid-W. York.	v.c. 64	2	"
N.W. York.	v.c. 65	1	"
Durham	v.c. 66	1	"
Westmorld. + N.Lancs	v.c. 69	3	"

Great Britain (contd)

Cumberland	v.c. 70	4 localities	
Edinburgh	v.c. 83	1	"
Mid Perth	v.c. 88	2	"
N. Perth	v.c. 89	2	"
N. Aberdeen	v.c. 93	1	"
Banff	v.c. 94	1	"
Dumbarton	v.c. 99	1	"
N. ~~Hebrides~~ Ebudes (Skye)	v.c. 104	1	"
Kerry	v.c. H.1 or H. 2	1 locality	
Wexford	v.c. H.12	1	"
W. Galway	v.c. H.16	1	"

Europe

France	1 locality	
Belguim	1	"
Andorra	1	"
Italy	1	"
Switzerland	1	"
Balearic Islds.	1	"
Spain	1	"
Germany	1	" , confirming Meyer (26,27).

Europe (contd)

Austria	1 locality
Sweden	2 "
Norway	1 "

Middle East.

Cyprus	1 locality
Turkey	1 "
Yemen	1 "

Asia

* Japan	2 localities

North America.

Ontario	1 locality
Vermont	1 "

South America

Basutoland	1 locality

Pacific.

Hawaiian Islands	2 localities

Australasia.

Western Australia	2	localities
Victoria	2	"
New South Wales	1	"
New Zealand	1	"

IMPORTANT DETERMINATIONS OF HERBARIUM MATERIAL.

Asia.

China : Fokien

U.S.S.R. : Tian-Schan Mts.

Afghanistan

Kashmir

North America.

West Virginia

Ohio

Vancouver Island

Africa

Algeria

Morocco

British Somaliland

Kenya

Atlantic

Azores

South America

Brazil : Santa Catharina

* Indicates the distinctive form known from Japan and China, and lacking any satisfactory name, although apparently it is known to Japanese workers as Asplenium anceps var. proliferum. As is indicated elsewhere (p.130 et seq.), there is as yet not sufficient evidence available to determine the true relationship of this plant to the typical form of the tetraploid cytotype. On the distribution map (fig. 2), this Japanese form is indicated by a rectangular symbol, in contrast to the circular symbol used for all other records.

DESCRIPTION OF THE DISTRIBUTION OF THE TETRAPLOID CYTOTYPE.

The tetraploid cytotype is clearly widely distributed, albeit discontinuously, throughout the Northern Hemisphere. This plant probably occurs somewhere in every vice county in the British Isles, and is almost as completely distributed over continental Europe, extending to Asian Turkey and

Cyprus in one direction, to the mountains of North Africa in another, and extending westwards out into the Atlantic to Madeira and the Azores.

The distribution of this plant in North America is in all probability only very incompletely known. It is known from several places in the east, but at present in the west it is only known from British Columbia.

The apparent very large discontinuities in distribution in Asia may not be real, since little material has been available for examination. Specimens have been seen from the Caucasus, and further east from Kashmir, Afghanistan, and the remote Tian-Schan ranges on the border of the U.S.S.R. Three thousand miles away to the east is the distinctive form found in Japan and Fokien province in China.

Equally isolated are the localities of this plant in the Hawaiian Islands, where it is found at considerable elevations (4-8,000'), on the three great volcanoes; Haleakala, on the island of Maui, Mauna Kea and Mauna Loa on Hawaii itself.

The distribution of the tetraploid cytotype in the Southern Hemisphere is discontinuous in the extreme. A notable feature is its absence from the Andes, though one specimen from South America, actually from the Santa Catharina region of S.E.Brazil, has been seen.

It is known from Sada in the Yemen, at 9,200 feet, and across the Red Sea, near the summit of Mt. Wagar in British Somaliland, at 6,500 feet. In South Africa, there are several records from Cape Province, Basutoland, and Natal. It is probable that the plant occurs in isolated localities at considerable elevation all along the main African mountain chain, although the only known locality between the South African and the Red Sea stations is Longonot Mt., in Kenya, at 8,000 feet.

In Australia, four localities are known in the extreme south-west of Western Australia, whilst on the east side of the continent there are about a dozen known isolated localities in Victoria and New South Wales. The plant has also been collected in Tasmania, and there is one station in South Australia. Across the Tasman Sea, a very few localities are known in the North Island of New Zealand.

GEOGRAPHICAL DISTRIBUTION (contd).

DISTRIBUTION OF THE HEXAPLOID CYTOTYPE (Figs 3 & 6).

CHROMOSOME COUNTS : n = 108.

New Zealand

North Island:	South Auckland	1	locality
" :	Hawke's Bay	1	"
South Island:	Nelson	5	"
" :	Marlborough	1	"
" :	Canterbury	11	"
" :	Otago	1	"

First recorded by Brownlie (3), the chromosome count being made on material from a locality in Canterbury Province.

This plant is endemic to New Zealand, which fact only increases interest in the problem of its origin. Over much the greater proportion of its range in New Zealand, it is the only representative of the species aggregate.

AUTECOLOGY. (Figs. 8-66).

INTRODUCTION.

The autecology of the three cytotypes, diploid, tetraploid, and hexaploid, will be described in turn, in that order. The accounts for the diploid and tetraploid cytotypes will commence with consideration of their autecology in the British Isles, followed by a similar account for Australia, and subsequently any information from elsewhere will be described.

Before proceeding to the description of the autecology of the individual cytotypes, in two further sections of this Introduction are considered the special problem of the gametophyte generation, and the characteristics of the rock crevice habitat.

THE SPECIAL PROBLEM OF THE GAMETOPHYTE GENERATION.

An investigation of the autecology of a fern presents an intrinsically more complex problem for analysis than a similar investigation on a flowering plant owing to the independent existence of the gametophyte generation of the life-cycle. As far as I am aware, no worker has yet succeeded in separating the ecological requirements of the prothallial stage of any species from those of the sporophyte, with the partial exception of Bracken, Pteridium aquilinum (Conway, 8), which presents a rather special case. Field investigation of the gametophyte is

very difficult, these difficulties being increased by the present lack of knowledge concerning the taxonomy of fern prothalli. Ecologists have generally found it necessary to consider any fern species as a single-stage organism for their purposes. Though an over simplification of this kind is evidently not desirable, I have perforce had recourse to the same approach in this investigation.

THE CHARACTERISTICS OF THE ROCK-CREVICE HABITAT.

The typical habitat of all three cytotypes of Asplenium trichomanes is crevices in or between rocks, although as is described below a variety of rock-types may provide the habitat.

Asplenium trichomanes thus grows in a very characteristic and specialised habitat. There is however little known concerning the ecology of the rock-crevice habitat. Our knowledge of the relative significance of the various factors involved is essentially of a superficial nature, and we are quite ignorant of the nature of the special characteristics which must surely be possessed by certain species, and which fit them for this peculiar habitat to the virtual exclusion of any other.

However, certain generalisations concerning the major factors involved in an analysis of this habitat-type are evidently largely true, and these must now be indicated

here, if the full significance of the field observations on the cytotypes of Asplenium trichomanes is to be clear to the reader.

The micro-climate of this habitat is subject to considerable extremes of variation wherever it occurs in exposed situations. For example, there may be great diurnal fluctuation in temperature at the rock-surface. In conditions of high insolation, the surface of exposed rock will attain a markedly higher temperature than the surrounding air. The temperature difference between the illuminated rock at the outside of a crevice and its shaded interior will become considerable, with a sharp gradient of transition. In such conditions the exposed rock at the surface will be dry, and the air about it correspondingly low in humidity, while at the same time in the depths of the crevice the rock surface may still be wet, and the surrounding air cool and moist. Although two individual plants may be growing within a foot of one another, their aerial portions may experience quite different micro-climates, according to their position in the crevice. Evidently, plants growing in exposed situations are subject to considerable diurnal fluctuation in both temperature and humidity. There may also be very great seasonal variation in environmental conditions, since situations subject to

long exposure to the sun are liable to gradual desiccation in spring and summer, and a condition of extreme drought may result in some years. Plants with their aerial portions obscured from the direct effects of sun and wind in the depths of a crevice will clearly be at an advantage in these conditions.

The aspect of the entire rock-face is clearly a factor of importance, and in connection with the present problem, it is important to emphasise that whereas the more exposed a section of a rock-face is to sun and wind, the more extreme will be the range in environmental conditions it will present to a colonising plant, the overall range in micro-climate becomes progressively less according to the degree of protection afforded by the aspect of the whole rock-face. As the degree of protection increases, a cooler, moister, and more equable micro-climate will prevail, subject to the condition that the precipitation of rain is sufficiently high, and of adequate frequency. The most extreme expression of this development is a shaded waterfall facing north (in northern latitudes), for the plants growing under the influence of its spray enjoy conditions of very uniform humidity and temperature in comparison with the conditions prevailing elsewhere even in the immediate

vicinity.

Aspect is thus a very significant factor in the ecology of rock-faces. Not merely is the gross aspect of the entire outcrop, with relation to the points of the compass, of great importance, but the situation and alignment of the crevices which figure the rock-face will affect the range of micro-climates to be found over the whole exposure. From the considerations discussed above, it is quite clear that the majority of rock exposures of any size will display a very wide range of environmental conditions.

Very often a considerable amount of soil is found to have collected about the roots of a plant growing in a rock-crevice. However analysis of the constitution of such soil may give misleading information about the true edaphic relations of these plants. Most important is the immediate environment of the living root tips, since both salt and water uptake are recognised to be associated with regions of active growth. Field observations of plants of Asplenium trichomanes have shown the striking feature that growing root tips are only found in contact with the rock surface, and in consequence, although the point is not susceptible of proof, it is very likely that salt-uptake proceeds very largely from the solution on the rock surface. The roots may be able to take up minerals which are only

present in minute quantities in the parent rock, and effectively absent from the available soil, as they appear in solution from the rock-face. Moreover the diffusion outwards of carbonic acid from respiring roots will accelerate the rate of solution of certain minerals from the rock. It is therefore clear that an apparent deficiency in the soil surrounding the older roots may not be a valid indication of a real deficiency to the plant. It also follows that certain characteristics of the soil, such as pH value, may not be the same as the corresponding characteristics of the solution at the soil-rock interface. It seems clear that in the case of Asplenium trichomanes, and also of other plants of similar habitat, the nature of the parent rock is of more importance to the investigator than are the properties of the soil. This is evident with particular force with reference to plants growing in igneous rocks, when only examination of a rock section under the polarising microscope can accurately indicate the composition of the rock, and in consequence the probable nature of the chemical environment of the root.

Finally, the "open" character of the majority of rock-crevice communities is very probably only apparent and not real. We have no means of measuring what proportion of

unoccupied rock-crevice area is in fact suitable for colonisation by a particular species. The majority of communities described as open are of a transitory nature, since they occupy habitats still in a primary or secondary stage of colonisation. The open character of rock-face is of a different nature, being usually relatively stable in terms of ecological succession, according to the extremity of the degree of exposure. Associations can be of considerable age, indicated by the evident age of individual plants included therein, maintenance of the open character of such associations being thus determined by the rigours of the environment. The essential difference between the two types of open habitats is that in the case of transitory communities, there is little or no competition between individual plants, but in relation to rock-crevice habitat associations, it is not possible to disregard the factor of competition between individuals, which may in fact be very important. In relation to the present investigation, this is a very significant point, since it directly concerns the problem of how important is the factor of competition for available space between the different cytotypes of Asplenium trichomanes in the determination of their different ecological distributions.

AUTECOLOGY (contd)

AUTECOLOGY OF DIPLOID CYTOTYPE (Figs 8-15 & 135-136).

BRITISH ISLES (Figs 8-12)

The diploid cytotype is rare in the British Isles, and so far only a few stations have been discovered. It is apparently confined to the mountainous parts of the country, which has made determination of its distribution a difficult task.

The known localities of this plant in the British Isles fall into four geographical groups: 1., the Lake District, 2., north-west Wales, 3., the eastern extremity of the Breadalbane hills, and 4., the serpentine belt of Aberdeen and Banff. The special features of each will be described in turn.

1. Lake District.

Only two localities are yet known in the Lake District; one a gully at the foot of Barf Fell, Thornthwaite, the other rocks near Castle Crag in Borrowdale. In both localities the plant is growing on base-poor rocks probably belonging to the Borrowdale series. The plant is present in very small quantity in both localities, the total number of individuals seen being less than a dozen.

Other localities must exist in other parts of the Lake District, but the amount of ground already unsuccessfully

covered in search of this plant makes it clear that the diploid cytotype is certainly distinctly rare there.

2.North-west Wales.

It is evident that the diploid cytotype is rather frequent in the mountains of Caernarvon and Merioneth wherever rainfall is high, i.e. above 60 inches per annum. The majority of the known stations are on damp rocks in shaded cool situations, most often on north-facing outcrops. This preference for damp shady situations is illustrated by the fact that the diploid cytotype though never actually found intermingled with the filmy fern Hymenophyllum wilsoni, is often found in the immediate vicinity of this species. The composition of the rock substratum seems to be of lesser significance than either rainfall or protection, since the diploid cytotype has been found in North Wales on a variety of igneous and sedimentary rocks, although never on rock of undoubted basic character.

3. Perth.

The most interesting feature of this region is that the diploid cytotype has been found growing on mica-schist on an outlier of the Breadalbane range, near Dunkeld.

This is a rock of very different character to any supporting this plant in the Lake District and North Wales, although paralleled by known occurrences on mica-schist in the Hardanger district of Norway.

It is notable that at this higher latitude, with lower summer temperatures, the limiting level for rainfall appears to be about 40 inches per annum. This is equally true of the Aberdeen region, and is in contrast to the 60 inches approximate limit for this plant in England and Wales.

4. Aberdeen.

Here the diploid cytotype has been found on two small outcrops of serpentine rock. There are other similar and apparently suitable outcrops of serpentine in the vicinity which do not support this plant, and the reason for this is not known. The distribution of other species of plants on these outcrops is equally inconsistent, so that the flora of no two outcrops is exactly the same. Until these outcrops are critically examined petrologically, this problem remains obscure.

These stations on Aberdeen serpentine are paralleled in Norway by localities on rocks of the serpentine group (probably in this case a form of Dunite), in the Sunnmore district (Figs. 136 & 137).

The main facts concerning the autecology of the diploid cytotype in Britain can be summarised. Essentially a lithophyte, it appears to be confined to the mountainous parts of the country, though not at any great altitude, but generally at submontane levels. The known altitudinal range is 200 - 1500 feet. The association of this plant with mountainous districts is in fact not related to altitudinal requirements, but is instead correlated with its preference for cool, sheltered, and perpetually humid habitats, which are to be found only in regions of high rainfall and notable relief. It can tolerate as sub-stratum a variety of rock-types, including mica-schist and serpentine, but it is more often found on rocks poor in bases. This plant completely avoids calcareous rocks, which is, as will be seen, in total contrast to the behaviour of the tetraploid cytotype.

AUSTRALIA (Figs 13 - 15).

Only one locality of this plant in Australia was visited, near Wulgulmerang in a rather remote part of the Gippsland region of eastern Victoria. The annual rainfall in this region ranges from 30 to 40 inches. The actual station is extremely restricted in area, consisting of a

small sheltered rock-face about fifty feet long, and not more than twenty feet high, composed of a conglomerate of volcanic origin. No other station is known in the vicinity, nor indeed for thirty miles in any direction. The site is subject to light shade from species of Eucalyptus. The ledges on the actual rock-face are colonised by grasses, especially Poa caespitosa s.l., but nevertheless this limited area supports a flourishing colony of the diploid cytotype of Asplenium trichomanes, composed of numerous individuals. Also present is A.flabellifolium, a rather distant relative.

The general aspect of this locality bears an extraordinary resemblance to the station for the diploid cytotype on Stenton Rock in Perthshire. The reader is invited to compare the photographs of the two localities (figs. 11 & 14). It may be unwise to draw conclusions from this comparison, but the remarkable resemblance is very striking.

It is clear that this plant is rare and most discontinuously distributed in south-eastern Australia. From a study of herbarium material, four other localities in Victoria are known, and two in New South Wales. One Victorian collection (from Mt. Buffalo), is stated by its

collector (Baron von Mueller), to be from slate rocks, and the specimen from Ebor, N.S.W., bears the description "on granitic rocks". It is not unreasonable to presume that this plant exhibits basophilic tendencies in all its Australian localities.

ELSEWHERE.

The only information available concerning the habitats of this plant in America is the comment by Britton (2), that the diploid plant cytologically examined by him from Ontario was found growing on granite.

No information is available concerning the localities of this plant in the Himalayas, save only that it has been found between 3,800 and 11,000 feet.

AUTECOLOGY OF TETRAPLOID CYTOTYPE.

BRITISH ISLES. (Figs 16 - 29).

The tetraploid cytotype is very generally distributed over the British Isles, and probably occurs in every vice-county. However, this complete distribution over the country cannot have existed before historical times, but is instead a consequence of the development of the stone-mason's art, for the plant is now found commonly on old mortared walls in districts far outside the range of its occurrence on natural rock.

The distribution of the tetraploid cytotype of Asplenium trichomanes in Britain must therefore be considered as if divisable into two separate parts; firstly as a native in the localities where it is truely indigenous, and secondly, as an invasive plant following the development of civilised communities. The natural distribution will be considered first.

This plant is characteristically a limestone fern, and as such is a prominent constituent of the flora of Carboniferous Limestone in Yorkshire, Derbyshire and Westmorland. It is also frequent on this rock in Brecon. It is most often to be found in crevices of rocks and on the faces of cliffs and scars, and also in the grikes of limestone pavements. It also occurs in breccia and on

scree slopes, but it is more common in this habitat on the limestones on Westmorland and Brecon, which receive more rainfall than those further east.

This fern also occurs, and in some places plentifully, on basic rocks in the mountainous regions of Britain. For instance, it is known from calcareous rocks in Snowdonia (e.g. Cwm Idwal, Cwm Llafar), from the Lake District (e.g. Borrowdale), and on metamorphic limestone on Falcon Clints in Upper Teesdale. Apart from calcareous rocks, it occurs on the same mica-schist face near Dunkeld that supports the diploid cytotype, and also on the same belt of serpentine in Aberdeen, although in this case not in direct association with the diploid.

There are no certain records of the occurrence of the tetraploid in Britain on other than base-rich rocks. Where the plant is recorded from districts where the rock is exclusively of a base-poor character, it has always been found that the occurrence is in a wall, usually built of local stone, but mortared or cemented, and thus providing the necessary base-rich sub-stratum. In such districts, the association of this plant with mortared walls is most striking, and affords a very clear demonstration of a real preference for calcareous rock irrespective of the climatic

factors operating in the area.

There are no authenticated records of the tetraploid cytotype either from the Chalk or from Magnesian Limestone, although it can occur in the vicinity of these rocks on mortared walls with a particularly favourable aspect. This absence must result from what bare rock exposures that do exist lacking crevices with a microclimate sufficiently amenable for the plant to survive, since evidently the general climatic conditions of such areas lie within the range of the plant's tolerance if adequate protection is available.

As has already been pointed out, man's device of cementing his buildings and walls with a calcium-rich mixture has resulted in the tetraploid extending its distribution from its native localities in the mountains and limestone hills into the lowlands. If the mortar is of a certain age and degree of weathering, and is consequently cracked and pitted with solution holes, it will be suitable for colonisation. Such conditions are frequently found on church-yard walls and the like. On such habitats the tetraploid has extended its range right across England into the driest and warmest counties of the south-east. Thus it is to be found in Kent, Surrey, and Sussex, and also in

Norfolk and Suffolk. However, its distribution in these easterly counties is scattered, and it is only found in very sheltered aspects. In fact in this part of the country only a minute proportion of the mortared walls available for colonisation support the plant. In the wetter parts of Britain, however, the tetraploid is frequently very abundant in such situations. It is thus a very familiar feature of the wall flora in North Wales and the Lake District. Occasionally in these two regions, and in the Craven district of the West Riding, the situation becomes confused, for in some places the plant is found on natural rock within a few hundred yards of a man-made habitat supporting a flourishing colony.

Thus in Britain the tetraploid cytotype differs strikingly from the diploid cytotype in edaphic preference and climatic tolerance, for not only is the tetraploid characteristically associated with calcareous rocks and apparently never found except on base-rich rock, but it is also distributed widely over those regions of the country with a rainfall much lower than the diploid cytotype can tolerate.

AUSTRALIA (Figs 30 - 39).

Six localities of the tetraploid cytotype in Australia have been studied. Three of these are situated in the far south-west of Western Australia, two in the Gippsland region of eastern Victoria, and one in the Blue Mountains of New South Wales. Since the eastern localities are separated by nearly 2,000 miles from the West Australian stations, the two groups will be described separately.

In the extreme south-west of Western Australia, a shallow peninsula extends for some sixty miles from Cape Naturaliste to Cape Leeuwin, bounded by a narrow belt of coastal limestone only a few miles wide. This limestone, which is of Pleistocene age, is of almost horizontal dip, with consequently few outcrops of bare rock, save for the sea cliffs. However, four stations for the tetraploid cytotype are known on this limestone, of which three were visited. One of these, at Yallingup, is on rock exposed at the confluence of two small creeks, and within sight of the Indian Ocean, half-a-mile away. Most of the plants of Asplenium trichomanes were growing under the shade of scrub dominated by Banksia grandis and Eucalyptus marginata(Jarrah). Twenty five miles due south of Yallingup is Mammoth Cave, not far from Margaret River. The fern is found growing on

the rocks about the partially collapsed entrance to the cave, which is now below ground level. Both this cave, and another, Lake Cave, three miles to the south, are situated in Jarrah forest. At Lake Cave, the roof of an immense cavern has at some time fallen in, leaving a great pit two hundred feet in diameter, and a hundred feet deep. Asplenium trichomanes is found amongst the tumbled rocks in the bottom of the pit, under the shade of Eucalyptus forest which is established amongst the boulders. Both of these stations at Mammoth and Lake Cave are situated within three miles of the sea.

Apart from these occurrences in the extreme south-west, only one other locality is known in Western Australia, almost two hundred miles further east, near Albany. Here Asplenium trichomanes is found "in shady wet spot in limestone, among rocks below waterfall, Mt. Many Peaks". This mountain, which is within two miles of the sea, is mainly composed of granite, but limestone of Miocene age outcrops in the vicinity.

These occurrences of the tetraploid cytotype in Western Australia are of great interest, not merely because of their extreme isolation, but also because the plant is here so evidently on the very fringe of its tolerance.

The coastal limestones extend up the west coast from Yallingup right to Perth, and far beyond, but apparently without Asplenium trichomanes. Although the annual rainfall of the far south-west is much the same as that of Perth, between thirty and forty inches, its distribution throughout the year is more even about Albany and in the extreme south-west than about Perth, where summer rainfall is very light, being usually less than three inches during the five months from November to the end of March. Albany receives more than twice this amount of rainfall during the same period. It would seem clear that the greater severity of the summer drought further north limits the distribution of Asplenium trichomanes.

In the eastern states, where the annual distribution of rainfall is more even over a larger area, the tetraploid cytotype is more widely distributed. There are numerous outcrops of limestones of Silurian and Devonian age scattered through Victoria and New South Wales. The three localities visited, Bindi and Buchan in Victoria, and Jenolan Caves in New South Wales, are all on localised outcrops of such limestone. Other stations at Yarrangobilly, Abercrombie Caves, and Bungonia Caves are also situated on such limestone outcrops. Information

concerning the rock-type of other recorded localities is not available, but it seems probable that all the habitats of this plant in Victoria and New South Wales are in fact on limestone.

The three localities studied all have one feature in common. In each case the rock outcrop supporting the plant has been exposed by the action of a small creek. The colony at Buchan is relatively exposed, although south-facing, the few shrubs present provide shade protection only for a minority of the individuals in the population. At Bindi and Jenolan, the colonies are in sheltered gullies with light Eucalyptus forest. The Bindi station is similar in all respects to the Wulgulmerang locality for the diploid cytotype save only the nature of the rock. This is a point of some interest, since these two localities are only twenty five miles apart, situated on either side of a high granitic plateau.

The Jenolan locality is noteworthy in that limestone walls have been built in one place to protect a path, only a few yards from where one colony of the tetraploid cytotype is growing on the natural rock. The shaded side of this wall is now supporting a much larger colony of Asplenium trichomanes than is anywhere to be seen in this locality

on natural rock. Evidently the tetraploid cytotype in Australia is just as capable of taking advantage of man-made habitats as is the tetraploid in Europe.

NEW ZEALAND (Figs 40 & 41).

This plant is very rare in New Zealand, and is unknown in the South Island. Only four records are known from the North Island. One of these is known only as a specimen in the Herbarium at Kew, briefly localised as "Wellington". The other three localities are all of recent discovery, and are all on limestones of Tertiary age in the Hawke's Bay region. These limestones are rather soft, and form rounded hills, with few outcrops of exposed rock.

Only two of the three localities have been studied. In both, the fern occurs on bare exposures of rock on low grass-covered hills. At Tangoio, there is clear evidence of relatively recent destruction of forest, which formerly covered the slopes of the hills in the area. Whether or not the plant grew in this station when it was forest-clad is not known.

In this part of the Hawke's Bay region, the mean annual rainfall lies between thirty and fifty inches. Since this is the driest part of the North Island, it is evident that the virtual restriction of the tetraploid cytotype to this

district is not determined by considerations of moisture. It is possible that competition with the hexaploid cytotype for the same ecological "niche" is responsible at least in part for the restricted distribution of the tetraploid, but this is a rather speculative point.

ELSEWHERE.

The only information available concerning the nature of the habitats of this plant in North America is the comment by Britton (2), that the plants cytologically examined by him came from localities on dolomitic limestone in Ontario.

The association of this plant with limestone, as is seen in Europe, Australia, and New Zealand, and perhaps also in North America, is certainly not universal throughout its range.

For information concerning Asplenium trichomanes in Hawaii I am indebted to Mr. Eugene Horner (in correspondence) who sent live plants from Mauna Loa and Mauna Kea, the two great volcanoes on the island of Hawaii. Both are more than 13,000 feet high. The fern is found on the slopes of the volcanoes from 4,000 to 7,500 feet. Mr. Horner writes that it is "usually in open forests.....clinging

to the sides of moss covered rocks". He also comments that "during long periods of dry weather the fronds may wither and dry, but in most cases the roots will respond to moisture and send forth new growth".

The isolated locality of this plant at the summit of Mt. Wagar, in British Somaliland, has been visited twice in recent years. Glover and Gilliland call the rock granite, while Bally describes it as gneiss. Whatever the truth may be, the rock is clearly not limestone.

For information concerning Asplenium trichomanes in South Africa, I am indebted to Dr. E. A. C. L. E. Schelpe (in correspondence). The species is decidedly uncommon in South Africa, hardly more than a dozen records being known. Dr. Schelpe has not personally met with this species in South Africa, but he had brought together details of records in the course of his studies on South African ferns. He writes that "its distribution in South Africa seems to be correlated with the occurence of a cool climate with fairly high summer rainfall in hilly or mountainous country", and in a previous letter, that "Its known altitude range here is from about 5,000 to 9,700 feet altitude. It occurs on stream banks in forest at its lower limit, and grows in rock crevices at its upper limit". There seems

to be no definite information concerning the parent rock in its localities in South Africa, for Dr. Schelpe writes that "I find no clear association with any particular type of parent rock.....its distribution in the eastern Cape Province straddles a whole variety of sedimentary rocks, outcropping around there".

AUTECOLOGY OF THE HEXAPLOID CYTOTYPE (Figs 42 - 66)

As has already been indicated, this plant is endemic to New Zealand. Furthermore, it is much more common in the South Island than in the North Island, where it is only known with certainty from a few localities in each of two limestone districts; about Waitomo and Te Kuiti in the South Auckland Land District, and about Napier in Hawke's Bay.

South Island.

The hexaploid is widely though discontinuously distributed throughout the South Island. Undoubtedly some of the discontinuities will be bridged by future records, but nevertheless it is unlikely that any major extension to its known range in the South Island will be discovered. It is therefore possible to make some attempt to interpret the ecological factors determining the limits of distribution of this fern in the South Island. Personal field observations have been made on twelve stations. Of these, three are situated in Nelson Land District, eight in Canterbury, and one in Otago. Useful information has been given by botanists who have collected this plant in other localities in Nelson, Marlborough, and

Canterbury. Various different aspects of the autecology of this plant will now be considered in turn.

Altitudinal range.

The altitudinal range is considerable. The author has seen this plant in localities ranging from near sea-level to 2,600 feet, while other workers have certainly found it above 3,000 feet.

Rock preference.

This fern has been found on three distinct rock types. Apart from the older and now metamorphosed Palaeozoic limestones of the Pikikiruna Range, Nelson, stations are found in several parts of Marlborough and Canterbury where limestones of Landon (Oligocene) age outcrop, e.g. Chalk Range, Castle Hill Waiau, and Mt. Somers. However, this plant is absent from many such outcrops.

Colonies are also found on Greywackes, rather featureless fine-textured but poorly stratified rocks, not rich in bases, which form the great bulk of the Southern Alps. Localities on this rock occur in Marlborough and Canterbury, e.g. Kaikouras, Cass, Hooker valley, and Waitaki.

The hexaploid cytotype is also found on mica-schist, as at Queenstown.

Protection.

The habitats of the hexaploid cytotype vary considerably in the degree of protection they provide. Most usually stations of this plant are bare of any tree cover. In most localities the majority of plants are sheltered only by the aspect of the rock, although some individuals enjoy the protection of isolated shrubs growing on the rock-face. However, since colonies are most often found on south-facing outcrops, they acquire considerable protection from the rock-face. Nevertheless, some colonies do exist in most exposed situations, with little or no shelter from the sun's radiation.

Associated flora.

Apart from native species of the flora, the open habitats favoured by the hexaploid cytotype always support a considerable proportion of introduced species, almost all of which are of Eurasiatic origin. This introduced element in the flora is valuable to the investigation since it affords some basis for comparison with European communities.

There is not room here to list all the various species found associated with the hexaploid cytotype in its different localities, nor is it necessary. Most of the indigenous

species found in association with the hexaploid cytotype are plants of relatively wide ecological distribution by no means confined to rock faces. The only important exceptions to this generalisation are a few species of fern which are confined to rock outcrops. Two of these are also species of Asplenium endemic to New Zealand, but neither is at all closely related to Asplenium trichomanes.

The alien species found with the hexaploid cytotype are mostly of a weedy character, and treat open rock habitats much the same as they do any other open habitat available for colonisation. The majority of these species are annuals, and as such are at some advantage in New Zealand, since this life-form is extremely rare amongst the indigenous rock-flora. The species most commonly found are Cerastium vulgatum, Arenaria serpyllifolia, Anthoxanthum odoratum, Linum catharticum, Verbascum thapsus, Dactylis glomerata, Aira caryophyllea, Holcus lanatus, and Crepis capillaris.

The occurrence of certain other introduced species in the same locality as the hexaploid is of rather greater interest. These are Myosotis hispida, Acinos vulgare, and Cerastium arvense, all of which occur at Castle Hill in Canterbury. Myosotis hispida has also been found with Asplenium trichomanes at Queenstown. In England, all of

these three species have distributions exactly contrasted to that of the tetraploid cytotype of Asplenium trichomanes in that they are more abundant in the drier south-east, and much less common in the west and north. The association of these plants with the hexaploid cytotype constitutes one type of evidence indicating that this latter plant is capable of tolerating drier conditions than the tetraploid cytotype. Evidence of a more direct nature has been gained by study of the relationship of the distribution of the hexaploid to the distribution of rainfall.

Relationship of distribution to rainfall.

The hexaploid cytotype rarely occurs in areas with more than eighty inches of rain a year. In no place does it transgress the eighty inches isohyet by more than a few miles. This correlation reflects the absence of this plant from the wet rain forest, owing to its intolerance of heavy shade or any conditions which permit luxuriant bryophyte growth over all rock surfaces.

The localities on the Pikikiruna Range present a rather special and instructive case. Here the annual rainfall is about eighty inches and the rock is metamorphic limestone. The stations known to the author are in places from which heavy mixed Podocarpus forest has not long since

been removed (see fig. 47). It is very likely that these stations are of recent colonisation. The range is dissected by steep gullies, and some of the precipitous bluffs can never have supported more than a light tree cover, and they are likely stations for Asplenium trichomanes, although owing to their inaccessibility, they have not been examined. However, thanks to Cockayne (6), it is known that Asplenium trichomanes was to be found on the top of the range prior to the clearance of the forest. He describes a "remarkable Nothofagus association", growing on very rocky ground composed of much weathered limestone. The dominant tree, Nothofagus cliffortioides, grew "with its slender trunks far apart, so that much light entered the association". Among the ground flora, Cockayne specifically mentions Asplenium trichomanes. Unfortunately, this association was later destroyed by fire. Evidently we have here a description of a locality, at that time still undisturbed, in which the hexaploid cytotype was at one limit of its ecological range. It is only with the destruction of the heavy forest on the lower slopes that the fern has become widespread over the district.

In contrast to these localities in a region of high rainfall, the hexaploid cytotype has been found in the most

arid region of New Zealand, namely Central Otago, where the mean annual rainfall is less than twenty inches. This region was not visited, but the locality seen at Waitaki, on the fringe of this area, is essentially comparable, for here the mean annual rainfall is 18.5 inches. The Waitaki habitat consists of bare greywacke rock, protected from the full force of the sun by its south-facing aspect. It is notable that in this station, Asplenium trichomanes is associated with Cheilanthes distans and Pleurosorus rutaefolius, two ferns only found in New Zealand in very dry situations. It is clear from this extreme example that the hexaploid cytotype is capable of flourishing in much more dry conditions than is known for either diploid or tetraploid cytotypes in any part of their ranges.

COMPARATIVE MORPHOLOGY. (Figs. 67-93).

The primary aim in this part of the investigation was to determine what differences in morphology existed between the three cytotypes, and which of such characters are most reliable for the identification of the three cytotypes in herbarium material, and could therefore be used as the basis for a taxonomic revision of the species aggregate.

The various characters investigated will be described in turn, commencing with qualitative characters such as the shape of the pinna and the form of the rhizome scale. Subsequently, various quantitative characters such as spore and stomatal size are considered, and finally, this section of the thesis is concluded with a synopsis of the morphological differences which exist between the three cytotypes.

The several techniques utilised in this part of the investigation are described under the sections to which they are appropriate.

COMPARATIVE MORPHOLOGY (contd)

QUALITATIVE DIFFERENCES.

There are unfortunately, no diagnostic macroscopic distinctions of this kind. If there were, it would not have been necessary to develop this part of the investigation in such detail.

PINNA SHAPE

Differences do exist between the three cytotypes in the shape of the pinna, but this character can display considerable variation. In the diploid the pinnae are usually rounded or orbicular in appearance, or at the most oval in outline. In contrast, the tetraploid cytotype (excluding the Japanese plant), is more oblong than oval in appearance. However, some stunted tetraploid plants cannot certainly be distinguished from diploid plants with respect to this character. In both diploid and tetraploid, the pinnae margin may either be entire or inconspicuously crenate.

The Japanese tetraploid differs markedly from the typical tetraploid in that pinnae are almost rhomboid, with a rather broadly crenate upper margin. This character is remarkably constant. In fact, the Japanese tetraploid more nearly resembles the hexaploid cytotype with regard to this character, for the pinnae of the hexaploid are

generally rather rhomboid in outline, but rather closely crenate on the upper surface. However, small specimens of the hexaploid are not distinguishable from the typical tetraploid form, with regard to this character.

RHIZOME SCALE (Figs 67 - 71).

The very short rhizome of these plants is densely covered with linear scales, each a few millimetres long, which serve as a protective covering for the delicate tissue of the growing point. Apart from the surface of the rhizome itself, identical scales are attached to the basal regions of the stipes. Since there is no morphological difference between these and the scales actually situated on the rhizome, for the sake of simplicity these are considered together here, and all are referred to as rhizome scales.

In preparing detached scales for microscopic examination, these are first dehydrated and de-aerated with 95% and Absolute Alcohol, transferred briefly in Xylol (overlong immersion will render the scales brittle), and then mounted in Canada Balsam.

Pen and ink drawings are prepared with the aid of a projection microscope.

Apart from the more plentiful fully-developed scales,

there are intermixed a range of smaller scales, showing all gradations from a chaffy state through increasing occlusion of the lumen of the cells to the typical degree of occlusion for the particular cytotype. In order to establish a truly sound basis for comparison, it is necessary to select only the best-developed and largest scales for examination.

DIPLOID (Figs 67 & 68).

Range of maximum scale length = 2.0 - 3.5 m.m.

Ratio length:breadth at base = 5 < 10 : 1

The scales of the diploid cytotype are lanceolate in shape, with a central occluded area, extending from the base of the scale for one-half to three-quarters of its length, but with a broad margin of unoccluded cells. The occlusion of the central cells is relatively thin, this region appearing pale red-brown in cleared preparations. (This character cannot of course be displayed in a pen and ink drawing). The margin of the scale is not regular, occasional multicellular projections being usually present. Such processes are only very rarely found on tetraploid scales, and never on the scales of the hexaploid cytotype.

There is quite considerable variation amongst diploid plants from different regions with respect to the character of the rhizome scale. The scales of the European diploid

are generally least easily distinguished from those of the tetraploid cytotype, since they tend to be rather larger, more slender, and with a somewhat greater area of occlusion than scales of diploid plants elsewhere. The scales of the Australian diploid are rather distinctive (see fig. 68), in that they are relatively small, with a markedly irregular margin, and possess a broad base in relation to the length of the scale.

TETRAPLOID (Figs 69 & 70).

Range of maximum scale length = 2.5 - 5.0 mm

Ratio length:breadth at base = $6 < 13 : 1$

The scales of the tetraploid cytotype are almost linear in shape. The central occluded region takes the form of a narrow conspicuous band extending about three-quarters the length of the scale, this occluded strip being of a dark-brown colour, and opaque, even in cleared preparations. There is a distinct wing of unoccluded cells, several cells broad.

The Japanese form is distinct from all other tetraploid forms with respect to the rhizome scale, which is in effect intermediate between the usual tetraploid type and the scale of the hexaploid cytotype, having a much larger band of occluded cells with a correspondingly smaller wing.

HEXAPLOID (Figs 71 & 72).

Range of maximum scale length = 2.5 - 5.0 mm

Ratio length : breadth at base = 7< 20 : 1

The scales of the hexaploid cytotype are linear or subulate in shape, and show occlusion of their cells over almost the entire area of the scale, only a narrow wing of unoccluded cells being present, from one to three cells broad. The occlusion is intense, being of a very dark brown colour, completely opaque, and generally extending right to the tip of the scale. In its most extreme development, the characteristics of the scale of the hexaploid cytotype are immediately distinctive from the scale of any tetraploid form.

SPORE SCULPTURE (Figs 73 - 84).

Preparations have been made by mounting spores direct in Gum Chloral, the slides being then placed on a hot-plate for a few hours to aid the clearing action of the Chloral hydrate. (See also p.70).

The exospore is regularly smooth and quite featureless in the spores of all three cytotypes. However, in this ~~genus~~ genus of ferns in common with many others, another layer known as the perispore is deposited outside the exospore.

This perispore may be very irregular in appearance, and shows characteristic patternings in each cytotype. A quite exceptional variability in perispore markings is present in the group under study, indeed the range of types found in the tetraploid cytotype has apparently never previously been encountered in one species.

The terminology used in the descriptions is that of Harris (16).

DIPLOID (Figs 73 - 75).

A range in perispore types is found, from a saccate and almost papillate condition, though a rugulo-saccate form, to an almost cristate sculpture. The rugulo-saccate condition is however most common. The perispore wing as seen in equatorial plane of focus is generally relatively large in comparison to the size of the spore.

TETRAPLOID (Figs 76 - 81).

Most often the perispore sculpture is rugulo-saccate, giving the impression of an irregular but wide wing to the spore. Less commonly, a cristate condition is found, with a conspicuous wing to the spore. Other perispore types are particularly frequent in the New Zealand tetraploid. A remarkable form is found in which the perispore is densely

or thinly marked with spinulose (echinulate) projections. In another form, the perispore approximates to a saccate condition, although individual projections could be described as papillate or echinate. Exceptionally, all these perispore types may be present in the one preparation.

HEXAPLOID (Figs 81 - 84).

Almost all the spores produced by the hexaploid cytotype have a cristate perispore. However, these cristate spores are quite distinct in appearance from cristate forms of the tetraploid and diploid, for quite apart from the size difference, in the hexaploid the perispore crests are long and shallow, producing a relatively even but narrow and inconspicuous wing to the spore. Occasionally, the crests are so shallow as to be barely detectable in side view, although quite evident in surface view, resulting in a superficially emarginate appearance to the spore.

QUANTITATIVE DIFFERENCES.

It is generally recognised that increase in level of polyploidy is very frequently accompanied by increase in the size of various parts of the plant. The comparative size of certain cells has often been found to provide a reliable character to distinguish members of a polyploid series from one another. In work on flowering plants in particular, very general use has been made of types of cells which are readily measured under the microscope, and which are not subject to great variation in size within an individual plant. The stomata and pollen grains are most often used, but the cells of the epidermis have also been utilised.

In the present study, the size of the spores, the size of the stomata, and the size of the cells of the sporangial annulus have all been investigated in order to discover whether these could be used as characters for the identification of the cytotypes in circumstances when a chromosome count is out of the question, as for instance, in the determination of herbarium material. It is not practicable to measure the cells of the epidermis in these ferns, because not only are these cells very sinuous in outline, but they are also variable in size in different parts of the pinna,

being generally longer where situated over the veins.

Certain other characters of the frond show size differences between the cytotypes, but these also are not readily measured. For example, the rachis of the frond is distinctly thinnest in the diploid, and thickest in the hexaploid. Similarly, it is clear that the thickness of the pinna increases in the grade of polyploidy, and the effect of this difference is visible in both living and dried material, although the expression of this character is strongly influenced by the conditions of the environment. Plants growing in deep shaded crevices tend to produce relatively thin broad pinnae, whilst others growing in very exposed situations have comparatively thick small pinnae. Nevertheless, the distinction between the pinnae of the cytotypes is usually clear. Those of the diploid are delicate, and sometimes almost membranous, in contrast to those of the hexaploid which are almost coriaceous. The tetraploid is intermediate with respect to this character.

PINNA SIZE AND PINNA SEPARATION. (Figs. 85 - 88).

These two characters are best examined in relation to one another in the form of scatter diagrams. The data utilised in the construction of these scatter diagrams have

been obtained by means of a certain uniform procedure. Only mature fertile fronds are chosen for examination. Both length and breadth of the pinna are measured for five or ten pinnae along one side of the rachis in the middle region of the frond. The mean length and mean area of the pinnae in this segment of the frond, and the mean distance between them, are thus calculated, and the resulting figures for each frond plotted as a point on a scatter diagram. In practice, it has been found that scatter diagrams of pinna area/pinna separation are essentially similar to scatter diagrams of pinna length/pinna separation, and in consequence apart from one example of the former type(fig. 85), all the scatter diagrams included in this thesis are of pinna length/pinna separation. A second example where pinna area/pinna separation and pinna length/pinna separation scatter diagrams are compared is illustrated in Lovis (21).

The construction of the frond, which is simply pinnate, is such that these statistics provide at the same time a reliable index of frond development. As the scatter diagrams show (figs 85 - 88), there is a very considerable range in frond size within each cytotype. Even individual plants are found to possess remarkable plasticity with regard to frond development.

Despite the very considerable variation in frond size, it is possible to detect discrete differences between the cytotypes with regard to the ratio between the size of the pinnae and their separation. Although some individual diploid fronds are indistinguishable on this basis from some tetraploid fronds, nevertheless pinna length is generally larger in relation to pinna separation in the tetraploid cytotype than in the diploid, the respective ratios being approximately 4 : 3 (1.3 : 1) in the tetraploid, and 1 : 1 in the diploid. It is interesting that with respect to this character, the hexaploid is intermediate, with a ratio of 6 : 5 (1.2 : 1). In spite of the extraordinary range in frond size, the axis of this ratio is clear on the scatter diagram (fig. 88).

However, the great variation in frond size makes these gross characters of the frond quite impossible as diagnostic criteria for identification, for the total range of variation in the hexaploid is confluent with the ranges of variation in the other two cytotypes.

SPORE SIZE (Figs 89 - 91, also 73 - 84)

All measurements have been made on spores mounted directly in Gum Chloral. This is a Gum Arabic mounting medium containing Chloral hydrate as a clearing agent.

This method is simple and rapid, and in comparison with some methods of preparation, mild in its action on the spore. The clearing action of the Chloral can safely be accelerated by placing the slide on a thermostatically controlled hotplate set not higher than 40° C.

Measurements have been confined to the length of the true spore or exospore, which is regular in shape, unlike the perispore layer, which may be most irregular. The boundary of the exospore is clearly visible within the perispore in Chloral mounts. Fifty spores are measured from each sample, rejecting any spores which are mis-shapen or imperfect in any way.

DIPLOID (figs 73 - 75 & 90a).

No. of samples of known chromosome number measured = 30

Mean exospore length = 31.90 μ

Absolute range in exospore length = 23 - 42 μ

Range in mean determinations of exospore length = 29 - 36 μ

TETRAPLOID (Figs. 76 - 81 & 90b).

No. of samples of known chromosome number measured = 40

Mean exospore length = 38.83 μ

Absolute range in exospore length = 27 - 50 μ

Range in mean determination of exospore length = 34 - 43 μ

HEXAPLOID (Figs. 82 - 84 & 91a).

No. of samples of known chromosome number measured = 20

Mean exospore length = 42.72 μ

Absolute range in exospore length = 33 - 55 μ

Range in mean determinations of exospore length = 40 - 46 μ

It is seen that although the absolute ranges in spore size in the three cytotypes overlap very extensively, comparison of mean exospore length for different samples is more informative. (See fig. 89).

Although the ranges of mean determinations for the three cytotypes are not mutually exclusive, there is very little overlap between the diploid and tetraploid ranges. In fact 95% of the determinations for diploid samples lie outside the range of the tetraploid, and it is clear that spore size is a very valuable character for detection of the diploid cytotype in herbarium material. The overlap between the ranges of mean determinations for tetraploid and hexaploid samples is more extensive, and consequently the character of spore size is less useful in this case. Nevertheless, 64% of the tetraploid samples measured have a mean spore size outside the ranges of both diploid and hexaploid. Mean spore length is of least usefulness for

identification of the hexaploid cytotype, since only 44% of the determinations made lie outside the range of the tetraploid.

ANNULUS OF THE SPORANGIUM (Figs 90 & 91a).

Manton (22), has shown that the number of indurated cells in the annulus is characteristic for each of the three cytotypes of Polypodium vulgare s.l., and the different levels of polyploidy can be distinguished in herbarium material by use of this character.

In Asplenium all the cells in the annulus are indurated, and in consequence the number of indurated cells is large in comparison with Polypodium. A further disadvantage is that the junction of annulus and stomium is not always as clear as in most Polypodiaceous ferns. Nevertheless, it was decided to examine this character in case useful differences existed.

NUMBER OF INDURATED CELLS IN THE ANNULUS.

DIPLOID

Absolute range : 14 - 23 indurated cells

Range in mean values: 17.3 - 19.2 " "

Overall mean : 18.1

TETRAPLOID.

Absolute range	:	15 - 24 indurated cells
Range in mean values	:	17.8 - 21.1 " "
Overall mean	:	19.4

HEXAPLOID.

Absolute range	:	12 - 24 indurated cells
Range in mean values	:	15.0 - 20.4 " "
Overall mean	:	17.6

It is clear that the number of cells in the annulus is variable, and that all three cytotypes display much the same range of variation. Rather unexpectedly, the hexaploid sporangium has perhaps less cells in the annulus than has the diploid.

It was a first thought that this might prove to be important, since the considerable differences in the size of diploid and hexaploid spores must result in an increase in the volume of the sporangium. If this increase in volume is not reflected in the number of cells in the annulus, then it must be compensated in the size of the cells of the annulus.

Although such a difference would not be a very convenient character in practical use, owing to inversion

of the annulus in dehiscence of the sporangium, some measurements were made, but the results were not encouraging. It was found that the annulus cells of the diploid are in fact decidedly smaller than those of the tetraploid or hexaploid, but there is some overlap between the size ranges found in the tetraploid and hexaploid cytotypes. (See figs 90 and 91a).

STOMATA.

It is not usually possible to obtain satisfactory edipdermis peels from the pinnae of Asplenium trichomanes, and it is necessary to make whole mounts of pinnae for examination of the epidermis.

If herbarium material is being used the pinnae are first soaked in water containing a liquid detergent. The pinnae are then cleared overnight in chlorine water. Usually, undiluted commercial "Domestos" was used as the clearing agent.

After clearing, the pinnae are thoroughly washed, mordanted in tannic acid, thoroughly washed again, stained in ferric chloride and mounted in glycerine jelly.

MEASUREMENTS OF STOMATAL LENGTH.

DIPLOID

Range of mean values : 38 - 43 μ

Overall mean : 41.3 μ

TETRAPLOID

Range of mean values : 40 - 49 μ

Overall mean : 45.3 μ

HEXAPLOID

Range of mean values : 41 - 51 μ

Overall mean : 45.9 μ

Evidently stomatal size is of no value as a character for morphological separation of the three cytotypes.

COMPARATIVE MORPHOLOGY (contd)

SYNOPSIS OF MORPHOLOGICAL DIFFERENCES BETWEEN THE THREE CYTOTYPES.

DIPLOID.

Pinnae: orbicular, little longer than broad, thin in texture, rather distant, scarcely more crowded at apex of frond.

Rachis: slender, flexible.

Rhizome scales: lanceolate, maximum length 3.5 mm., with a red-brown central occluded area, hardly extending more than half the length of the scale, with a broad wing of unoccluded cells, multicellular processes often present on margin of the scale.

Spores: mean length of exospore 29-36 μ, perispore usually rugulo-saccate, but sometimes saccate or cristate, wing of perispore evident.

TETRAPLOID.

Pinnae: sub-orbicular or oblong, much longer than broad, rather thin in texture, rather close, distinctly more crowded at apex of frond.

Rachis: slender, rather flexible.

Rhizome scales: sub-linear or linear, maximum length 5 mm, with a

Rhizome scales:(contd) conspicuous but narrow dark brown occluded strip extending three-quarters of the length of the scale, with a broad wing of unoccluded cells.

Spores: mean length of exospore 34-43 μ, perispore usually rugulo-saccate, but otherwise saccate, cristate, or echinulate, perispore wing conspicuous.

The Japanese form of the tetraploid cytotype differs in its sub-rhomboid pinnae, the greater area of occluded cells in the rhizome scale, and in the rachis being more conspicuously winged.

HEXAPLOID.

Pinnae: oblong or sub-rhomboid, much longer than broad, thick almost coriaceous in texture, rather distant, distinctly more crowded at apex of frond.

Rachis: stout, almost rigid.

Rhizome scales: linear or subulate, maximum length 5 mm., completely occluded except for a narrow inconspicuous wing.

Spores: mean length of exospore 40-46 μ, perispore

Spores:(contd) uniformly shallowly cristate, perispore wing narrow, inconspicuous, sometimes apparently emarginate.

GENETICAL RELATIONSHIPS. (Figs 94 - 132).

An account is first given of the manner in which the gametophyte and sporophyte generations have been cultured, followed by a description of the technique involved in hybridisation attempts. Subsequently, details are given of the cytological techniques employed. This is followed by the main portion of this section of the thesis, in which an account is given of the cytogenetical investigation of the cytotypes of *Asplenium trichomanes*. This account is confined to a brief statement of the aims of the investigation, and description of the results obtained. Discussion of these results is deferred until the next section of the thesis.

CULTURE AND HYBRIDISATION TECHNIQUES

CULTIVATION OF ADULT SPOROPHYTES.

Asplenium trichomanes is not an easy plant to grow in cultivation. In particular, it has proved very difficult to maintain the diploid cytotype under greenhouse conditions.

Experience seems to indicate that perhaps the most important factor in pot cultivation of these plants is unimpeded drainage. In consequence, the pots to be used are "crocked" generously, and a light potting compost employed. Reasonable success was obtained with a mixture containing approximately two parts of coarse sand, two parts of loam, one part of leaf mould, and one part of old mortar or limestone chippings. The limestone fraction is omitted from compost intended for diploid plants.

In planting, it is necessary to ensure that the rhizome apex is just above the surface of the soil. Growing points cover over by soil tend to stagnate and eventually rot.

GERMINATION AND CULTURE OF GAMETOPHYTES.

All soil to be used for cultivation is first sterilised by heat. Sterilisation of the soil is necessary not only as a preventive measure against parasitic fungi, but also

because the number of potential competitors to the gametophytes which are present in the soil is very high. Treatment does mean that the soil used for sowings is at least initially virtually free from algae, fungi and moss protonemata, although as soon as the sowing surface is exposed to the air spores of these organisms will settle, and growth commence.

Subsequent growth of algae and moss protonemata can generally be controlled with reasonable success by weeding. Fungal attacks present by far the greatest problem, and to date the only effective method of dealing with such pathogens has been removal of the affected area to prevent spreading of the infection.

If fern spores are scattered fairly thickly over the surface of a sowing pot, the resultant growth of prothalli will form a closed community. Competition for both space and light results in the individual prothalli growing upwards rather than sideways, with increased relative growth of the antheridia-bearing region of the prothalli. In consequence, prothalli grown under these conditions generally provide good cultures for use as the male parent in a hybridisation experiment.

As a prothallus grows older, the apical region becomes

much broader and the number of archegonia increases. Eventually a stage is reached when the prothallus bears a large number of immature, mature, and old archegonia, while at the same time the male region is senescent. This stage of development, when the prothallus can be used as the female parent in a hybridisation attempt, is obtained more easily and quickly when the prothallus is growing free from competition. In consequence, prothalli intended for use as females are separated out from a pot of young prothalli and placed singly in concentric circles on the surface of a pot of fresh soil, at approximately one centimetre intervals.

Watering of gametophytic cultures must always be effected from the bottom upwards, otherwise release of antherozoids and self-fertilisation would result. It is also necessary to cover the pots (inverted clock-glasses are suitable), as a precaution against stray water, and also to prevent stray spores from reaching the soil surface.

HYBRIDISATION TECHNIQUE.

No way has been found of obtaining entirely female prothalli, and thus completely eliminating the self-fertilisation of females. It is necessary to accept as inevitable the occurrence of a certain proportion of self-fertilised

plants amongst the progeny, however carefully the female prothalli are selected.

When carrying out a hybridisation attempt, first of all about one square centimetre of a thick antheridia-bearing culture is teased out in a little water in a small clock-glass. After a few minutes, this is examined for swimming antherozoids and bursting antheridia. If liberation is considered adequate, about three square centimetres more of the culture is added. About half a dozen female prothalli are then taken from their pot and carefully cleaned, any juvenile prothalli which may have germinated amongst the rhizoids being removed. These prothalli are also inspected for signs of a embryonic sporophyte, in case self-fertilisation may already have occurred. The selected and cleaned prothalli are then placed ventral surface downwards in the glass of male prothalli. The clock-glass is then covered to prevent drying out, placed in bright light, and left for at least one hour.

At the end of the period of hybridisation, the females are removed, washed, once again cleaned thoroughly, and are then transferred to a fresh pot. The first signs of hybrid sporophytes appear in from four to six weeks. Sporophytes which appear as soon as two weeks after the

hybridisation attempt will prove to be the result of self-fertilisation having occurred prior to the date of hybridisation.

GENETICAL RELATIONSHIPS (contd)

CYTOGENETIC INVESTIGATION OF THE RELATIONSHIPS OF THE CYTOTYPES OF ASPLENIUM TRICHOMANES.

CYTOLOGICAL AND PHOTOGRAPHIC TECHNIQUES.

CYTOLOGICAL TECHNIQUES.

MEIOTIC SQUASHES

All meiotic squashes have been made using Aceto-Carmine. Fixation is effected in Acetic Alcohol (Glacial Acetic Acid 1: Absolute Alcohol 3), the young sporangia being removed from the frond by a scalpel prior to fixation. It was found to be helpful to the intensity of the stain to add a few drops of a solution of Ferric Acetate in Glacial Acetic Acid to the fixative. Fixed material was left overnight in fixative in order to harden before use.

A few sporangia are broken up in a drop of Aceto-Carmine (saturated solution of Carmine in 45% Acetic Acid), on a slide, and heated under a cover-slip until the Carmine bubbles gently. Considerable pressure is then applied to flatten the preparation, allowing no lateral movement of the cover slip. Preparations are subsequently made permanent according to the variant of McClintock's method described by Manton (22).

MITOTIC SQUASHES.

Mitotic squashes have been made from root-tips according to the method of Tjio and Levan (33). Root-tips are first treated for four hours in a saturated aqueous solution of 8-Hydroxy-Quinoline, and then fixed overnight in Acetic Alcohol. Root-tips are macerated and stained in a watch-glass with a freshly-made mixture of two drops of N/1 Hydrochloric acid and eighteen drops of Acetic Orcein (a 1% solution in 45% Acetic Acid). This mixture is heated until it bubbles, and is then left to cool. The root-tips are then mashed in a drop of fresh stain on a slide. Preparations are made permanent in the same manner as for meiotic squashes (see above).

PHOTOGRAPHIC TECHNIQUES

Preparations have been photographed on a horizontal camera-microscope combination apparatus at a magnification of X 1000, using a Watson 2 mm. N.A. 1.28 Panchromatic objective and a Watson X 7 Holoscopic eyepiece. Monochromatic green light is used, under conditions of critical point illumination. A "Point-o-lite" Lamp is used as the light source. Subjects of normal contrast are photographed on Ilford Special Rapid Panchromatic plates,

whereas Ilford Thin Film Half-Tone plates are used for poorly stained specimens. Development is usually effected in a one-third dilution of Ilford's ID2 formula, but other dilutions and other developers may be used. Analytical diagrams are made from X 2 enlargements on Matt Bromide paper by the method described by Manton (22).

CYTOLOGY OF THE THREE CYTOTYPES (Figs 94 - 104).

The basic chromosome number in Asplenium is x = 36, and the somatic chromosome numbers of the cytotypes of Asplenium trichomanes consist of different multiples of this number. Thus the diploid cytotype has 2n = 72 chromosomes, the tetraploid has 2n = 144, and the hexaploid has 2n = 216. The triploid hybrids occasionally found wild have 2n = 108. Aneuploidy does not appear to exist in these plants, for none of the considerable number of individuals cytologically examined in the course of this investigation has shown any departure from a straightforward multiple of the base number.

In all three cytotypes, meiosis is uniformly regular and only bivalent associations are formed, multivalents and univalents being absent. The sole exception to this rule is a single example of an apparent multivalent in one cell

from a plant of the hexaploid cytotype from the Hooker valley, Canterbury, N.Z, (see Figs 103a & 104). Although a considerable number of cells from hexaploid plants from various localities have been examined, no other such irregularity has been observed. All other hexaploid cells showed a completely regular meiosis with 108 bivalents, and thus to all intents and purposes, this plant is functionally a diploid.

Meiosis has been studied carefully in many plants of the tetraploid cytotype, but no sign of multivalent formation has been detected. Invariably, 72 bivalents are seen, so that this plant also is functionally a diploid.

It is thus found that meiosis in the tetraploid and hexaploid cytotypes provides no evidence of autopolyploid origin.

EXPERIMENTS DESIGNED TO TEST THE HYPOTHESIS OF AUTOPOLYPLOID ORIGIN OF THE TETRAPLOID CYTOTYPE.

Experiments were designed which, if successful, would give information which would materially assist in deciding whether or not the tetraploid cytotype is an ancient autotetraploid derived directly from the diploid cytotype by doubling of the chromosome complement. Since examination of meiosis in the tetraploid cytotype show multivalents to be absent, then if the hypothesis of autopolyploid origin is correct, the tetraploid must have evolved some type of genetic mechanism which either directly or indirectly inhibits the formation of multivalents. An autotetraploid of this type can aptly be termed a cryptic autopolyploid.

The presence in nature of a mechanism inhibiting multivalent formation is theoretically not unlikely, since should it once arise, natural selection would ensure its rapid increase in the population. A newly formed autopolyploid is likely to have very low fertility, since the formation of multivalents tends to depress fertility on account of irregular segregation at the first meiotic anaphase. Inhibition of multivalent formation will consequently raise fertility, and therefore any type of mechanism which reduces multivalent formation will be

favoured by natural selection. This mechanism may either be in the nature of a genic system directly inhibiting multivalent formation, or else be a consequence of genetic divergence between the two genomes of the reduced complement of the tetraploid producing a real loss of cytogenetic homology between the two genomes.

The basic aim in the design of the experimental procedures was to break down any system which might be preventing synapsis between the chromosomes of the two genomes represented in the reduced (gametophytic) complement. This was attempted in three quite different ways, described below.

1. The production of a diploid sporophyte from the tetraploid by induction of an apogamous sporophyte from a "tetraploid" prothallus. (Such a prothallus would in fact itself be diploid since it would carry the reduced gametophytic chromosome complement.) Meiosis in this sporophyte with a reduced chromosome number would be examined for any signs of bivalent formation.

This has been successfully accomplished in Asplenium scolopendrium* (Manton 22, as Scolopendrium vulgare), and for two species of Dryopteris (Manton and Walker, 23).

* Footnote overleaf.

However, there is no way of forcing a prothallus to produce an apogamous sporophyte. It is only possible to prevent a prothallus from self-fertilising itself, and to hope that the ageing prothallus will produce the desired apogamous sporophyte. Unfortunately, Asplenium trichomanes has not shown any signs of being amenable to the production of such abnormal sporophytes, all attempts having been unsuccessful.

2. The artificial induction of an autotetraploid plant of Asplenium trichomanes from the diploid cytotype, and the subsequent hybridisation of this plant with the natural tetraploid cytotype. Meiotic pairing would be analysed in the resultant hybrid.

*FOOTNOTE. Asplenium scolopendrium L.
Synonyms = Phyllitis scolopendrium (L.) Newman, Scolopendrium vulgare Sm.. Copeland (9), gives cogent reasons why the recognition of Phyllitis and Scolopendrium as genera distinct from Asplenium is not justified. The occurrence of Asplenophyllitis hybrids (Alston, 1), gives additional weight to his contention, since intergeneric hybrids are an anachronism, and should be interpreted as evidence that the two genera concerned are not truly distinct. In consequence the Linnean name of Asplenium scolopendrium is used here, although not at present generally accepted.

Two techniques can be applied in the attempted synthesis of an autopolyploid fern. One method is of special application to ferns, the other is of very general use.

The first method involves the induction of apospory in the detached first or second leaves from young sporophytes of the diploid cytotype. Aposporous prothallial outgrowths are thus obtained, and an autotetraploid could, in theory at least, be obtained from such prothalli by normal sexual processes. Aposporous prothalli can be quite readily induced in this way from the diploid cytotype, but these prothalli are abnormal in form, and it has not proved possible to raise one of these prothalli to maturity.

The second method entails the use of a spindle-arresting substance such as colchicine, deriving autopolyploid growths from the restitution nuclei formed as a consequence of its use. This technique has proved immensely valuable in work with flowering plant material, but has not yet been successfully applied to ferns.

In the present investigation, various periods of treatments have been applied to prothalli subsequent to self-fertilisation in the hope of affecting early divisions of the zygote, but with complete lack of success.

3. The synthesis of hybrids between the tetraploid cytotype of Asplenium trichomanes and other species of Asplenium with which it has no close affinity, followed by analysis of meiosis in the resultant hybrids.

Alston (1), reviewed the records of natural occurrences of such hybrids, and reaches the conclusion that though such hybrids are intensely rare, there is good reason to believe that such combinations as Asplenium trichomanes x ruta-muraria (=A.x clermontae Syme), and Asplenium trichomanes x scolopendrium have actually been found.

The attempt has been made to synthesise some such hybrids, but so far without success. This is perhaps not altogether surprising, since in the course of the hybridisation programme on this group of ferns it has been found that even hybrids which are relatively frequent in nature, and which one would expect could be synthesised with ease, are in fact only obtained with a low percentage of succes.

Details of hybrids attempted.

Female parent	Male parent	No.female used.	Hybrids obtained
A.trichomanes 4x	A.ruta-muraria	107	0
A.trichomanes 4x	A.scolopendrium	74	0
A.ruta-muraria	A.trichomanes 4x	18	0
A.scolopendrium	A.trichomanes 4x	7	0

GENETICAL RELATIONSHIPS (contd)

SYNTHESIS OF HYBRIDS BETWEEN ASPLENIUM ADULTERINUM AND CYTOTYPES OF ASPLENIUM TRICHOMANES, WITH ANALYSIS OF MEIOSIS (Figs 105 - 110).

ASPLENIUM ADULTERINUM X DIPLOID CYTOTYPE.

As is described in the Supplement to this thesis (See also Lovis, 20), a cytogenetic investigation of Asplenium adulterinum has shown that there is sufficient homology between the chromosomes of European plants of the diploid cytotype of Asplenium trichomanes and half of the chromosome complement of Asplenium adulterinum (which is a tetraploid species), for them to pair together at prophase of meiosis, forming 36 bivalents, in the triploid hybrid formed between these two plants. It is also known that the other chromosomes of Asplenium adulterinum are quite distinct, since these will pair completely with the chromosomes of Asplenium viride.

Since the affinities of both of the genomes present in Asplenium adulterinum are known, it is possible to use the chromosomes of the trichomanes - type genome in Asplenium adulterinum as a standard genome by which to compare the chromosomes of different strains of the diploid cytotype of Asplenium trichomanes. By determining the number of bivalents formed at meiosis in hybrids between different

forms of the diploid cytotype and Asplenium adulterinum, an indirect measure is obtained of the degree of cytological divergence existing between various strains of the diploid.

This information is of considerable value, since it is not possible to obtain a direct measure of divergence between strains of the diploid cytotype by hybridising them all with one another, since in the absence of suitable marker genes, there are no morphological characters by which to separate hybrids from self-fertilised plants.

In presentation of the results of the hybridisation experiments, figures are given of the number of females used, the number of hybrids obtained, the number of self-fertilised plants produced, and the number of sporophytes which died before their identity could be established. The ratio given, female prothalli used: hybrids produced, is obtained by correction of the number of prothalli used so as to exclude all prothalli which subsequently produced sporophytes by self-fertilisation and also those prothalli which produced sporophytes which died before they could be identified. The figure given for percentage success is calculated from this ratio, and corrected to the nearest 0.25% for values between 0% and 10%, and to the nearest

0.5% for values between 10% and 100%. The procedure just described is standard throughout the thesis from this point onwards.

A.ADULTERINUM X DIPLOID : EUROPE. (Fig.105, also 145 & 146).

Details of hybridisation attempts:

	No.♀.	H.	S.	D.	No.♀ : H
ADULTERINUM ♀ x EUROPE ♂	332	1	12	6	314 : 1
EUROPE ♀ x ADULTERINUM ♂	116	6	9	4	103 : 6
					417 : 7

Percentage success = 1.75%

Analysis of meiosis:

36 bivalents and 36 univalents always found.

A.ADULTERINUM X DIPLOID : ASIA. (Fig. 106).

Details of hybridisation attempts:

	No.♀.	H.	S.	D.	No.♀ : H
ADULTERINUM ♀ x ASIA ♂	91	11	12	7	72 : 11
ASIA ♀ x ADULTERINUM ♂	-	-	-	-	- -
					72 : 11

Percentage success = 15%

Analysis of meiosis:

36 bivalents and 36 univalents always found.

A.ADULTERINUM X DIPLOID : NORTH AMERICA (Fig.107).

Details of hybridisation attempts:

	No.♀.	H.	S.	D.	No.♀ : H.
ADULTERINUM ♀ x N.AMERICA ♂	5	2	1	2	2 : 2
N.AMERICA ♀ x ADULTERINUM ♂	23	5	2	0	21 : 5
					23 : 7

Percentage success = 30%

Analysis of meiosis:

36 bivalents and 36 univalents always found.

A.ADULTERINUM X DIPLOID : AUSTRALIA (Fig.108).

Details of hybridisation attempts:

	No.♀.	H.	S.	D.	No.♀ : H.
ADULTERINUM ♀ x AUSTRALIA ♂	65	0	8	1	56 : 0
AUSTRALIA ♀ x ADULTERINUM ♂	98	1	4	4	90 : 1
					146 : 1

Percentage success = 0.75%

Analysis of meiosis:

Only a limited number of exact analyses have yet been obtained. These indicate that only 32 or 33 bivalents are formed, leaving 44 or 42 chromosomes unpaired, which appear as univalents.

ASPLENIUM ADULTERINUM X TETRAPLOID CYTOTYPE.

Some hybrids have also been obtained between forms of the tetraploid cytotype of Asplenium trichomanes and Asplenium adulterinum.

A.ADULTERINUM X TETRAPLOID : EUROPE. (Fig.109).

Details of hybridisation attempts:

	No.♀.	H.	S.	D.	No.♀ : H.
ADULTERINUM ♀ x EUROPE ♂	137	14	6	14	117 : 14
EUROPE ♀ x ADULTERINUM ♂	36	1	6	2	28 : 1
					145 : 15

Percentage success = 10%

Analysis of meiosis:

III	II	I
3	32	71
3	33	69

Therefore approximately 36 bivalents and 72 univalents are found, modified by the presence of a few trivalents.

A.ADULTERINUM X TETRAPLOID : N.AMERICA. (Fig.110)

Details of hybridisation attempts:

	No.♀.	H.	S.	D.	No.♀ : H.
ADULTERINUM ♀ x N.AMER ICA ♂	-	-	-	-	- : -
N.AMERICA ♀ x ADULTERINUM ♂					34 : 2
					34 : 2

Percentage success = 6%

Analysis of meiosis:	III	II	I
	2	34	70
	2	33	72

Therefore approximately 36 bivalents and 72 univalents are found, modified by the presence of a few trivalents.

GENETICAL RELATIONSHIPS (contd)

SYNTHESIS OF HYBRIDS BETWEEN THE CYTOTYPES OF ASPLENIUM TRICHOMANES, AND ANALYSIS OF MEIOSIS (Figs 111 - 130).

As will become apparent, chromosome pairing in these hybrids is rather complex and variable. In consequence, it is difficult to obtain cells with their chromosomes separated so perfectly as to enable a complete and unequivoca analysis of chromosome pairing to be made. For this reason, the cytological results for most hybrids are dependent on a very few analyses, and for four hybrids only a single analysis is given. However, for each one of these hybrids several analyses have been made which though themselves imperfect, demonstrate that the single complete analysis is typical.

DIPLOID X TETRAPLOID (= TRIPLOID HYBRIDS).

Two main series of synthetic triploid hybrids have been attempted. The first is a series of hybrids of all the available diploid stocks with a single tetraploid stock. The second series is complementary to the first, being a series of hybrids of all the available tetraploid stocks with a single diploid stock.

VARIOUS DIPLOIDS X TETRAPLOID : EUROPE.

DIPLOID : EUROPE X TETRAPLOID : EUROPE (Figs 111 - 116).

Details of hybridisation attempts:

	No.♀.	H.	S.	D.	No.♀	:	H
2x EUROPE ♀ X 4x EUROPE ♂	95	22	1	3	91	:	22
4x EUROPE ♀ X 2x EUROPE ♂	108	41	19	7	92	:	41
					183	:	63

Percentage success = 34.5%

In Europe, the diploid and tetraploid cytotypes not infrequently grow together, and where this happens, wild triploid hybrids may be found. In the course of investigation, wild triploid hybrids have been detected from four localities. These localities are:- 1. Tyn-y-Groes, near Dolgelley, Merioneth, 2, Dunkeld, Perthshire, 3, Mundheim, Hardanger district, Norway, and 4, Ronco, Tessin, Switzerland.

Analyses of meiosis:	IV	III	II	I
Tyn-y-Groes, Merioneth			36	36
" "		1	34	37
" "		4	31	34
" "	1	0	34	36
" "	1	2	34	30

Analysis of meiosis: (contd).	IV	III	II	I.
Mundheim, Norway.		1	36	33
" "		2	34	34
" "		4	32	32

DIPLOID : ASIA X TETRAPLOID : EUROPE (Fig.117).

Details of hybridisation attempts:

	No.♀.	H.	S.	D.	No.♀ : H
2x ASIA ♀ x 4x EUROPE ♂	74	9	2	4	68 : 9
4x EUROPE ♀ x 2x ASIA ♂	30	11	7	0	23 : 11
					91 : 20

Percentage success = 22%

Analysis of meiosis:	III	II	I
	1	35	35
	2	33	36
	2	33	36
	2	35	32

DIPLOID : NORTH AMERICA X TETRAPLOID : EUROPE. (Fig.118).

Details of hybridisation attempts:

	No.♀.	H.	S.	D.	No.♀ : H
2x N.AMERICA ♀ x 4x EUROPE ♂	48	14	3	10	35 : 14
4x EUROPE♀ x 2x N.AMERICA ♂	27	8	0	3	24 : 8
					59 : 22

Percentage success = 36.5%

Analyses of meiosis:	III	II	I
		35	38
	2	34	34
	2	34	34

DIPLOID : AUSTRALIA X TETRAPLOID : EUROPE (Fig.119)

Details of hybridisation attempts:

	No♀.	H.	S.	D.	No.♀ : H
2x AUSTRALIA ♀ x 4x EUROPE ♂	102	2	2	0	100 : 2
4x EUROPE ♀ x 2x AUSTRALIA ♂	72	0	12	0	60 : 0
					160 : 2

Percentage success = 1.25%

Analyses of meiosis:	II	I
	33	42

VARIOUS TETRAPLOIDS X DIPLOID : EUROPE.

TETRAPLOID : EUROPE X DIPLOID : EUROPE.

See page 101 , and figs 111-116.

TETRAPLOID : NORTH AMERICA X DIPLOID: EUROPE (Fig.120).

Details of hybridisation attempts:

	No.♀.	H.	S.	D.	No.♀ : H
4x N.AMERICA ♀ x 2x EUROPE ♂	48	4	11	3	34 : 4
2x EUROPE ♀ x 4x N.AMERICA ♂	25	0	5	0	19 : 0
					53 : 4

Percentage success = 7.5%.

Analysis of meiosis:

III	II	I
1	35	35

TETRAPLOID : SOUTH AFRICA X DIPLOID : EUROPE (Fig.121).

Details of hybridisation attempts:

	No.♀.	H.	S.	D.	No.♀:
4x SOUTH AFRICA ♀ x 2x EUROPE ♂	35	10	2	4	29 :
2x EUROPE ♀ x 4x SOUTH AFRICA ♂	-	-	-	-	- :
					29 :

Percentage success = 34%.

Analysis of meiosis:

III	II	I
1	35	35

TETRAPLOID : HAWAII X DIPLOID : EUROPE (Fig.122).

Details of hybridisation attempts:

	No.♀.	H.	S.	D.	No.♀ : H
4x HAWAII ♀ x 2x EUROPE ♂	102	29	2	7	93 : 29
2x EUROPE ♀ x 4x HAWAII ♂	-	-	-	-	- -
					93 : 29

Percentage of success = 31%.

Analyses of meiosis:	III	II	I
	1	36	33
	2	35	32

TETRAPLOID : AUSTRALIA X DIPLOID : EUROPE. (Fig.123)

Details of hybridisation attempts:

	No.♀.	H.	S.	D.	No.♀. : H
4x AUSTRALIA ♀ x 2x EUROPE ♂	-	-	-	-	- -
2x EUROPE ♀ x 4x AUSTRALIA ♂	13	1	1	6	6 : 1
					6 : 1

Percentage success = 13.5%

Analysis of meiosis:	III	II	I
	1	35	35

TETRAPLOID : JAPAN X DIPLOID : EUROPE. (Fig.124).

Details of hybridisation attempts:

	No.♀.	H.	S.	D.	No.♀ : H
4x JAPAN ♀ x 2x EUROPE ♂	99	0	22	11	66 : 0
2x EUROPE ♀ x 4x JAPAN ♂	27	20	4	5	23 : 20
					89 : 20

Percentage success : = 22%

Analyses of meiosis:	III	II	I
	1	35	35
	2	32	38

DIPLOID X HEXAPLOID (=TETRAPLOID HYBRIDS)

DIPLOID : EUROPE X HEXAPLOID : NEW ZEALAND. (Figs.125-127)

Details of hybridisation attempts:

	No.♀.	H.	S.	D.	No.♀	: H
2x EUROPE ♀ x 6x N.Z. ♂	-	-	-	-	-	-
6x N.Z. ♀ x 2x EUROPE ♂	50	11	1	11	38	: 11
					38	: 11

Percentage of success:=28%

Analyses of meiosis:	III	II	I	III + II
		55	34	55
	4	48	36	52

continued overleaf.

Analyses of meiosis:(contd)	III	II	I	III + II
	3	45	45	48
	3	44	47	47
	1	46	49	47

TETRAPLOID X HEXAPLOID (= PENTAPLOID HYBRIDS)

TETRAPLOID : EUROPE X HEXAPLOID : NEW ZEALAND. (Figs 128-130)

Details of hybridisation attempts:

	No.♀	H.	S.	D.	No.♀. : H
4x EUROPE ♀ x 6x N.Z. ♂	27	24	2	1	24 : 24
6x N.Z. ♀ x 4x EUROPE ♂	26	13	0	2	24 : 13
					48 : 37

Percentage success = 79%

Analyses of meiosis:	IV	III	II	I	III + II
		2	71	32	73
	1	7	62	31	70
		5	64	37	69
		3	64	43	67

DIPLOID X DIPLOID.

It has generally not been possible to make hybrids

between forms on the same level of polyploidy because of the difficulty in distinguishing the resulting hybrids from self-fertilised plants. However, as is indicated below, it has fortunately been found that hybrids produced by crosses between the European and Australian diploids can be distinguished with certainty from the parents.

DIPLOID : EUROPE X DIPLOID : AUSTRALIA.

Details of hybridisation attempts:

	No.♀.	H.	S.	D.	No.♀ : H
2x EUROPE ♀ x 2x AUSTRALIA ♂	-	-	-	-	- : -
2x AUSTRALIA♀ x 2x EUROPE ♂	82	7	5	7	70 : 7
					70 : 7

Percentage success = 10%

Analysis of meiosis:

Meiosis appears normal, 36 bivalents usually being found, but there is some evidence of occasional univalents.

Other characteristics:

Although meiotic pairing is normal, and tetrads of normal appearance are formed, the spores abort during the last stages of development (see fig. 93b). The probable reason for this sterility is considered later, during the Discussion (p.134).

A further notable feature of these hybrids is very obvious positive heterosis, in total contrast to the parents, which are maintained in cultivation only with difficulty.

DISCUSSION & CONCLUSIONS.

In the preceding pages of this thesis, a considerable body of facts have been presented relating to the various sections of the investigation. It is now necessary to discuss the interpretation of this evidence, and to attempt correlation of the several aspects of this study. Much of the evidence, particularly that relating to the cytogenetical investigation, is difficult to interpret, and it may not always be possible for final conclusions to be drawn. In order to keep this section of the thesis within reasonable proportions, the arguments involved will be presented as concisely as possible, and discussion will be restricted to matters strictly relevant to the actual investigation, avoiding any lengthy consideration of the theoretical concepts which are involved.

The main body of this discussion will be divided into two sections. In the first section, the facts of geographical distribution and of ecology will be discussed. The second section concerns the cytogenetical investigation.

DISCUSSION AND CONCLUSIONS (contd)

GEOGRAPHICAL DISTRIBUTION AND ECOLOGY.

DISCUSSION.

Earlier in this thesis (pp. 14 - 24), the very extensive ranges over the surface of the world possessed by the diploid and tetraploid cytotypes were described. Their distributions are also illustrated in figures 1 - 7. Such extremely wide distributions are the exception rather than the rule amongst the ferns. Indeed there are very few ferns which show the same type of extreme range over the temperate regions of the world as do the diploid and tetraploid cytotypes of Asplenium trichomanes. Most nearly comparable are Cystopteris fragilis s.l., Asplenium adiantum-nigrum s.l., and Anogramma leptophylla. The first two are both aggregate species; the last-named species belongs to a unique genus with perennial prothalli, but producing sporophytes which are only annual.

It is relevant here to emphasise that the same principles of plant geography which are valid for the flowering plants are also applicable to the ferns. It is not true that ferns are ubiquitously and rapidly distributed over long distances by wind-dispersal of their spores. Indeed the ferns show the same individuality in the distribution of the species as do the flowering plants, characteristic fern floras being

found in different vegetational and geographical regions. For example, long distance wind-dispersal of the disseminules is probably hardly any more important in the distribution of the ferns than it is in the distribution of the Orchidaceae.

The question arises as to how the diploid and tetraploid cytotypes acquired their present very wide distributions. In consideration of this problem, it is first necessary to decide whether or not man can have played a significant although involuntary role in the dispersal of these two plants. We shall see that the evidence appears to demonstrate conclusively that such has not been the case.

In relation to the distribution of these plants in the Pacific region, it should first be emphasised that these plants can in no way be regarded as comparable to the pan-tropical weeds demonstrated by Merrill (25), to have been widely distributed over the Pacific by human agency in pre-European times. It seems probable that none of the various races believed to have migrated through this part of the world in prehistoric times originated from regions where these ferns are found today, and moreover the general distribution and the nature of the habitats of these ferns in the Pacific region make such accidental pre-European human interference improbable in the extreme. We can therefore consider that the earliest possible date for

introduction by man of these ferns into various parts of the Pacific region is the date of first European penetration.

With regard to the status of the tetraploid cytotype in the Pacific region, very valuable information is obtained from the collections of David Nelson, now incorporated in the British Museum Herbarium. David Nelson accompanied Cook on his third voyage, (1776 - 1780), and specimens of the tetraploid cytotype collected by him on this voyage exist labelled "Owhyee" (=Hawaii), and "Nutka, N.W.coast of America" (Vancouver Island). In Australia, the tetraploid cytotype was collected by von Mueller at Buchan in Gippsland in 1853, at which date this part of eastern Victoria had only been settled by Europeans for a few years. There can be no doubt that the presence of the tetraploid cytotype in these three places is in no way related to the spread of European influence, and that this plant is truly indigenous in these localities.

The tetraploid must also be native in its stations near Sada in the Yemen, on Mt. Wagar in Somaliland, on Mt. Longonot in Kenya, and on Mont-aux-Sources in the Natal Drakensberg, all of which are isolated localities at high altitudes and in the most remote places.

We may conclude that there would appear to be no

grounds for considering man to have played any significant part in the dispersal of the tetraploid cytotype over its immense world range.

Concerning the diploid cytotype, there is no reason to doubt that it is native in Europe, the Himalayan region, and China. It is known from a few localities in south-east Australia. In one of these, Mt. Buffalo, it was collected at a very early date, 1853, by von Mueller. All the other known localities of this plant in the south-west Pacific are in extremely remote places at great altitudes. In New Guinea, it is known from the Owen Stanley Ranges between 9,000 and 12,000 feet. It has been found on Mt.Tatamailau, in Timor, at 9,000 feet, and on Mt. Rindjani, in Lombok, at 6,500 feet. It is difficult to conceive of any three localities in this region which are more remote from the influence of European colonisation. There is then no reason to believe that the remarkable distribution of the diploid cytotype has any other than a natural origin.

Since the distributions of diploid and tetraploid cytotypes are natural, and cannot be explained in terms of recent long distance wind-dissemination of spores, it follows that both the diploid and tetraploid cytotypes must be of ancient origin, a conclusion of considerable influence.

The problem of how these two plants acquired their present distribution can probably now never be entirely solved. It is however possible with considerable confidence to interpret the present localities of both diploid and tetraploid cytotypes in the Tropics and in the Southern Hemisphere as being relics of a former more continuous distribution during certain phases of the Pleistocene period.

In order to substantiate this last assertion, it is necessary to consider briefly the conditions and climatic changes during the Pleistocene period. The broad outline of events during the Pleistocene in the Northern Hemisphere is well known, and the profound effect which the great glacial advances had upon the vegetation is generally accepted, but it is not always realised that the occurrence of extended glaciation in the Pleistocene was by no means solely a Northern Hemisphere phenomenon. In the Southern Hemisphere the glaciation of the Antarctic continent waa more extreme than at present, and alpine glaciations of considerable extent occurred in the southern Andes, in Tasmania, and in New Zealand. (See Flint, 13, 14).

There is also ample evidence of a marked lowering of the snowline and increased glaciation on tropical mountains. In East Africa, the great mountains of Kilimanjaro (19,700'),

Mt. Kenya (17,100'), and Ruwenzori (16,900'), all show evidence of former glaciation at least as low as 10,000 feet, in other words, at least 3,000 feet lower than the existing snowline on these mountains (Nilsson, 28). In Hawaii, the volcano Mauna Kea (13,780'), was glaciated down to 10,500 feet (Stearns, 29). There is also evidence in the form of cirques of former glaciation in the high mountains of New Guinea, where today only a single ice-cap is found, on Ngga Poeloe (16,600'), (Dozy, 11).

In many parts of the world not directly affected by glacial conditions, pluvial phases occurred, identifiable today by the existence of saline basins, and by evidence that existing lakes were of far greater extent. These pluvial phases, which were probably synchronous with the glacial phases, were characterised by cooler, wetter conditions brought about by decrease in evaporation and by increase in the level of precipitation (Flint 13, 14). There is evidence of pluvial conditions during some part of the Pleistocene in two regions which are of great importance for this discussion. Nilsson (28), has described the former expansion of the great East African lakes, and information concerning the Lake Eyre system of South Australia is given by David (10).

Further evidence of a totally different kind indicating that a lowering of temperature did occur at certain times in the Pleistocene, not only in regions directly affected by glaciation, but also elsewhere, and moreover, in equatorial latitudes, has been obtained by study of foraminifera in cores taken from equatorial deep sea sediments (Emiliani, 12).

In view of all these facts, there would appear to be little reason to doubt that temperate species would have been able to exist at lower altitudes in tropical latitudes during certain phases of the Pleistocene than they can today. In tropical regions where Asplenium trichomanes s.l., is now confined to high altitudes on isolated mountains, it must surely have occurred in many more localities and at lower altitudes during the pluvial phases than at present. One may thus deduce a former chain of localities of the tetraploid cytotype along the main African mountain chain. Equally, the diploid cytotype must have been more widespread and more continuously distributed in the mountains of New Guinea and Indonesia.

In Australia, glacial conditions are known to have occurred during some part of Pleistocene time in Tasmania and on the Kosciusko plateau of New South Wales. Elsewhere

on the Australian continent there is clear evidence of the existence of pluvial conditions in South Australia in the compounded expansion of the Lake Eyre system to form the great Pleistocene Lake Dieri. Fossil bones of giant herbivorous marsupials (e.g. Diprotodon, Nototherium), comparable in size to the rhinoceros have been found here in the lake sediments (see David, 10). Clearly,the vegetational cover of this region must have been utterly different when these animals were alive in comparison with the present.

In Australia today, the tetraploid cytotype occurs not only on limestone rocks in the temperate parts of the eastern states, but also in Western Australia, where however it is restricted to a very few localities in coastal limestone in the extreme south-west, being found here nowhere more than a few miles from the sea. All but one of these localities in Western Australia are on limestone rock formed during the Pleistocene period, and presumably these stations were not colonised until the end of the Pleistocene period at the earliest, or else in Recent time. However, the colony at Mt. Many Peaks is on limestone of Miocene age, and it is reasonable to presume that during the pluvial phases, the distribution of this plant must

have been effectively continuous across Australia from west to east, with stations for the plant throughout the Miocene limestones of the Nullarbor Plain.

The distributions of the diploid and tetraploid cytotypes today represent the result of the influences of the glacial and pluvial phases of the Pleistocene in both Northern and Southern Hemispheres, together with subsequent climatic changes in Recent times. The fluctuations of the Pleistocene period undoubtedly produced the most drastic phytogeographic changes, and there can have been no lack of opportunity for many temperate fern species in both hemispheres to radically extend their range along mountain ranges in the direction of the equator.

Why then are the extensive ranges of the diploid and tetraploid cytotypes so unusual amongst the ferns? The answer to this problem must largely rest in the relative ability of a plant to maintain an expanded range not merely in the face of subsequent regional climatic change, but also in spite of the ecological and vegetational changes that will inevitably accompany such a change in climate. In this respect, the peculiar habitat of the cytotypes of Asplenium trichomanes may be of importance, since an exposed rock-face is much the same the whole world over. For

example, one limestone rock is not necessarily intrinsically very much different from another on the other side of the world. Should aspect and climate be comparable, the ecological conditions presented will be very alike. Indeed, the habitat characteristics preferred by the diploid and tetraploid cytotypes in Australia are extraordinary similar to those obtaining where the same cytotypes are found in Britain. (Compare figs 11 and 14, figs 22-23 and 34).

The surmise that the type of habitat occupied by the diploid and tetraploid cytotypes of Asplenium trichomanes is an important factor in their successful maintenance of their extremely wide geographical ranges is considerably strengthened by the fact that two of the other ferns mentioned as having very extensive ranges occupy very similar habitats. These are Asplenium adiantum-nigrum s.l., and Cystopteris fragilis s.l., both of which may be found in Britain in association with the tetraploid cytotype of Asplenium trichomanes.

As is illustrated in the scatter diagrams (figs 85-88), and in the field photographs (figs. 20 & 23, 34 & 38, 43-45, 48 & 50), the cytotypes of Asplenium trichomanes possess remarkable plasticity of form in response to different climatic conditions. This capacity of a single individual

to display a great range of phenotypic expression in response to variation in its environment has been termed "phenotypic flexibility" by Thoday (32), and has been argued by him to be an important component of fitness. It seems very possible that the extreme plasticity or phenotypic flexibility possessed by these plants may have been a significant contributory factor in the maintenance of their extremely wide distributions.

A further factor of potential importance, but one concerning which we have no evidence, is the degree of genetic homeostasis possessed by these plants. By this term is usually understood the capacity of a population of plants to maintain over numerous generations an optimal internal physiological balance in spite of the pressure of fluctuating environment changes. This is obviously an important property to be possessed by a species if it is to succeed in persisting in a particular station throughout a period of great climatic fluctuation. Unfortunately we have no knowledge of the extent to which the cytotypes of Asplenium trichomanes possess this faculty.

It remains only to consider the comparative aspect of the ecology of the three cytotypes of Asplenium trichomanes. An obvious and significant feature is their different

tolerance of increasing aridity of the habitat, for increase in level of polyploidy is found to be correlated with tolerance of drier habitat conditions. A study of the distributions of the diploid and tetraploid in Britain shows that the tetraploid can flourish in regions of lesser rainfall wherein the diploid cannot grow. Similarly, comparative study of the tetraploid and hexaploid cytotypes in Australia and New Zealand has clearly demonstrated that the hexaploid can grow in more arid conditions than the tetraploid can tolerate.

A comparison of the edaphic tolerances of the three cytotypes, based on personal observations in Europe and Australia, is also interesting. The diploid cytotype is found on a variety of rock-types, including some basic rock-types, but never on calcium-rich rocks. In contrast, the tetraploid is predominantly a plant of limestone, although found on other basic rocks. The hexaploid to a certain degree combines the preferences of the other two cytotypes, for it is found not only on limestones and mica-schist, but also on Greywacke, a base-poor rock.

With its greater tolerance of aridity, and more generalised edaphic preference, the hexaploid would appear to be superficially better equipped than the other two

cytotypes successfully to meet a range of habitat conditions. For this reason, it seems improbable that the hexaploid is found only in New Zealand as a last refugium. It is more likely that this plant is of relatively recent origin within New Zealand, and that the relative scarcity of the tetraploid there is attributable to it having been replaced in its stations by the better fitted hexaploid. This however is only speculation, for this hypothesis lacks any direct supporting evidence.

CONCLUSIONS.

The very wide world distributions of both diploid and tetraploid cytotypes are natural in origin, and are not attributable to any significant extent to accidental dispersal by man. It follows that both plants are of ancient origin, and it is believed that both were more continuously distributed through certain parts of their ranges during the glacial and pluvial phases of the Pleistocene period than they are today. Possible reasons why these ferns are exceptional in possessing such extremely extensive distributions are discussed.

A comparative analysis of the autecology of the three cytotypes shows that there is a correlation in these plants

between increased level of polyploidy and increased tolerance of dry habitat conditions, and also that the hexaploid to a certain extent combines the edaphic preferences of the other two cytotypes. In consequence, it is considered to be less probable that the hexaploid is present in New Zealand only as its last refugium than that it has originated there relatively recently.

DISCUSSION AND CONCLUSIONS (contd)

GENETICAL RELATIONSHIPS OF THE THREE CYTOTYPES.

INTRODUCTION TO THE DISCUSSION.

In this investigation, the pairing of chromosomes at meiosis in hybrids has been studied as a source of information concerning cytological relationships. This technique has been much used in studies of polyploidy and the like, and has proved extremely valuable, but it is subject to certain limitations which must be clearly indicated.

Stated precisely, the basic assumption invoked in the interpretation of these studies is that the degree of synapsis which occurs at meiosis between the chromosomes of two genomes may be interpreted as an approximate measure of the degree of comparative homology between these two genomes.

Considered literally in this form, the assumption is surely not exceptionable. However, should two genomes synapse completely, it is not justifiable to state that the two genomes are therefore homologous, since all that has been demonstrated is that they are sufficiently homologous cytologically to enable all the chromosomes to find partners in the zygotene stage of meiosis. It clearly does not mean that there is complete cytological homology,

and even less does it mean total genetic homology.

It is evident that unless great precision is exercised in the choice of language for description of this type of cytogenetic work, some confusion or ambiguity may result, especially since the term homologue is generally used to describe one chromosome's meiotic partner in the corresponding genome. I therefore propose to introduce the phrase "synaptic homology", on the grounds that no exact alternative exists in general usage, and that use of this phrase, once defined, will lend greater precision to this discussion. The introduction of a new term is considered to be justified, since in this context the word homology is subject to different possible interpretations. Where two genomes in a hybrid pair completely, they may be said to show complete "synaptic homology". It follows that where two genomes pair incompletely, they may be said to show partial "synaptic homology".

It is necessary to use considerable caution in discussing the parentage of allopolyploids. The study of chromosome pairing is very generally used in order to investigate the relationships of polyploids. The example may be considered of two distinct diploids, each found to possess complete synaptic homology with a different genome

present in a certain tetraploid. This does not necessarily mean that the two diploid stocks used in the experimental investigation are in fact the diploid parents of the allotetraploid. It may however be taken to indicate that two plants essentially closely related to this pair of diploids were the actual parents of the tetraploid.

On the basis of such evidence, Asplenium viride and the diploid cytotype of Asplenium trichomanes are referred to as the apparent parents of Asplenium adulterinum. This is admittedly a rather loose description of the facts, but what is meant by this phrase is that it is known that two diploids bearing genomes essentially the same (at least in terms of synaptic homology), as the genomes of Asplenium viride and the diploid Asplenium trichomanes were the actual parents of Asplenium adulterinum. The phrase "apparent parents" is more easily comprehended, but is slightly inaccurate.

Before it can be permissible to be more positive or dogmatic concerning the relationship of these two diploids to Asplenium adulterinum, it will be necessary to produce an artificial allotetraploid between them, and to be able to demonstrate that this synthesised plant is effectively identical with Asplenium adulterinum. Only then will it

be legitimate to refer to Asplenium viride and the diploid cytotype of Asplenium trichomanes as the parents of Asplenium adulterinum.

DISCUSSION

ORDER OF DISCUSSION.

Much of the cytogenetical information obtained during the course of this investigation is rather complex. In consequence, the interpretation of the evidence is correspondingly difficult, and as will be seen, it is not always possible to make a final decision as to the correct explanation of the facts.

In order that this discussion shall be as straightforward in procedure as possible, it has been sub-divided under six headings, under which are considered in turn the six main problems which require solution.

These six problems, in the order in which they will be discussed, are:-

1. Are all the diploid forms investigated essentially the same?
2. Are all the tetraploid forms investigated essentially the same?
3. How much divergence exists between the diploid forms?

4. How much divergence exists between the tetraploid forms?
5. What is the nature of the ancestral genetical relationship between the diploid and tetraploid cytotypes?
6. What is the nature of the ancestral genetical relationship between the hexaploid and the diploid and tetraploid cytotypes?

1. ARE ALL THE DIPLOID FORMS INVESTIGATED ESSENTIALLY THE SAME?

By "essentially the same" it is meant that they still belong to the same broad genetic unit, in a sense of a common genetic origin.

It is clear from the evidence obtained from the series of hybrids between the various diploid forms and Asplenium adulterinum that all four diploid forms investigated are essentially the same, since all show more or less complete synaptic homology with the "trichomanes" genome in Asplenium adulterinum. It is possible to make this statement with confidence, since the nature of the unpaired genome in this series of triploid hybrids is known, being the "viride" genome derived from Asplenium adulterinum. This illustrates well the vital importance for the

investigation of the series of hybrids with Asplenium adulterinum, because the cytological analyses from the comparable series of hybrids between the various diploid forms and a single tetraploid cytotype are not conclusive, since in this latter case there is no guarantee that in each of the triploid hybrids it is the same genome from the tetraploid which pairs with the diploid genome, whereas the information from the adulterinum series of hybrids is not subject to this objection.

2. ARE ALL THE TETRAPLOID FORMS INVESTIGATED ESSENTIALLY THE SAME?

With regard to this problem the cytogenetical evidence is inconclusive. All that can be said on the basis of the series of hybrids of various tetraploid forms against a single diploid is that the meiotic pairing is approximately the same in all these triploid hybrids.

It might be considered likely that all the tetraploid forms investigated are essentially genetically alike, but this need not necessarily be so. The Japanese form of the tetraploid is morphologically distinct from all the other tetraploid forms investigated, although these others, which may collectively be termed the "typical" form, are themselves

all extremely alike. This Japanese plant is therefore the most likely to be of separate origin.

For the sake of clarifying the point under consideration, and without prejudice to the later discussion of the relationship between the diploid and tetraploid cytotypes, let it be assumed for a moment that in all cases the bivalents found in the triploid hybrids are formed by pairing between the genome supplied by the diploid parent and one of the genomes supplied by the tetraploid parent. Let it further be assumed that the genomes of the diploid are AA, and those of the typical tetraploid are AABB. In these circumstances, the Japanese tetraploid could conceivably be AACC, without doing violence to the available cytogenetic evidence. In other words, in the absence of any information concerning the nature of the second tetraploid genome, it is quite possible for the typical tetraploid and the Japanese tetraploid to be two different allotetraploids, or for that matter, for one of them to be an autopolyploid. Thus, on the limited cytogenetic information available, we are not able to exclude the possibility that the Japanese tetraploid is of totally distinct origin.

All the available cytogenetical and morphological evidence suggests that all the "typical" forms of the tetraploid (i.e. exclusive of the Japanese plant) are in fact essentially the same, and subsequently in the course

of this discussion, in order to simplify the argument, it will be assumed that this is in fact true. Nevertheless it must be clearly pointed out here that this is an assumption, and that we lack sufficient evidence to demonstrate that the assumption is correct. However, in view of the considerations above it would clearly be unwise to include the Japanese form within this assumption, and therefore future discussion will be based solely on consideration of the typical tetraploid form, to the exclusion of this Japanese plant, which could conceivably be of quite distinct origin.

3. HOW MUCH DIVERGENCE EXISTS BETWEEN THE DIPLOID FORMS?

The evidence of the series of hybrids with Asplenium adulterinum (pp. 94 - 97 , figs 105-108), clearly shows that the forms of the diploid cytotype from Europe, N.America, and Asia, are all very alike. All show complete synaptic homology with the "trichomanes" genome of Asplenium adulterinum, 36 bivalents being formed in these triploid hybrids.

It is equally clear that the Australian diploid shows some degree of divergence from the typical diploid form. This is indicated by both cytological and genetical evidence.

In the hybrid with Asplenium adulterinum only 32 or 33 bivalents are formed, in contrast with the 36 bivalents found in the other hybrids in this series. There is evidently some slight loss of synaptic homology. The hybrid with the European tetraploid seems also to show less pairing than other hybrids of that series (pp. 101-103, figs 111-119), but in this case the analysis is less certain, since it is based on inadequate evidence.

In relation to the divergence of the Australian diploid, the percentages of success obtained in the hybridisation experiments are of considerable interest (figs 131-132). Hybrids involving the Australian diploid proved very much more difficult to obtain than hybrids involving any other form of the diploid cytotype. Indeed, in the experiments involving synthesis of triploid hybrids between all forms of the tetraploid and diploid cytotypes, the overall percentage success using the Australian diploid was 1.25%, in contrast to the 25% success obtained in experiments involving the other three diploid forms used, a factor of X 20. This result would seem to provide evidence of genetic divergence as between the Australian diploid and the other diploid forms, inasmuch as the Australian form is clearly less compatible with the tetraploid genotypes

than are the other diploids.

Evidence of the greatest importance is provided by the hybrid between the Australian and European diploids, which displays genic sterility in the F1 generation. Meiosis appears to be virtually normal, only one or two occasional univalents being found, but the resulting spores are abortive (p. 108 , fig. 93b). Presumably the diploid hybrid nucleus owes its viability to the presence of one complete genome from each parent. Whereas the independent assortment of chromosomes at meiosis results in genic unbalance owing to each tetrad nucleus receiving two part-genomes, which evidently do not comprise a combination which is sufficiently harmonious to be viable.

4. HOW MUCH DIVERGENCE EXISTS BETWEEN THE TETRAPLOID FORMS?

Apart from the special case, discussed above (p. 130), of the Japanese form, which is morphologically distinct, there is no positive evidence of divergence amongst the various tetraploid forms. This by no means demonstrates that such divergence does not exist. The cytogenetical investigation gives no certain information with regard to this question, and the argument concerning the Japanese tetraploid applies equally here, i.e. the possibility cannot

be excluded that one of the tetraploid forms studied is really of quite distinct origin to the others.

5. WHAT IS NATURE OF ANCESTRAL GENETICAL RELATIONSHIP BETWEEN THE DIPLOID AND TETRAPLOID CYTOTYPES?

It has been established that all the diploid forms investigated are essentially similar, at least in origin. We may also assume similar fundamental uniformity for all the typical tetraploid forms investigated, excepting only the distinctive Japanese form, which is excluded from this section of the discussion. It will therefore be possible to simplify this section of the discussion by referring to the diploid cytotype and the tetraploid cytotype as two units.

The only direct evidence concerning the relationship between the diploid and tetraploid cytotypes is obtained from a study of meiotic pairing in the triploid hybrids formed between them. A number of such hybrids have been cytologically studied, and analyses generally show approximately 36 bivalents and 36 univalents, modified by the presence of from one to four trivalents. Rarely, an apparent quadrivalent is seen.

The most likely interpretation is that the genome provided by the diploid synapses more or less completely

with one of the genomes supplied by the tetraploid. The obvious explanation of this complete synaptic homology is that some form of the diploid cytotype is part-parental to the tetraploid. However, this explanation is not necessarily entirely correct, since it presupposes that the tetraploid is of allopolyploid origin, whereas the close morphological resemblance between diploid and tetraploid raises the possibility of autopolyploid origin.

The most straightforward method of obtaining an unequivocal answer to this problem would be acquired by study of meiosis in a sporophyte with the reduced (gametophytic) chromosome number resulting from the tetraploid cytotype by apogamy. Right from the commencement of the investigation, the extreme importance of such a reduced "tetraploid" sporophyte has been recognised, but regrettably it has not been possible to produce such a plant.

The absence of multivalents in the tetraploid cytotype does not preclude an autopolyploid origin, since either the two genomes of the reduced complement of the autotetraploid may have diverged under the pressure of natural selection, or else some genic system may have been evolved, inhibiting multivalent formation. Either event could conceivably

occur, but probably not both.

A study of the distributions of the diploid and tetraploid cytotypes shows that both of these plants must be of relatively ancient origin (see p.114). In view of its antiquity the tetraploid, if of autopolyploid origin, may subsequently over such a great period of time have evolved so as to differentiate its constituent genomes, thus largely concealing its autopolyploid origin.

There are two reasons why the presence of some trivalents in the triploid hybrids does not invalidate the second suggestion made above; that some genic system directly inhibiting multivalent formation may have been evolved. Firstly, it is likely that the inhibitor mechanism may not be fully effective in a hybrid nucleus. Secondly, the so-called trivalents seen may in any case be caused by the presence in the hybrid nucleus of segmental interchanges in the heterozygous state. The genetical mechanism against multivalents would not affect multiple associations of this origin since multiple homologies are not concerned in their formation. The occasional detection of quadrivalents would seem to support this second contention,

It must now be pointed out that it is hypothetically possible to interpret the cytological analyses in the triploid hybrids without invoking any direct relationship between the diploid and tetraploid cytotypes. If the tetraploid should in fact be an autotetraploid, but derived from a diploid quite unrelated to the diploid cytotype of Asplenium trichomanes, then the bivalents present in the triploid would then be produced by pairing between the chromosomes of the two genomes contributed by the tetraploid, the chromosomes contributed by the diploid parent remaining unpaired. Admittedly this hypothesis is clumsy and unattractive, but there is nothing in the behaviour of the triploid hybrids which excludes such an interpretation. However, it will be seen below (p.145), that the cytogenetical evidence from the hybrids involving the hexaploid cytotype presents very serious difficulties in the way of acceptance of this alternative.

We may therefore conclude that it is probable that some form of the diploid cytotype has at some time in the past played some direct role in the origin of the tetraploid cytotype. The diploid cytotype may have given rise to the tetraploid either directly by autopolyploidy, or by hybridisation with some other diploid, still unknown,

followed by amphidiploidy. This unknown other parent may have no synaptic homology with the diploid cytotype, in which case the tetraploid cytotype is a true allopolyploid. It is also possible that two forms of the diploid cytotype may have combined to produce the tetraploid cytotype, which would then be an autoallopolyploid according to the categories of Stebbins (30, 31). It is not possible to distinguish between these three alternatives on the basis of the evidence available at the present time.

6. WHAT IS THE NATURE OF THE ANCESTRAL GENETICAL RELATIONSHIP BETWEEN THE HEXAPLOID AND THE DIPLOID AND TETRAPLOID CYTOTYPES?

In the pentaploid hybrids obtained by crossing together the hexaploid and tetraploid cytotypes, and also in the tetraploid hybrids obtained by hybridisation between the hexaploid and diploid, the pairing of chromosomes in meiosis is complex, and complete analyses are only obtained from the most favourably squashed cells. Only four cells from the pentaploid hybrids and five cells from the tetraploid hybrids have been successfully analysed. Complete accuracy cannot be claimed even for these nine analyses, but it can nevertheless be confidently asserted

that the degree of error in interpretation is small. The results obtained are consistent, and in spite of their complexity, may reasonably be regarded as a sound basis for discussion. (See pp.106 -107 , and figs. 125-130).

It is immediately apparent that there is a considerable degree of relationship between the genomes present within both pentaploid and tetraploid hybrids. The analyses are complicated by the presence of a few trivalents, and the total number of trivalents and bivalents are considered as a whole in terms of the total number of associations present. In which case the results obtained may be expressed thus:-

Tetraploid hybrid (hexaploid x diploid)

Range in analyses: 47 - 55 associations + 34 - 50 univalents

= 36 II + 11 - 19 II & III + 34 - 50 I

Pentaploid hybrid (hexaploid x tetraploid)

Range in analyses: 67 - 73 associations + 31 - 46 univalents

= 36 II + 31 - 37 II & III + 31 - 46 I

The interpretation of these results in terms of cytogenetical relationships is difficult, but it is nevertheless possible to deduce some important conclusions.

We may first draw attention to the high number of associations present in the tetraploid hybrids. Even if

it is assumed that the genome contributed by the diploid parent is pairing completely with one of the genomes from the hexaploid parent, it remains an incontrovertible fact that at least eleven to nineteen associations are being formed between the other two genomes contributed by the hexaploid parent. There is clearly a significant degree of synaptic homology within the genomes of the hexaploid cytotype, which therefore cannot be an allopolyploid according to the restricted sense of Stebbins (30, 31), but is instead presumably an autoallopolyploid. This conclusion is of importance, because with the sole exception of Doodia caudata (Manton, 22), it represents the first demonstration amongst the ferns of any polyploid form other than an orthodox allopolyploid with no synaptic homology between its constituent genomes.

A further conclusion which inevitably follows from consideration of the pairing found in this tetraploid hybrid is that the genome of the diploid cytotype must have at least partial synaptic homology with one of the genomes of the hexaploid, since even if two of the hexaploid genomes pair completely, the remaining eleven to nineteen associations must be formed between chromosomes of the third hexaploid genome and chromosomes of the genome from

the diploid cytotype. We can be certain therefore, that the diploid genome has at least partial synaptic homology with one of the genomes of the hexaploid cytotype; but it is furthermore likely that in fact it possesses complete synaptic homology with one of the hexaploid genomes, and is in some way involved in the ancestry of the hexaploid cytotype.

A high degree of pairing is found at meiosis in the pentaploid hybrid. The associations formed are roughly equivalent to complete synapsis between two pairs of genomes. An obvious possible interpretation of this fact is that the two genomes contributed by the tetraploid parent show virtually complete synaptic homology with two of the genomes from the hexaploid, and in consequence on the face of the evidence it would appear likely that the tetraploid cytotype is in fact one parent of the hexaploid. However, the obvious and most attractive hypothesis is not necessarily the most nearly correct, and the origin of the associations seen at meiosis in this pentaploid can be interpreted quite differently.

From this point on, it is only possible to further the interpretation of the cytogenetic evidence by correlation of the cytological results obtained from the study of meiosis in the different synthesised hybrids. However,

such correlated analysis of the cytogenetic evidence tends to become extremely complicated, and it is desirable to utilise symbols for genomes in order to clarify the reasoning involved. The symbols to be used must first be defined:-

1. Capital letters, A,B, etc., are used to indicate genomes, where A, B, etc., have no synaptic homology.
2. Use of an apostrophe, e.g. A,A', indicates partial synaptic homology between genomes.
3. III = trivalent, II = bivalent, I = univalent.
4. 2x = diploid, 4x = tetraploid, 6x = hexaploid cytotype.

Let us first consider the variations of the simplest hypothesis, which is that forms of the diploid and tetraploid cytotypes are the actual parents of the hexaploid cytotype.

A. Where 2x = AA

4x = AABB = related allotetraploid

6x = AA x AABB = AAAABB

Then 4x (6x/2x) hybrid = AAAB = 36 AA II + some A B II ?

∴ this hypothesis clearly fails, because observed pairing in 4x hybrid would require synapsis between A and B, which is not possible, by definition.

B. Where 2x = AA

4x = AAA'A' = ancient autotetraploid or autoallo-tetraploid

6x = AA x AAA'A' = AAAAA'A'

Then 4x (6x/2x) hybrid = AAAA' = 36AAII + some AA'II

5x (6x/4x) hybrid = AAAA'A' = 36AAII + 36A'A'II + AI

The trivalents found in both 4x and 5x hybrids (from one to eight in number, see p.107, figs 125 - 130), are presumably of AAA composition, but their number is not high enough to prove the existence of the A genome in triplicate. However, this hypothesis conforms with the cytogenetical evidence, and is therefore tenable.

It is clear from the reasoning set out above, that if the known diploid is parentally related to the known tetraploid cytotype, and these two together are directly parental to the hexaploid, then in order to satisfy the cytogenetical evidence not only must the hexaploid be an autoallopolyploid, but the tetraploid must either also be an autoallopolyploid, or else an ancient autotetraploid in which some divergence of the constituent genomes has occurred.

We may now consider the hypothesis that the diploid and tetraploid cytotypes are not related, but are nevertheless together parental to the hexaploid.

C. Where 2x = AA

4x = BBCC = unrelated allotetraploid

6x = AA x BBCC = AABBCC

Then 4x (6x/2x) hybrid = AABC = 36AA$\amalg$ + some BC$\amalg$?

∴ this hypothesis clearly fails, since the observed pairing in the 4x hybrid would require synapsis between A and B, which by definition is not possible.

D. Where 2x = AA

4x = BBB'B' = unrelated autotetraploid or autoallotetraploid

6x = AA x BBB'B' = AABBB'B'

Then 5x (6x/4x) hybrid = ABBB'B' = 36BB$\amalg$ + 36B'B'$\amalg$ + AI

3x (4x/2x) hybrid = ABB' = 36BB'$\amalg$ + AI

4x (6x/2x) hybrid = AABB' = 36AA$\amalg$ + 36BB'$\amalg$ X ?

In fact, in the 4x hybrid only 47 - 55 associations are formed, not 72, as would be required if B and B' were to behave consistently in the 4x and 3x hybrids. It may be considered that such an inconsistency is not enough to completely invalidate this hypothesis, but it does render it distinctly improbable.

One further hypothesis must be considered, which is consistent with the cytogenetical evidence. This demonstrates that it is possible for the known tetraploid to be an allotetraploid and still be one parent of the hexaploid cytotype. In this case, it is necessary that the known diploid is not directly parental to the hexaploid cytotype.

E Where known 2x = AA

unknown 2x= BB

4x = AABB

$$6x = BB \times AABB = AABBBB \xrightarrow[\text{divergence of B genomes.}]{\text{subsequent}} AABBB'B'$$

Then 4x (6x/2x) hybrid = AABB' = 36AAII + some BB'II.

5x (6x/4x) hybrid = AABBB'= 36AAII + 36BBII + B'I

It is seen that on the basis of the cytological facts, this hypothesis is tenable.

There is little to be gained by construction and examination of further more complex hypotheses concerning the inter-relationships of the three cytotypes, since it is already clear that it is not possible to arrive at a final conclusion with regard to this problem on the basis of the evidence at present available.

It would be tedious to enumerate and invalidate in turn all the possible alternative hypotheses to explain the cytogenetical facts without assuming the known tetraploid cytotype to be one parent of the hexaploid cytotype, and it is presumed that the reader is prepared to accept the statement that it is not possible to construct any such hypothesis which is consistent with the cytological evidence.

We may conclude this rather involved discussion of the relationship of the hexaploid to the other two cytotypes by stating that it is probable that one of two (i.e. B and E), of the hypotheses which have been examined is essentially correct. Either the known diploid is parental to the tetraploid, which may be autopolyploid or autoallopolyploid in origin, and these two together are the parents of the hexaploid cytotype, or, the known diploid is one parent of the tetraploid, which is an allopolyploid, the tetraploid together with its other diploid parent being themselves parental to the hexaploid. It is not possible on the basis of the available cytogenetical evidence to differentiate between these two hypotheses.

In both hypotheses, the known diploid is in some manner parental or part-parental to the tetraploid, and the tetraploid is one parent of the hexaploid cytotype. In

consequence, it can be said that it is very likely that both these last statements are correct.

CONCLUSIONS.

This discussion will now be terminated with a summary of the conclusions obtained through consideration of the cytogenetic evidence, together with a statement of the lines which future research must follow.

Although all the diploid forms investigated have been found to be essentially the same, in the sense that they evidently belong to the same broad genetic unit, with a common genetic origin, it is clear that the Australian diploid has diverged to an appreciable extent away from the Northern Hemisphere forms of this cytotype.

It is believed that at least all the typical forms of the tetraploid cytotype belong to the same broad genetic unit, but this has not been positively demonstrated to be true. There is no cytogenetic evidence that the Japanese form of the tetraploid does not belong to this same genetic unit, but nevertheless its inclusion therein is accepted only with certain reservations.

The most important hypotheses concerning the relationship of the three cytotypes have been described and considered in

relation to the meiotic behaviour of the pentaploid, tetraploid and triploid hybrids which have been formed between them.

It has already been stressed that it is evidently not possible on the basis of the available genetical evidence to determine the precise genetical relationships which exist between these three cytotypes, but it is clear that they are all mutually inter-related as members of an autoallopolyploid complex.

It has demonstrated that the hexaploid cytotype cannot be an allopolyploid, and is therefore presumably of autoallopolyploid origin. It is probable that some form of the tetraploid cytotype is of direct parental relationship to the hexaploid, and that the diploid is also related to the hexaploid, either indirectly through the tetraploid, or directly as an ancestral diploid parent.

The exact genetical relationship between the diploid and tetraploid cytotypes is not known, but it is probable that the diploid is in some parental or part-parental to the tetraploid.

An interesting subsidiary conclusion may be drawn from the results of the investigation. From a study of their distributions, it has been shown (p.114), that both the diploid and tetraploid cytotypes must be of relatively

ancient origin. The two series of triploid hybrids formed between them show that apparently little divergence, in terms of synaptic homology, has occurred between the different forms of the two cytotypes. That this should be so, in spite of the evidently very long separation, in terms of both space and time, of the Australian and European forms of either cytotype, indicates that synaptic homology may persist over very considerable periods of time if there is no direct selection pressure favouring its elimination, such as exists within an autopolyploid.

Finally, it remains only to indicate the course which subsequent investigation of this species complex must follow. Further experimental investigation of the tetraploid cytotype is needed, with the intention of determining, if possible, whether or not this plant is of autopolyploid origin. Discovery of a successful method for the production of an artificial autotetraploid from the diploid cytotype would do much to further investigation of this problem. The successful synthesis of a series of hybrids between the tetraploid cytotype of Asplenium trichomanes and other relatively distantly related species of Asplenium would also result in information of great value.

For rather similar reasons, a comparable series of hybrids between the hexaploid cytotype and other species of Asplenium might also yield information of importance concerning the genetic constitution of this plant.

A less specific but none the less important aim should be a search for other entities which may be involved in this species complex. These might be found anywhere in the world, though perhaps the most likely regions are Asia and the Pacific region.

TAXONOMIC CONCLUSIONS.

Throughout this thesis the different members of the Asplenium trichomanes species aggregate which have been investigated in this study have been referred to as the diploid, tetraploid, and hexaploid cytotypes respectively. However, the nomenclature of these three plants cannot be left in this state indefinitely, and more conventional names must be attributed to them for general use.

In my opinion, there need be no doubt that all three cytotypes are entitled to separate specific status since they possess the basic characteristics of distinct natural species. They possess distinctive geographical and ecological distributions, and are genetically isolated from one another by sterility in the first generation hybrids. Although the morphological problem involved is exceptionally difficult, it is possible to distinguish the three cytotypes on the basis of morphological grounds alone, without recourse to cytology.

The argument might be introduced that on practical grounds it is not feasible to recognise these plants as three distinct species in view of the fact that it is not possible to distinguish them with complete confidence without reference to microscopic characteristics.

However, practical considerations must surely not be regarded as adequate reason for obscuring the natural status of a taxon. Once two or more plants are demonstrated to be distinct natural species, there is no alternative to classifying them as such.

Having come to the conclusion that the three cytotypes must be recognised taxonomically as distinct species, it is necessary to decide what names should properly be applied to them, which in the case of the diploid and tetraploid cytotypes presents a most difficult nomenclatural problem.

Asplenium trichomanes is a Linnean name (19). However, at the time when he drew up his not very adequate diagnosis, Linnaeus might have had material of either or both the diploid and tetraploid cytotypes in his possession, since both are frequent in Scandinavia and on the continent of Europe. At present, only one Linnean specimen of Asplenium trichomanes is known to me. This is in the possession of the Linnean Society in London. The spore and rhizome scale characters of this specimen have not yet been examined, but nevertheless it can be said with fair confidence that it belongs to the diploid cytotype. If in fact no other Linnean specimen of Asplenium trichomanes can be found, then clearly this Linnean Society specimen must be considered as

the type of Asplenium trichomanes. However, if it eventually transpires that Linnean specimens of both diploid and tetraploid cytotypes exist, then deciding which of these two plants should take the name of Asplenium trichomanes will prove to be a very nice point.

Although the diploid and tetraploid cytotypes thus still present an awkward nomenclatural problem, fortunately the situation with regard to the hexaploid is quite straightforward. In 1888 Colenso (7), published the description of a new species of Asplenium from New Zealand, which he called Asplenium melanolepis. It is not completely clear from this paper exactly how Colenso interpreted his species, but the specimen in the Herbarium of the Dominion Museum, Wellington, labelled by Colenso as Asplenium melanolepis, and cited by him with his description of this species, undoubtedly belongs to the hexaploid cytotype. Colenso's name is however a later homonym of Asplenium melanolepis Franchet and Savatier published in 1879 (15), and is therefore an illegitimate name according to the present International Rules of Botanical Nomenclature (18).

It will therefore be necessary to propose a new name for the hexaploid cytotype, preferably with a fresh description and diagnosis, and based on a holotype specimen

of known chromosome number, since Colenso's description, though fulsome, is not entirely accurate and would require amendment, while his specimen is incomplete, consisting of three fronds only, without any portion of the rhizome.

SUMMARY.

The aggregate species Asplenium trichomanes is found to consist of three races characterised by different levels of polyploidy. There is thus a diploid cytotype with n = 36 chromosomes, a tetraploid with n = 72, and a hexaploid with n = 108.

Studies have been made on their geographical distributions, ecology, and comparative morphology, and on the cytogenetical relationships of these three cytotypes.

GEOGRAPHICAL DISTRIBUTION.

The world distributions of the three cytotypes have been constructed on the basis of chromosome counts and the examination of herbarium material.

The diploid cytotype is widely distributed in Europe, continental Asia and North America. It is also found in south-east Australia and on a very few high mountains in the Indonesian region.

The tetraploid cytotype is widely distributed in Europe, North America, and some parts of continental Asia. It also occurs in Japan, Hawaii, Australia, and New Zealand. Isolated localities exist in the central African mountain chain as far south as Cape Province, and there is a single

locality known in south-east Brazil.

It is believed that accidental dispersal by man has not been a significant factor in the establishment of the very wide world distributions of the diploid and tetraploid cytotypes and that these distributions are therefore natural in origin. It is further concluded from consideration of their extremely wide distributions that both diploid and tetraploid are of ancient origin, and must both have been much more continuously distributed through their ranges in the Southern Hemisphere and the mountains of the Tropics during the glacial and pluvial phases of the Pleistocene period than they are today.

In contrast to the other two cytotypes, the hexaploid is endemic to New Zealand, and even there is mainly found in the South Island.

AUTECOLOGY.

Field observations have been made in the United Kingdom, Norway, Australia, and New Zealand.

All three cytotypes are essentially plants of crevices in rock outcrops.

In the United Kingdom the diploid cytotype is confined to regions of relatively high rainfall and is therefore

restricted to the mountainous districts of North Wales, the Lake District, and Scotland.

In contrast the tetraploid is very completely distributed over the country, owing to its capacity for colonising old mortared walls. Its natural distribution is however confined to the outcrop of the hard Carboniferous limestones in the north and west of the country and to basic rocks generally in mountainous districts.

It is clear that in the United Kingdom, the diploid cytotype is much less tolerant than the tetraploid of relatively dry conditions. The two cytotypes also differ in their edaphic preference, the diploid being found on various base-poor rocks, and also on non-calcareous base-rich rocks such as serpentine and mica-schist, whereas the tetraploid is essentially a limestone plant, although also found on other base-rich rocks.

The same distinction in the edaphic preferences of the diploid and tetraploid is also found in Australia, and it is notable that there is considerable similarity in the general ecological characteristics of the localities of the corresponding forms of the same cytotype in Australia and the United Kingdom.

The hexaploid cytotype appears to combine the edaphic

preferences of the other two cytotypes, since it is not only found on Greywacke, a base-poor rock, but also on limestone and mica-schist.

The hexaploid is also capable of withstanding more arid habitat conditions than the tetraploid cytotype can apparently tolerate.

Comparative ecological study of these three cytotypes has thus indicated that in this species complex, increase in level of polyploidy is correlated with increase in tolerance of dry habitat conditions.

COMPARATIVE MORPHOLOGY.

The three cytotypes are very similar in general appearance and cannot be satisfactorily distinguished on the basis of macro-characters alone. Study has been made of a number of micro-characters in order to determine characters suitable for general use in distinguishing the three cytotypes from one another in herbarium material.

The three cytotypes are found to differ most conspicuously in the shape and texture of the pinnae, the size and sculpture of the spores, and the structure of the rhizome scales.

In the diploid cytotype the pinnae are orbicular in shape and thin in texture, in the tetraploid the pinnae are

sub-orbicular or oblong in shape and rather thin in texture, and in the hexaploid the pinnae are oblong to sub-rhomboid in shape and usually almost coriaceous in texture.

Determinations of mean length of the exospore range from 29 - 36μ in the diploid, 34 - 43μ in the tetraploid, and 40 - 46μ in the hexaploid cytotype.

In both diploid and tetraploid cytotypes the perispore is usually rugulo-saccate in sculpture, with an evident but irregular wing. In the hexaploid the perispore is usually shallowly cristate, with an inconspicuous but relatively even wing.

The rhizome scale of the diploid is lanceolate, with a relatively small region of occluded cells in the central region, and that of the tetraploid is sub-linear to linear in shape with a conspicuous band of occluded cells, whereas that of the hexaploid is linear to subulate in shape, and almost completely occluded.

The Japanese form of the tetraploid differs from other forms of this cytotype in the sub-rhomboid shape of the pinnae and in a greater proportion of occluded cells in the rhizome scale.

A more detailed synopsis of the morphological differences between the cytotypes is given in the text (pp.76 - 78).

GENETICAL RELATIONSHIPS.

All three cytotypes show a regular meiosis, only bivalents being formed, and all behave functionally as diploids.

Several series of artificial hybrids have been raised. These include a series of hybrids between different geographical forms of the diploid cytotype of Asplenium trichomanes and Asplenium adulterinum, a comparable series of hybrids between forms of the diploid cytotype and a single form of the tetraploid cytotype, and a series of hybrids between several geographical forms of the tetraploid and a single form of the diploid. Hybrids have also been synthesised between the hexaploid cytotype and both the diploid and tetraploid cytotypes. Wild triploid hybrids formed between the diploid and tetraploid cytotypes have been available from localities in different parts of Europe.

The chromosome pairing found at meiosis in these hybrids has been studied as a source of information concerning cytogenetical relationships. The results of the cytological studies, which are mostly of a rather complex nature, are set forth in the text. The problem of their interpretation is discussed at some length, and certain conclusions drawn.

All the geographical forms of the diploid cytotype investigated are found to belong to the same broad genetic unit, with evidently a common genetic origin and affinity, although the Australian form does show some degree of both cytological and genetical divergence away from the Northern Hemisphere forms.

There is no positive evidence of any gross cytological or genetical heterogeneity amongst the various geographical forms collectively referred to as the tetraploid cytotype, but the possibility of the existence of such heterogeneity cannot be excluded.

The inter-relationships of the three cytotypes are still uncertain. The diploid is probably parental or part-parental to the tetraploid, but whether this plant is of allopolyploid, autopolyploid, or autoallopolyploid origin is unknown.

The ~~diploid~~ hexaploid is found to be an autoallopolyploid, and it is probable that some form of the tetraploid cytotype is one of its parents. The diploid cytotype is believed to be related to the hexaploid either indirectly through the tetraploid or directly as an ancestral diploid parent.

TAXONOMIC CONCLUSIONS.

Reasons are given why it will be necessary to treat the three cytotypes taxonomically as distinct species, and the nomenclatural problems involved are briefly discussed.

SUPPLEMENT: ASPLENIUM ADULTERINUM.

An account is given of the distribution of this species, which is endemic to rocks of the serpentine group in Europe.

Cytogenetic evidence is described supporting the assumption made in the main body of the thesis that Asplenium adulterinum is an allotetraploid, with Asplenium viride and the diploid cytotype of Asplenium trichomanes as its apparent parents.

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APPENDICES.

LISTS OF RECORDS OF THE THE THREE CYTOTYPES OF *ASPLENIUM TRICHOMANES* S.L., FORMING THE BASIS OF THE DESCRIPTION OF THEIR DISTRIBUTIONS.

ABREVIATIONS USED.

B.M.	British Museum (Natural History,) London.
Mel. N.H.	Melbourne National Herbarium.
N.S.W.N.H.	New South Wales National Herbarium, Sydney.
R.B.G.K.	Royal Botanic Gardens, Kew.
U.Cal.	University of California Herbarium.
U.Cal., Copeland.	E.B.Copeland Herbarium, lodged in University of California.

APPENDIX I.

DIPLOID CYTOTYPE : LIST OF CHROMOSOME COUNTS.

GREAT BRITAIN

CAERNARVONSHIRE v.c. 49.

Aber Falls. On shaded rocks on either side of the waterfall, 750-1000'. J.D.L. 1952.

Pass of Llanberis. In rock crevices, at foot of n.w. precipices of Glyder Fawr massif, 500-1000' J.D.L. 1953.

Cwm Idwal. On rocks, 1350-1800' F.J.Taylor, 1953.

Cwm Llefrith. (Further details not known) T.Pritchard 1954.

MERIONETHSHIRE v.c. 48.

Cader Idris. On rocks at base of the north-facing escarpment, Cyfrwy, 1200-1500 ' J.D.L.1952.

Coed y Gribin, W. of Dolgelley. Shaded rocks by streamside in light oak wood, 3 miles S.W.W. of Dolgelley, 200'. J.D.L. 1952.

Tyn-y-Groes, N. of Dolgelley. Rock crevices on slopes on west side of Ganllwyd valley, ¾ mile S.W. of Gelligemlyn, 250-500'.J.D.L.1952.

CUMBERLAND v.c. 70.

Barf Fell, Thornthwaite. In shaded or sheltered rock crevices along stream by east face of Barf Fell, 500-1000'. J.D.L. 1953.

CUMBERLAND v.c. 70 (continued)

Castle Crag, Borrowdale. Sheltered rock crevices just south of Castle Crag, 800'. J.D.L. 1953.

STIRLING v.c. 86.

Loup of Fintry v.c. 86. Rocks in the valley of the Endvick Water. B.W.Ribbons 1954.

PERTHSHIRE v.c. 89

Stenton Rocks. On shaded rocks in light birch wood, 450'. J.D.L. 1954.

Dunkeld. In crevices of mica-schist rocks north of Dunkeld, 750'. J.D.L. 1954.

ABERDEEN v.c. 93.

Craig Dorney, 5 miles N. of Cabrach. In deep crevices in scree on serpentine rock in exposed situation on south-facing slope, 1150 - 1300'. J.D.L. 1954.

Craigs of Succouth, 6 miles N.N.E. of Cabrach. In deep crevices in scree on serpentine rock in exposed situations, 1250'. A.viride occurs on same outcrop. J.D.L. 1954.

EUROPE.

NORWAY.

Norddal, Norddalsfjord, Sunnmore. With Asplenium adulterinum on serpentine rock in exposed situation on S.W. facing slope, 150'. J.D.L. 1953.

Rodbergvik, Sunnylvenfjord, Sunnmore. With Asplenium adulterinum on serpentine rock in an exposed situation on west facing slope, at sea level. J.D.L. 1953.

Bjorkedalen, N. of Nordfjordeid. With Asplenium adulterinum and Asplenium viride on serpentine rock, amongst tumbled boulders on S.W. facing slope, at sea level. J.D.L. 1953.

Volda, Vollsfjord, Sunnmore. With Asplenium septentrionale in crevices at foot of south facing precipice, 200'. J.D.L. 1953.

Mundheim, Hardangerfjord. In crevices of rocks at sea level. J.D.L. 1953.

Moldegarde, near Os, Bjornafjord. On mica-schist rocks with Asplenium viride, at sea-level. J.D.L. 1953.

FRANCE.

St. Nectaire, Puy-de-Dome, Auvergne. E.W.Davies 1949.

SWITZERLAND.

La Ravoère, Mont d'Ottan, Canton de Valais. 3000'.
A.H.G.Alston no. 11802 1952. B.M.

near Basel.
(Further details not known)

GERMANY

(Material sent by D.E.Meyer, locality not stated).

ASIA.

INDIA.

Parbati valley, Kulu district, Punjab (Kangra Himalaya)
Occasional around tree bases at tree line, 11,000'. E.A.C.L.E. Schelpe, no.3542, 1952. B.M.

Walong, in Lohit valley, Assam. 3800'
F.Kingdon-Ward, no. 19172. 1952. B.M.

TIBET.

Kongbo, S.E.Tibet. At foot of Nyoto Chu Glacier, 12,000'
Ludlow, Sherriff, & Elliot, no.15629. 1947. B.M.

Gyadzong, Yigrong Chu, Pome, S.E.Tibet. On boulders in pine and shrub forest, 8,500'. Ludlow, Sheriff & Elliott, no. 12163. 1947. B.M.

NEPAL.

(Details of locality not known). Rocks in grass in cedar forest, 7000'. Polunin, Sykes & Williams, no. 3906. B.M.

(Details of locality not known). Polunin, Sykes, & Williams, no. 5283.

AMERICA.

CANADA.

Gordon Bay, L.Joseph,(Parry Sound), Ontario. Growing in sod on granite. D.M.Britton.

AUSTRALIA.

Bare Rocks of Wulgulmerang, Gippsland, Victoria. Growing in cracks in rock-face (conglomerate of igneous origin), above stream in open Eucalyptus forest near Bare Rocks. N.A.Wakefield & R.Melville 1953, N.A.Wakefield & J.D.L. 1955.

APPENDIX II.

DIPLOID CYTOTYPE : IMPORTANT DETERMINATIONS OF HERBARIUM MATERIAL.

ASIA

CHINA.

Yunnan. 1910. U.Cal., Copeland 11993.

Western Hupeh.E.H.Wilson, no. 2659. Arnold Arboretum Exp. China, 1907. B.M.

Shen-si : Lao-y-San. Fr. Hugh 1899. B.M.

Shen-si : Chen-hai-jao. E.Licent, no. 2453. 1916 B.M.

Shen-si : Seian-Kio. Fr.Hugh, 1895. B.M.

NORTH AMERICA.

U.S.A.

Washington,D.C. & vicinity. ex. Herb., Bureau of Science, Manila. U.Cal., Copeland, no.6215.

Maine. U.Cal., Copeland no. 6205.

North Carolina: mountains, Broad River. 1841. B.M.

Georgia : Whitfield County, top of Rocky Face Mt., 1900.B.M.

Oklahoma : Wichita Mts., Comanche Co. Moist cove on rocky slope near summit of Mt. Scott, 2350'. 1947. U. Cal. 965718.

Colorado : near Canon City, Fremont Co..U.Cal.123193.

U.S.A. (contd)

Arizona : Graham Co. Pinaleno Mts. 1944. U.Cal. 904935.

Arizona : Coconinio Co. 1947. U.Cal. 749817.

Washington ? : confluence of Columbia. David Douglas 1825. B.M.

PACIFIC REGION.

INDONESIA.

Lombok, Lesser Sunda Islands : Mt. Rindjani. 6500'. C.N.A. de Voogd, no. 2645, 1936. R.B.G.K, ex. Herb. Bogor.

TIMOR.

Portugese Timor : Mt. Tatamailau. Mostly on rocks or in damp places, in stream courses and clefts of rock, 8500-9500'. C.G.G.J.van Steenis, no. 18443, 1954. B.M., ex Herb Bogor.

NEW GUINEA.

Morobe. On rock on grassy hill, 9,000-10,000'. M.S.Clemens. no. 10075. 1939. U.Cal., Copeland 16650.

Rawlinson Ranges. 12,000 (?), M.S.Clemens, 1941. U.Cal., Copeland 19058.

AUSTRALIA.

Mt. Aberdeen, Victoria. Mueller 1853. R.B.G.K.

AUSTRALIA (continued)

Buffalo Ranges, Victoria. "On the waterfalls towards the large gap of the Buffalo Ranges on mossy perfectly shaded slate rocks with Osmunda rhodarctica (=Dicksonia antarctica), Pteris (Pellaea) falcata, Asplenium flabellifolium co-associated". Mueller 1853. Mel. N.H.

Tawonga, Kiewa River, Victoria. A.J.Tadgell 1924. Mel.N.H.

Delegate district, on border of N.S.W. and Victoria. W. Bauerlen no. 165. Mel. N.H.

near Molong, (N.W. of Bathurst), N.S.W. Jones 1879 Mel.N.H.

Ebor, (New England Ranges), N.E. New South Wales. On granite. N.A.Wakefield, 1941. Mel.N.H.

(NEW ZEALAND)

? Petane, Hawke's Bay. A. Hamilton 1881. Dom.M.Well.

One sheet in the Herbarium of the Dominion Museum, Wellington (no.353), contains three plants of Asplenium trichomanes s.l.. Of these specimens, one plant is the hexaploid cytotype, while the other two plants undoubtedly belong to the diploid cytotype. The label indicates that two separate gatherings are mounted on the one sheet, thus:- "Auckland E.C. b. Petane 4/3/81 A.H." From their position

on the sheet, it is likely that the Petane portion of the label refers to the two diploid plants, but unfortunately this is not specifically indicated, and it is not possible to exclude the possibility that "Auckland E.C." refers to the diploid plants. This latter label in Hamilton's own hand is known to indicate a specimen purchased by him from Eric Craig, a curiosity dealer of Auckland. The collections bought from Craig included foreign material. It is therefore not possible to be certain that these two diploid specimens are of New Zealand origin, although it is more probable that they were in fact collected somewhere in the Hawke's Bay region by Hamilton during his early residence in Petane. No other specimens of the diploid cytotype are known from New Zealand.

APPENDIX III.

TETRAPLOID CYTOTYPE : LIST OF CHROMOSOME COUNTS.

GREAT BRITAIN.

CORNWALL. v.c. 1 & 2.

Trevarno, 3 miles N.W. of Helston. On a mortared wall, bordering a metalled road, shaded by a copse, 200'. J.D.L. 1953.

Delabole. On a low mortared roadside wall, by the slate quarry, 600'. J.D.L. 1953.

St. Austell. B.D.Harrison 1952.

DEVON v.c. 3

near Plymouth S.Saunders.

SOMERSET v.c. 5.

Porlock. On mortared walls, 200-900'. J.D.L. 1953.

SUSSEX v.c. 13.

Midhurst. On old mortared walls, right inside the town, 100'. J.D.L. 1952.

KENT v.c. 16.

Hayes. On sheltered side of old mortared churchyard wall, 250'. J.D.L. 1952.

Strood. D.J.Hambler 1953.

SURREY v.c. 17.

Godalming. On old mortared walls in town, 200'.J.D.L. 1952.

SURREY v.c. 17 (continued)

Compton, S.W. of Guildford. On old mortared walls bordering main street of the village, 200'. J.D.L. 1952.

NORTHAMPTON v.c. 32.

Rockingham. In crevices in the old stone walls of the castle.R.W.Painter 1950.

GLOUCESTERSHIRE v.c. 34.

Coleford. D.J.Hambler 1953.

BRECONSHIRE v.c. 42.

Crickhowell. Old mortared garden walls in town, 300'. J.D.L. 1952.

Craig-y-Cilan, 3 miles S.W. of Crickhowell. In rock crevices and amongst scree on Carboniferous Limestone, 1000-1350'. J.D.L. 1952.

Pen Cerig-Calch,3 miles N. of Crickhowell. In rock crevices of limestone band at summit, 2300'. J.D.L.1952.

Clydach,5 miles S. of Crickhowell. In rock crevices along gorge of Clydach River, in Carboniferous Limestone, under shade, 700-1000'. J.D.L.1952.

MERIONETH. v.c. 48.

Cader Idris. On basic rocks about Llyn y Gafr, below north escarpment, 1200-1500'. J.D.L. 1952.

MERIONETH v.c. 48 (continued)

Tyn-y-Groes, N. of Dolgelley. Rock crevices on slopes on west side of Ganllwyd valley, S.W. of Gelligemlyn, 250' and on roadside walls, 100'. J.D.L. 1952.

CAERNARVON v.c. 49.

Aber Falls On rocks on east side of waterfall, 1000'. Also on mortared stones of bridge at Bont Newydd, 1½ miles to the north, 250'. J.D.L.1952.

Pass of Llanberis. On walls along metalled road, near Gwastadnant, 400'. J.D.L. 1953.

Cwm Idwal. On basic rocks below Devil's Kitchen, 1500' J.D.L. 1953.

Cwm Llafar. A.W.Westrup, 1953.

ANGLESEY v.c. 52.

Gors Goch. On roadside wall, 300'. J.D.L. 1953.

DERBYSHIRE v.c. 57.

Backdale. On limestone rocks. F.B.Lovis 1952.

YORKSHIRE. v.c. 61 - 65.

Malham. On limestone rocks, 1250'. J.D.L. 1954.

Grassington. In crevices of limestone rocks about Cove Scar, 750'. J.D.L. 1954.

Buckden. On mortared walls in village, 750'. J.D.L.1957.

YORKSHIRE v.c. 61 - 65 (continued)

Fountains Abbey, 3 miles S.W. of Ripon. In crevices between stone blocks of ruined abbey, and on nearby walls, 250'. J.D.L. 1957.

Aysgarth. On mortared roadside walls, 650'. J.D.L. 1954.

DURHAM v.c. 66.

Falcon Clints, Upper Teesdale. In solution crevices in metamorphic limestone blocks at foot of south-facing crags, 1350'. J.D.L. 1954.

WESTMORLAND v.c. 69.

Wray Castle & Ferry House, Windermere. On mortared walls, 150'. J.V.Lovis 1952.

Langdale. On mortared roadside walls near the Old Dungeon Ghyll Hotel, 350'. J.D.L. 1954.

Hutton Roof. In crevices of rocks and between scree fragments on Carboniferous Limestone, 750'. J.D.L. 1954.

CUMBERLAND v.c. 70.

Rosthwaite, Borrowdale. On basic rocks in narrow gorge near Yew Crag, 900', and on mortared walls in village of Rosthwaite, 300'. J.D.L.1953.

Seatoller, Borrowdale, On mortared stonework of bridge, 400'. J.D.L. 1953.

CUMBERLAND v.c. 70 (continued)

Loweswater. From old mortared wall by Maggie's Bridge, near foot of Loweswater Lake. J.D.Hinde, 1954.

EDINBURGH v.c. 83.

Arthur's Seat. On roadside rocks, 300'. J.D.L. 1954.

PERTHSHIRE v.c. 88 & 89.

near Lawers, on Aberfeldy road. On mortared walls at roadside, 500'. J.V.Lovis 1952.

Lochan na Lairige, 2½ miles S.W.W. of Ben Lawers, 1600' J.V.Lovis 1952.

Stenton, 2½ miles S.E.E. of Dunkeld. On road side wall, between mortared stones, 200', and in natural rock on Stenton Rocks immediately above, 300'. J.D.L. 1954.

Dunkeld. In crevices of mica-schist rock north of Dunkeld, and between tumbled boulders, 750'. J.D.L. 1954.

ABERDEEN v.c. 93.

Craig Dorney, 5 miles N. of Cabcach. In crevices of serpentine rock in a steep gully, 1000'. J.D.L. 1954.

BANFF v.c. 94.

(Same locality as the preceding).

DUMBARTON v.c. 99.

Glenarbuck, Bowling. In crevices of stone wall, B.W.Ribbons 1954.

SKYE v.c. 104.

Loch Brittle. On walls, D. Leaver 1953.

KERRY v.c. H1

Glencar. M.G.Shivas 1951.

WEXFORD v.c. H.12.

nr. Mt. St. Benedict. Growing in stonework of bridge over River Bann. A.H.G.Alston no.11578. B.M.

GALWAY v.c. H.16.

Ballynahinch. Other data not known.

EUROPE.

FRANCE.

Villard-de-Lans, Dept. Isère. Road on south slope of Gorge de Bourne. A.H.G.Alston. 1949. B.M.

Passenans, French Jura. E.Walter 1941. B.M.

Besse-en-Chandesse, Puy-de-Dome, Auvergne. E.W.Davies 1949.

BELGUIM.

Namur, Yvoir. A.H.G.Alston no. 9875 B.M.

ANDORRA.

(Other data not known).

ITALY.

near Florence. R.A.Pichi-Sermolli, 1953.

BALEARIC ISLANDS.

Col de Sollya, Majorca. E.O'Nians.

SWITZERLAND.

Commugny, Vand. On walls, A.H.G.Alston no.11750 1952. B.M.

SPAIN.

Puerto de Pico, Sierra de Grados, Avila. In shaded and fairly moist crevass (sic), in granite face with N. aspect, 5000'. A.C.Jermy. 1955.

SWEDEN.

Runmaro, near Stockholm. G. Haglund & R. Rydberg. 1948 .

Uto Island, Stockholm Archipelago. J.E.Dandy & G.Taylor, 1950. B.M.

NORWAY.

Moldegarde, near Os, Bjornafjord. On mica-schist rocks J.D.L. 1953.

GERMANY.

(Material sent by D.E.Meyer, locality not stated).

AUSTRIA.

Kranbath, Styria. On serpentine rocks. A.H.G.Alston 1952.B.M.

MIDDLE EAST.

CYPRUS.

Makhaeras. On base-rich igneous rocks in river gorges on north-facing slopes. F.Merton.

TURKEY.

between Yaruzkemal and Karinca.

SAUDI-ARABIA.

Soda, S.W. Saudi-Arabia (Yemen?). In cranny of rocks of Wadi back below Soda, 9200'. Tothill, no. 166. B.M.

NORTH AMERICA.

CANADA.

Rocky Saugeen River, Ontario. 100 miles N.W. of Toronto D.M.Britton.

Rattlesnake Point, Holton Co., Ontario. 10 miles N. of Hamilton On dolomitic limestone. D.M.Britton.

Stokes Bay, Ontario. 130 miles N.W. of Toronto. M.L.Heimburger. 1952.

U.S.A.

East Montpelier, Vermont. K. Boydston.

Mt. Belvedere, Vermont, growing on ledges where asbestos could be picked out, 2160'. let. K.Boydston.

SOUTH AFRICA.

Tsanatalana valley, Basutoland. Rare in crevices of moist cliff, 8000'.D.J.B.Killick, no.1981. 1953.

PACIFIC REGION.

JAPAN.

Honsyu. (No other details known. Sent by National Science Museum, Tokyo).

Mt. Seburi, Fukuoka Prefecture (Kyushu). T. Haga 1953.

HAWAII.

Mauna Kea, Hawaii. 6,000'. E. Horner 1954.

Mauna Loa, Hawaii. 4,000'. E. Horner 1954.

N. of Papaloa, Captain Cook, Kona, Hawaii.
On old stone wall in forest, 5100' O.Degener & T. Murashige, no. 20373. 1949. R.B.G.K., & N.S.W.N.H.

Haleakala Crater, Maui Island. W.E.Bonsey 1954.

AUSTRALIA.

Mc Keon's Creek, Jenolan Caves, N.S.W. In crevices of Silurian Limestone rock in gully under light shade, . E.F.Constable,1954. & J.D.L.1955.

Buchan, Gippsland, Victoria. On Palaeozoic Limestone rocks outcropping on grassy slopes, . N.A.Wakefield 1954; and J.D.L. 1955.

Bindi Creek, Gippsland, Victoria. In crevices of Palaeozoic Limestone Rock on south-facing bank of stream, under light shade, . N.A.Wakefield & J.D.L. 1956.

AUSTRALIA (continued)

Yallingup, Western Australia. In crevices of Pleistocene limestone rock in exposed situations and more abundantly in sheltered shaded situations, . J.D.L. 1956.

Mammoth Cave, Margaret River, Western Australia. On Pleistocene Limestone rock about collapsed entrance to Cave, J.D.L. 1956.

NEW ZEALAND.

near Tangoio, 10 miles N. of Napier, Hawke's Bay. In crevices of limestone rock of Tertiary age in exposed situations on grassy slopes, . L.B.Moore & J.D.L. 1955.

APPENDIX IV.

TETRAPLOID CYTOTYPE : IMPORTANT DETERMINATIONS OF HERBARIUM MATERIAL.

ASIA.

U.S.S.R.

Province Kutais, Caucasus. Alexeenko 1902. U.Cal., Copeland 6206.

Western Tian-Schan mountains. On calcareous rocks about confluence of the Kok-su and Tschotkal rivers (Trans.) Vatolkina 1928. Mel.N.H.

AFGHANISTAN.

Vama, Nuristan. Edelberg no. 392, 1948. 3rd Danish Expedition to Central Asia, ex. Bot. Mus. Univ. Copenhagen.

Arrandz.(Waigel-dal) 5,500'. 1953. Botanical Museum of the University of Copenhagen, E2405.

KASHMIR.

Gauhan, Bringhi valley. 1946. B.M.

CHINA.

Central Fokien. Dunn 1905. U.Cal., Copeland 6203. ex. Hong Kong Herb. (Corresponds to Japanese form).

NORTH AMERICA.

CANADA.

Nutka, N.W. coast of America. (Vancouver Island, B.C.)
David Nelson (1778) B.M.

Vancouver Island, B.C. Mt. Edinburgh, District of
Renfrew. 1902. B.M.

Chippewa River, Algoma district, Ontario. leg. W.H.Wagner,
1953.

U.S.A.

Ohio : Springfield. B.M.

West Virginia : Lost River, 3m. W. of Wardensville.
leg. W.H.Wagner.

SOUTH AMERICA.

BRAZIL.

Santa Catharina : Lages, Campo Bello.
Spannagal ? 1910. U.Cal. 441829.

ATLANTIC ISLANDS.

AZORES.

Santa Maria. B.F.C. Sennitt. B.M.

AFRICA.

NORTH AFRICA.

Morocco : High Atlas, Tadelert (Marrakesh - Our zarat
road) 1937. B.M.

Morocco : Djebel Amadour. E.K.Balls 1936. B.M.

AFRICA. (continued)

Algeria: Chiffa Gorge, Blida Atlas. A.H.G.Alston. 1937.B.M.

BRITISH EAST AFRICA.

Somaliland Protectorate : Wagar Mt. In crevices of gueiss (granite?) rocks, under summit, 6200'. P.R.O. Bally no. 10259, 1954. Glover & Gilliland No. 483. 1944. R.B.G.K.

Kenya: Longonot Mt. (N.W. of Nairobi). On lava boulders in Crater, 8000'. R.A.Dummer 1922.R.B.G.K.

SOUTH AFRICA.

Spitzkop, Transvaal. (now in Cape Province?). Lydenburg 1877. B.M.

PACIFIC REGION.

HAWAII.

"Owhyee, in arboribus emortium". David Nelson (1779) B.M.

AUSTRALIA.

Bungonia Caves, (S.E. of Goulburn), N.S.W. On limestone rocks. E.F.Constable 1956. N.S.W.N.H. P.7247.

Abercrombie Caves, (S. of Bathurst) N.S.W. Very occasional in (Palaeozoic) limestone rock crevices, 2000'. E.F.Constable 1955. N.S.W.N.H, P7136.

AUSTRALIA (Continued)

"Coolo Creek, near Paramatta", N.S.W. A.Cunningham, 1817. R.B.G.K.

Yarrangobilly, (S.W. of Canberra), N.S.W. W.Forsyth, 1901 N.S.W.N.H., P6087.

Limestone Creek, at source of Murray River, N.E.Victoria. J.H.Willis 1946, Mel. N.H.

Wairewa, E.Victoria. A.J.Tadgell 1935. Mel.N.H.

Mt. St. Bernard, Barry Mts. Victoria. 5000' G.T.Hollins, 1916, Mel.N.H.

Keegan's Bend, Glenelg River, S.W.Victoria. C.Beauglehole, 1948. Mel.N.H.

Dartmoor. (on Glenelg River), S.W.Victoria. C.Barrett, Mel.N.H.

Franklin River and Acheron (S. of Queenstown), Tasmania. Gunn no. 1532, 1845. N.S.W.N.H., R.B.G.K.

Mt. Gambier, South Australia. 1896. South Australian State Herbarium, Adelaide.

Mt. Many Peaks, about 20 miles E. of Albany, W.A. Shady wet spots in limestone (of Miocene age), among rocks below waterfall. C.A.Gardner, no. 3312. 1935. West Australian State Herbarium.

AUSTRALIA (continued)

Jarrahdene, Karridale, W.A. Morrison. B.M., R.B.G.K.

Lake Cave, near Margaret River, W.A. In crevices and between tumbled boulders of Pleistocene limestone on sides and floor of large collapsed cave, under shade of Eucalyptus trees. J.D.L. 1956.

NEW ZEALAND. (NORTH ISLAND)

Pukeora Sanatorium, W. of Waipukurau, Hawke's Bay. R.Green 1954.

upper reaches of Tukipo River, Hawke's Bay, R.Green.

Wellington (Province ?) sea to 1000', H.H.Travers, 1906, R.B.G.K.

APPENDIX V.

HEXAPLOID CYTOTYPE : LIST OF CHROMOSOME COUNTS.

(THIS PLANT IS ENDEMIC TO NEW ZEALAND).

NORTH ISLAND, N.Z.

SOUTH AUCKLAND.

Waitomo Hostel. In wall along back entrance to Hostel grounds. J.D.L. 1955.

HAWKE'S BAY.

N. of Napier, D.Holt 1955.

SOUTH ISLAND, N.Z.

NELSON.

Owen River Gorge. In crevices of limestone rock at bottom of gorge near waterfall. W.Byrom 1956.

Rameka Gorge, Pikikiruna Range. In crevices of Palaeozoic metamorphic limestone along course of creek. V.M.Scott & J.D.L. 1955,1956.

Canaan Track, Pikikiruna Range. In crevices of Palaeozoic metamorphic limestone on site of relatively recently ~~developed~~ cleared rain forest. V.M.Scott & J.D.L. 1955, 1956.

Canaan Road, Takaka. V.M.Scott 1955.

Rangihaieta Headland, Takaka. In crevices of limestone rock. V.M.Scott 1955 & J.D.L. 1955.

NELSON (continued)

Takaka Hill, Pikikiruna Range: In crevices of Palaeozoic metamorphic limestone. V.M.Scott. K.Marshall and J.D.L. 1956.

MARLBOROUGH.

Molesworth Station. In crevices of Greywacke rock. M. Simpson 1956.

CANTERBURY.

Waiau Marble Quarry. In crevices of limestone rock of Tertiary age. J.D.L. 1956.

Bourne Stream, Lower Waiau. In crevices of limestone rock of Tertiary age, in exposed situations.

Cass. In crevices of Greywacke rock near Canterbury University College Biological Station. J.D.L. 1955, 1956.

Mt. Somers. In crevices and grikes of pavements and rock-faces composed of limestone of Tertiary age, in open situations. J.D.L. 1956.

Halpin's Gorge. In crevices of Greywacke rock at head of gorge. M. Scott & J.D.L. 1956.

Avoca. In cultivation at Otari, Wellington.

Ashburton River Gorge. In crevices of Greywacke rock-face above Ashburton river. J.D.L. 1956.

Castle Hill, Broken River Basin. Rare in crevices of

Tertiary limestone rock, in exposed situations. J.D.L. 1955.

Waitaki Hydroelectric Lake. In crevices of dry Greywacke rock, associated with Pleurosorus rutaefolius and Cheilanthes distans. J.D.L. 1956.

Glencoe Creek, Hooker valley. Rock crevices in Greywacke rock on sites of gully in sub-alpine scrub. J.D.L. 1956.

Monument, Hooker valley. Growing between Greywacke blocks and fragments in old terminal moraine. J.D.L. 1956.

OTAGO.

Ben Lomond massif, near Queenstown. In crevices of mica-schist rock. J.D.L. 1955.

SUPPLEMENT.

ASPLENIUM ADULTERINUM.

INTRODUCTION.

As was described earlier in the main body of the thesis (pp. 94 - 97), Asplenium adulterinum Milde has been used in a series of hybrids with the diploid cytotype of Asplenium trichomanes as a standard by which to obtain an indirect measure of the degree of synaptic homology (see P. 126), existing between forms of this diploid obtained from different parts of its world range. The interpretations drawn from study of chromosome pairing at meiosis in this series of triploid hybrids are only valid if it is true that the reduced complement of Asplenium adulterinum consists of one trichomanes-type genome, and one viride-type genome, or in other words, if it is true that Asplenium viride and the diploid cytotype of Asplenium trichomanes are the apparent parents of Asplenium adulterinum, this plant being an allotetraploid.

The cytogenetic evidence justifying this assumption must now be presented. This will be preceded by a summary of the distribution and comparative morphology of Asplenium adulterinum.

DISTRIBUTION. (Fig. 133).

This plant is endemic to Central Europe and Scandinavia, and even within this restricted range, it is strictly confined to rocks of the serpentine group. Recent discoveries of the plant in Switzerland and the Hardanger district of Norway have not altered the essential pattern of its distribution, which consists of a limited number of isolated localities widely scattered over its range. Though known from several stations in eastern Germany, Silesia, and Austria, and from perhaps a dozen localities in Norway, it is otherwise recorded from only two areas in Central Finland, and one locality in both Sweden and Switzerland.

It is clear that the extremely discontinuous nature of the distribution of Asplenium adulterinum is occasioned by its absolute preference for "serpentine" rocks. It would appear that this lithological factor limits its distribution in Central Europe to the extent that other factors of the environment have comparatively little practical significance, since it appears to be found almost everywhere where chemically suitable rock outcrops in this region. This is in contrast to its distirbution in Scandinavia, where its occurrences are further restricted by microclimatic considerations. There is general agreement amongst the

authors describing Scandinavian serpentine vegetation (Bjorlykke, 2 , and Rune, 9), that Asplenium adulterinum shows distinct thermophilic tendencies in Scandinavia. There it is never to be found at any great altitude, and usually only on rocks fully exposed to the south, which may sometimes exhibit quite arid conditions in the height of summer. In August of 1953, I had the opportunity to visit some of the localities in the Sunnmore district of Norway which were first described by Bjorlykke (2), and my own conclusions only endorse his opinion (see figs 134-137). On the evidence before them, both Scandinavian authors logically consider the occurrences with them of Asplenium adulterinum to be of Central European origin.

COMPARATIVE MORPHOLOGY.

In morphology this plant is intermediate between Asplenium trichomanes and Asplenium viride, with one or both of which species it is usually found growing. The most characterisitc feature of Asplenium adulterinum is the point on the rachis at which the latter changes in colour from brown to green (see fig. 138). In Asplenium trichomanes the rachis is brown-black to the tip, whereas in Asplenium viride the brown colouration does not extend beyond the first pair

of pinnae. In Asplenium adulterinum the rachis is brown to within approximately an half-inch or four or five pinna-pairs of the frond apex. This is the only feature of Asplenium adulterinum which can serve to immediately and conclusively distinguish it from Asplenium trichomanes and Asplenium viride. With regard to certain other characters, such as the form of the pinnae, and the distribution and form of the sori, concerning which Asplenium trichomanes and Asplenium viride are obviously distinct, Asplenium adulterinum is intermediate in form, but without resulting in a particularly characteristic appearance.

CYTOLOGY AND CYTOGENETICS (Figs 140-146).

CYTOLOGY OF ASPLENIUM ADULTERINUM. (Fig. 140).

Chromosome counts for Asplenium adulterinum have been published for material from Kraubath, in Austria (Manton, 5, Meyer 7, and Lovis 4), and from Zoblitz, in Saxony (Meyer,6). Manton and Lovis both studied meiosis, and report that this is regular, 72 bivalents being formed. Meyer has made chromosome counts from root-tips, and reports that 2n = 144.

In the course of this investigation, thanks to the assistance of several kind collectors whose help is gratefully acknowledged, it has been possible to make chromosome counts

on material from several localities in various parts of the range of Asplenium adulterinum. A complete list is given below. All counts were made at meiosis, and in all cases it was found that n = 72.

1. Kraubath,Austria.....collector:A.H.G.Alston.
2. Bosco,Canton Tessin,Switzerland..supplied by Prof. M. Geiger-Huber.
3. Rauholmane Islands, near Lindas,N.of Bergen,Norway.. ....supplied by Prof. ~~Kurt~~ Knut Faegri.
4. Halandsdalen, S.E. of Bergen,Norway.....collector: Prof.Ivar Segelberg.
5. Norddal, Sunnmore, Norway.....collector: J.D.L.
6. Rodbergvik, Sunnylvenfjord, Sunnmore,Norway..... ..collector : J.D.L.
7. Bjorkedalen, Sunnmore, Norway.....collector J.D.L.
8. Rodon Island, Tjotta, Nordland, lat.66° Norway.... ..collector: Dr. Olof Rune.
9. Mt. Taberg, s.w. end of Lake Vattern, Smaland, Sweden, ..collector Dr. Olof Rune.

It is therefore possible to confidently conclude that Asplenium adulterinum is tetraplcid throughout its range with a regular meiosis producing 72 bivalents. This is in contrast to its supposed parents, Asplenium viride, and the diploid cytotype of Asplenium trichomanes.

Asplenium viride has been found to be diploid and regular in meiosis, producing 36 bivalents, whenever it has been examined (Manton 5, Meyer, 7, 8, Britton, 3, and Lovis, 4). These reports include examination of Asplenium viride from three localities where Asplenium adulterinum also grows, namely Kraubath, Austria, Bjorkedalen, Norway, and Zoblitz, Saxony. (See fig. 141).

The cytology of the diploid cytotype of Asplenium trichomanes is described in the main section of this thesis (p. 87 , figs 94-97a).

CYTOGENETICS OF WILD HYBRIDS.

Plants intermediate between Asplenium adulterinum and Asplenium viride are often found where these two species grow together. Such plants have long been suspected to be of hybrid origin, especially since their spores were known to be abortive, an observation first made by Ascherson in 1913 (1), and this hybrid is known as A.xposcharskyanum (Hoffmann) Dorfler. (See fig. 139).

The cytology of A.xposcharskyanum was first investigated by Manton (6), in plants brought back to this country by Mr. A.H.G.Alston from Kraubath, Austria, and subsequently by Lovis (4), in the same plants from Kraubath, and also in plants personally collected at Bjorkedalen, Norway.

It is found that A. xposcharskyanum from both of these localities is triploid, and that 36 bivalents and 36 univalents are constantly formed at meiosis (figs 142 & 143).

Meyer (8), has counted chromosomes in root-tips of plants of A.xposcharskyanum from both Kraubath, Austria and Zoblitz, Saxony, and confirms that this plant is triploid, with 2 n = 108.

CYTOGENETICS OF SYNTHESISED HYBRIDS.

Triploid hybrids have been successfully synthesised between Asplenium adulterinum and both Asplenium viride and the diploid cytotype of Asplenium trichomanes.

A.ADULTERINUM X A.VIRIDE (Fig. 144)

Details of hybridisation attempts:

	No.♀.	H.	S.	D.	No.♀ : H
A.adulterinum (Austria)♀ x A.viride ♂	22	0	2	0	20 : 0
A.viride ♀ x A.adulterinum(Austria) ♂	121	5	10	0	111 : 5
					131 : 5

Percentage success = 3.75%

Analysis of meiosis:

36 bivalents and 36 univalents constantly formed.

This cytological result is exactly the same as was obtained for the wild examples of this hybrid (see above). The synthesised hybrids are also identical in morphology with the wild hybrids.

A.ADULTERINUM X A.TRICHOMANES : DIPLOID (Figs 145 & 146).

Details of hybridisation attempts:

	No.♀.	H.	S.	D.	No♀:H
A.adulterinum(Austria)♀ X A.trichomanes^{2x}(Europe)♂	188	0	6	1	
A.adulterinum(Switzerland)♀ X A.trichomanes^{2x}(Europe)♂	33	1	0	4	
A.adulterinum(Norway)♀ X A.trichomanes^{2x}(Europe)♂	63	0	5	0	
A.adulterinum(Sweden)♀ X A.trichomanes^{2x}(Europe)♂	48	0	1	0	
	332	1	12	5	315:1
A.trichomanes^{2x}(Europe)♀ X A.adulterinum(Austria)♂	116	6	9	4	103:6
	448	7	21	9	418:7

Percentage success = 1.75%

Analysis of meiosis:

36 bivalents and 36 univalents are constantly formed. Hybrids incorporating material of Asplenium adulterinum from Switzerland and Austria give the same meiotic analysis.

A.VIRIDE X A.TRICHOMANES : DIPLOID.

Attempts have also been made to synthesise hybrids between Asplenium viride and the diploid cytotype of Asplenium trichomanes. These attempts have been unsuccessful.

Details of hybridisation attempts:

	No.♀ used.	Hybrids
A.trichomanes 2x ♀ X A. viride ♂	61	0
A.viride ♀ X A.trichomanes 2x ♂	201	0
	262	0

Experiments where female prothalli treated with colchicine within a few hours after hybridisation.

	No.♀ used	Hybrids.
A.viride ♀ X A.trichomanes 2x ♂	58	0

DISCUSSION AND CONCLUSIONS.

It has been demonstrated above that Asplenium adulterinum is a tetraploid species with regular pairing of its chromosomes at meiosis. It has also been shown that in both the triploid hybrids between Asplenium adulterinum and Asplenium viride and the triploid hybrids between Asplenium adulterinum and the diploid cytotype of Asplenium trichomanes, two of the three genomes present pair completely at meiosis. The logical conclusion from these cytological facts is that of the two genomes present in the reduced complement of Asplenium adulterinum, one genome has complete synaptic homology with the genome of Asplenium viride, and the other genome has complete synaptic homology with that of the diploid cytotype of Asplenium trichomanes. A further conclusion follows that Asplenium adulterinum is an allotetraploid, and the two diploids are its probable ancestral parents. This conclusion is strongly supported by the morphological evidence, which indicates that Asplenium adulterinum is in

fact intermediate between A.trichomanes and A.viride.

Not all the facts so clearly support the hypothesis that in Asplenium viride and the diploid cytotype of Asplenium trichomanes we have the ancestral parents of Asplenium adulterinum. To demonstrate beyond all reasonable doubt that the hypothesis is true, it is necessary to achieve the artificial re-synthesis of Asplenium adulterinum, by first securing a diploid hybrid between the two supposed parents, which hybrid would presumably be sterile, and then inducing doubling of the chromosome number in this diploid hybrid, thus restoring fertility by bringing the hybrid to the tetraploid level. As has been described above, it has not yet been possible to synthesise such a diploid hybrid between Asplenium viride and the diploid cytotype of Asplenium trichomanes. However the fact that this has not been achieved should not lead to the assumption that it is not possible. Reference to the figures for percentage success in attempts to synthesise A.xposcharskyanum will show that even hybrids which occur commonly in a state of nature may be difficult to produce experimentally. Extraordinary efforts may be necessary to achieve the artificial synthesis of a hybrid which evidently must be very rare even in the wild.

Assuming that the present hypothesis concerning the origin of Asplenium adulterinum is true, it is surprising

that an allotetraploid formed from two such widely spread and successful species as are Asplenium viride and the diploid cytotype of Asplenium trichomanes should itself be restricted to such a very narrow range of habitat. One would expect that an allotetraploid formed from Asplenium viride and Asplenium trichomanes would prove to be a vigorous and successful plant. The explanation of this paradox may be that Asplenium adulterinum arose from physiologically adapted serpentine races of Asplenium trichomanes and Asplenium viride, and that Asplenium adulterinum owes its restriction to serpentine to the nature of its origin.

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ACKNOWLEDGMENTS.

During the course of the investigation, I have been the fortunate recipient of a great deal of assistance from diverse sources, and it is my great pleasure to acknowledge this help here.

I wish first to record my appreciation and gratitude to the Nature Conservancy and the New Zealand Department of Scientific and Industrial Research for their financial support. A Nature Conservancy Research Studentship was held from July 1952 to June 1955, and a New Zealand National Research Fellowship from August 1955 to August 1956.

My thanks are due to Professor W.R.Philipson, who kindly made facilities available to me in the Botany Department at Canterbury University College, Christchurch, during tenure of the New Zealand Fellowship.

I wish to thank the authorities of various Herbaria who allowed me access to their collections, and who granted me study facilities in their Institutions, including the British Museum (Natural History,) Royal Botanic Gardens, Kew, Melbourne National Herbarium, New South Wales National Herbarium, West Australian State Herbarium, Auckland Museum, Dominion Museum, Wellington, and the Canterbury Museum, Christchurch. I would also like to express my gratitude to members of the staffs of these Institutions who helped me in my work. I cannot mention them all by name here, but I

must record my indebtedness to the late Mr.A.H.G.Alston, whose generously given advice and assistance were of the greatest value to the investigation.

It is not practicable to mention all those in different parts of the world who have given help in the work on distribution and ecology, either by assisting me to visit various localities, or by personally collecting material. However, I must mention my good friend Mr. Norman Wakefield of Noble Park, Victoria, who provided transport and accompanied me to the three localities of Asplenium trichomanes visited in Victoria, a total travelling distance of well over one thousand miles.

I wish to acknowledge with gratitude the valuable advice of Mr. Bryan Clarke with regard to the photographic techniques used in this investigation.

Finally, it is my great pleasure to record my grateful thanks to Professor Manton for help and encouragement. It goes without saying that without her invaluable advice and support this investigation could never have been begun, let alone be brought to a successful conclusion.

AN EVOLUTIONARY STUDY OF THE FERN *ASPLENIUM TRICHOMANES*.

J. D. LOVIS.

Thesis submitted for the Degree of Doctor of Philosophy in the University of Leeds.

Department of Botany

1958.

VOLUME II : FIGURES.

LIST OF FIGURES.

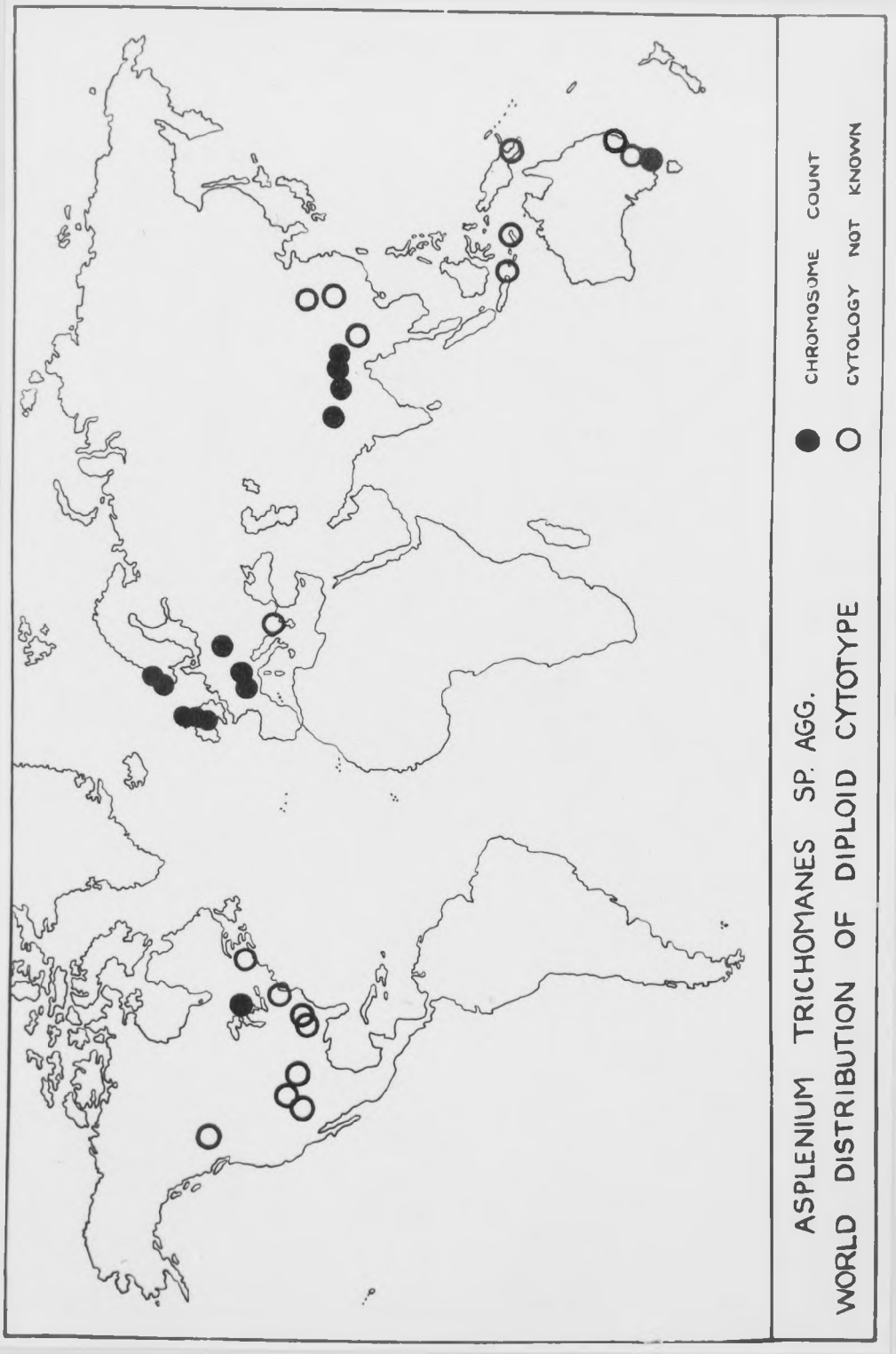
ASPLENIUM TRICHOMANES.

LIST OF FIGURES (CONT.)

ASPLENIUM ADULTERINUM (SUPPLEMENT)

DISTRIBUTION MAPS

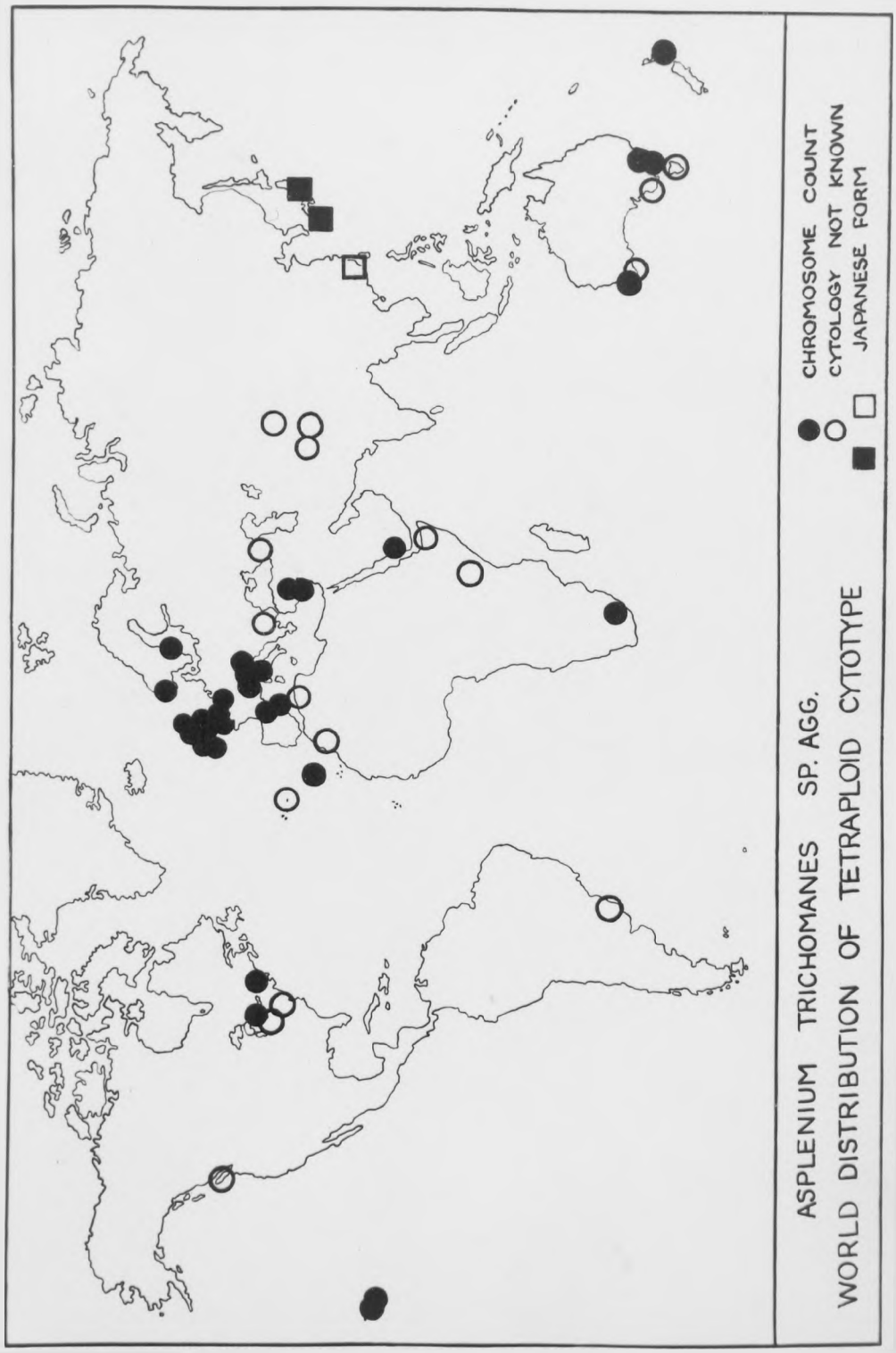
Figs. 1 - 7.



Asplenium trichomanes sp. agg.

World distribution of the diploid cytotype.

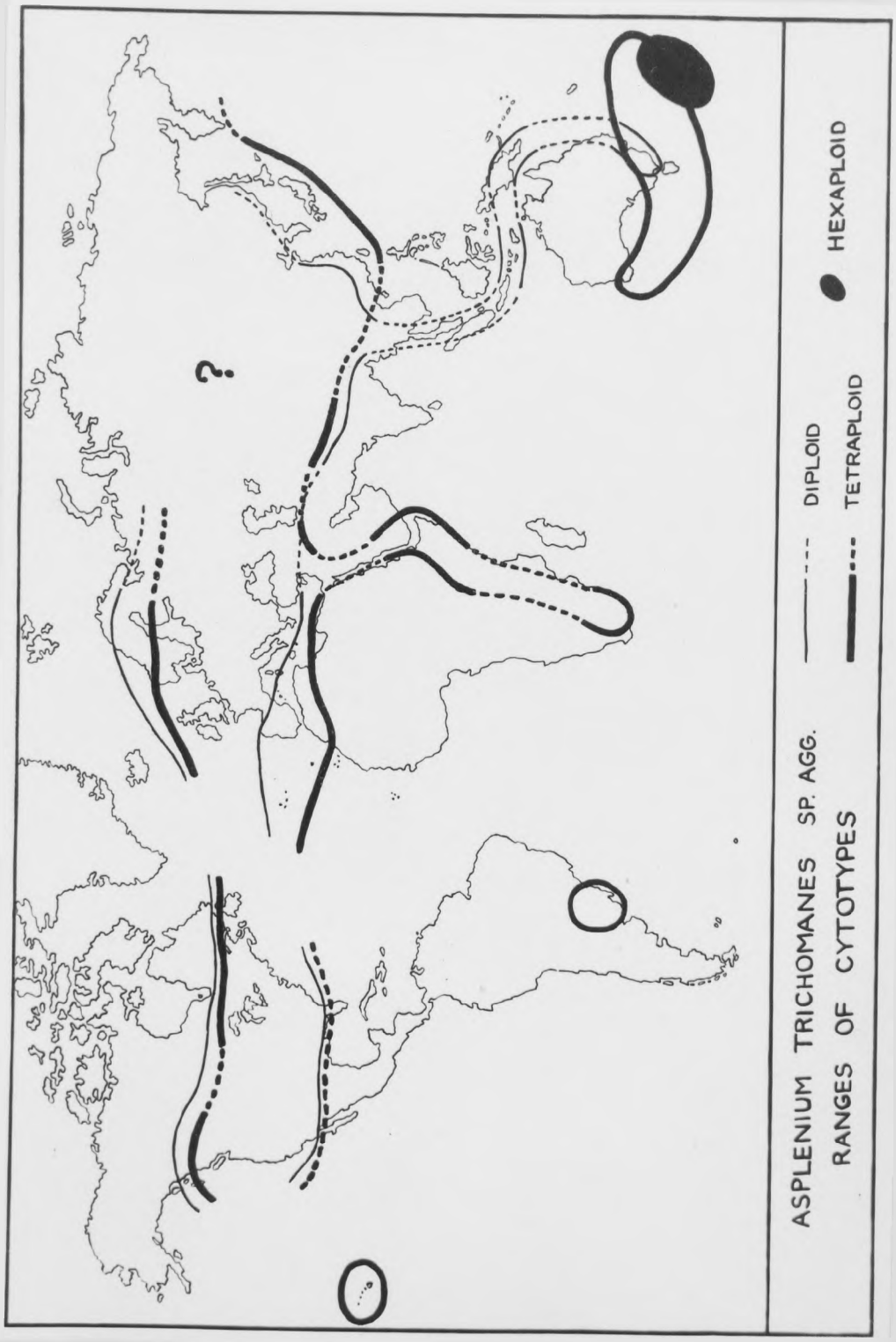
Distribution map. Fig. 2.



Asplenium trichomanes sp. agg.

World distribution of the tetraploid cytotype.

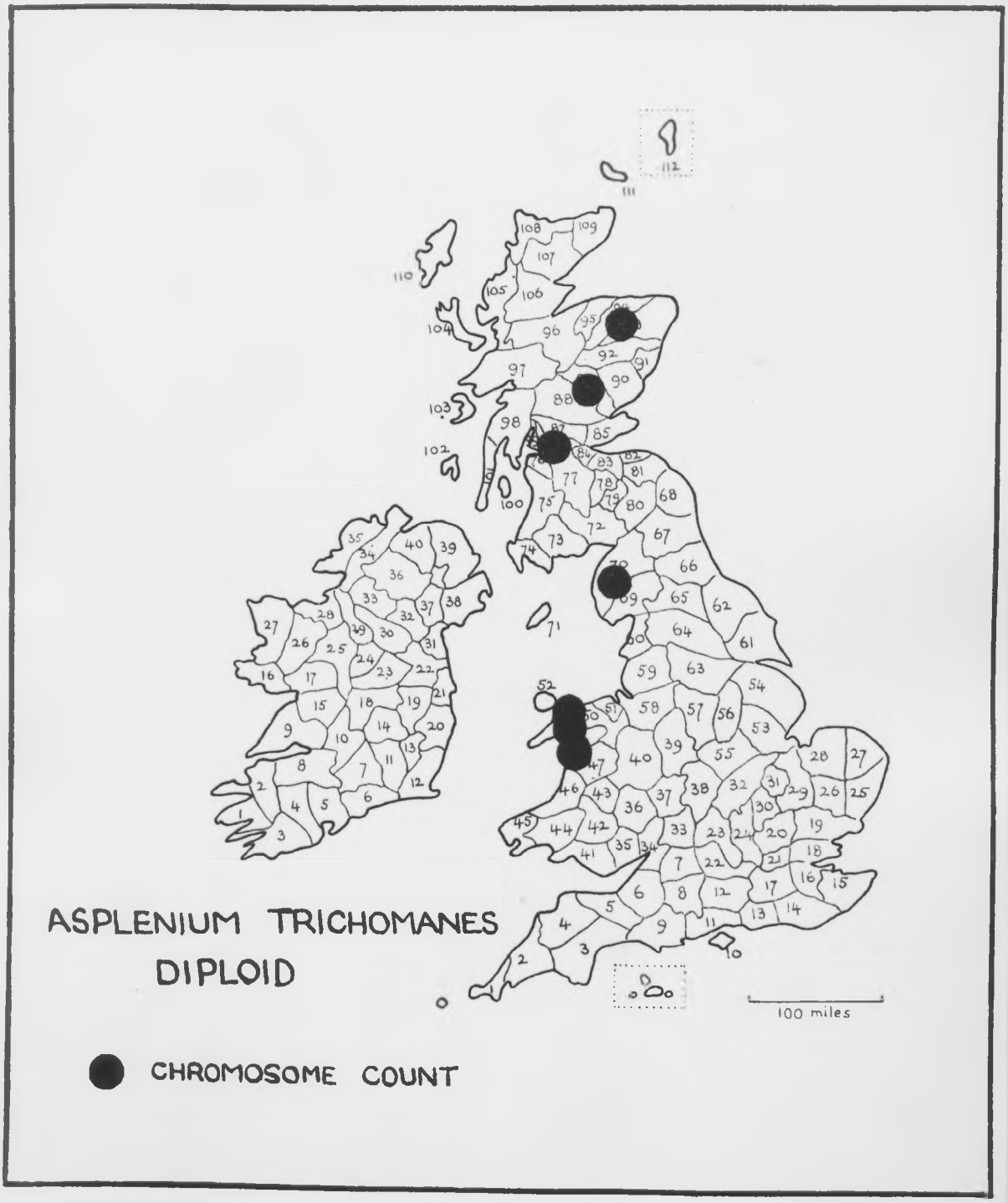
Distribution map. Fig. 3.



Asplenium trichomanes sp. agg.

World ranges of the three cytotypes.

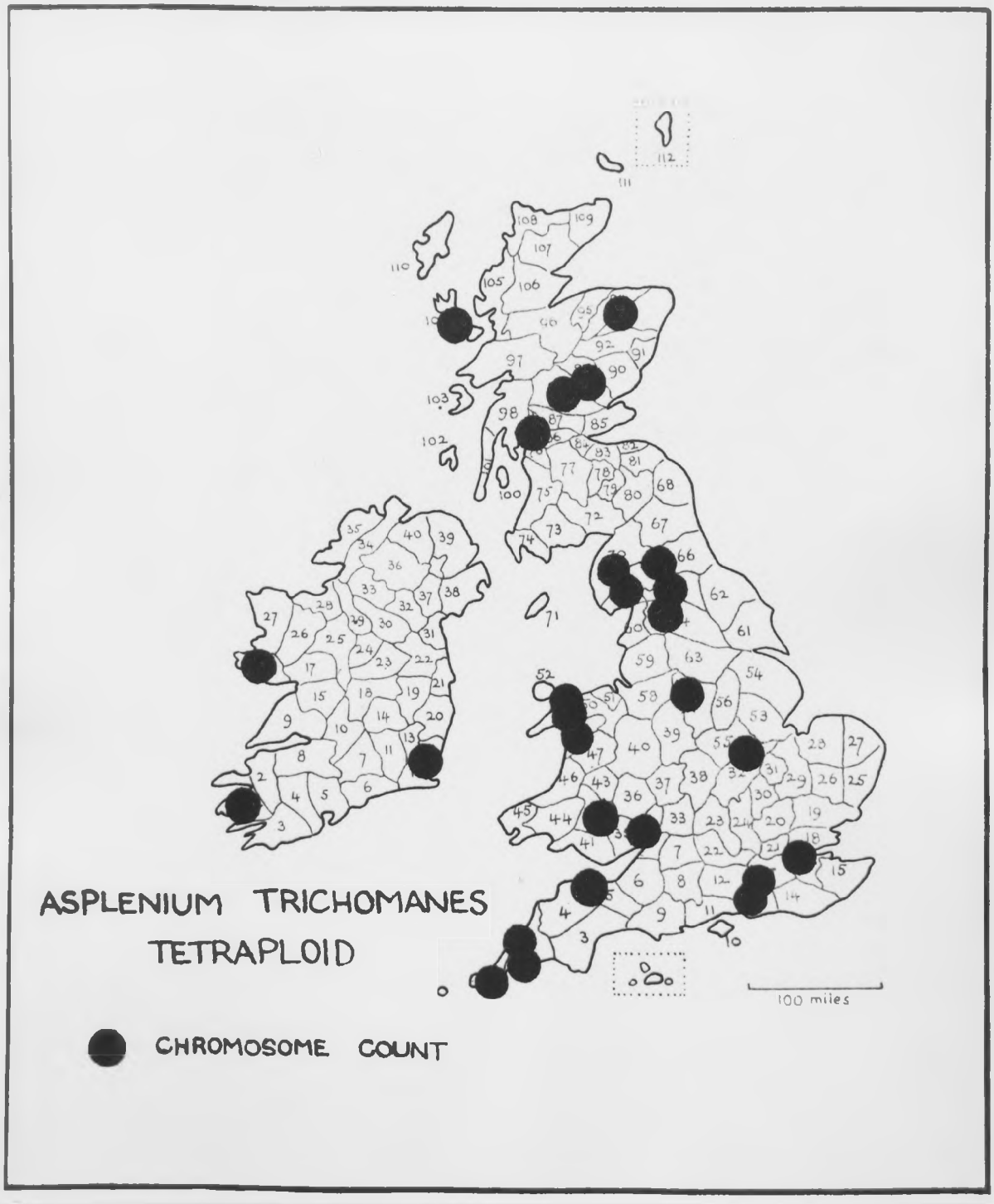
Distribution map. Fig. 4.



Asplenium trichomanes sp. agg.

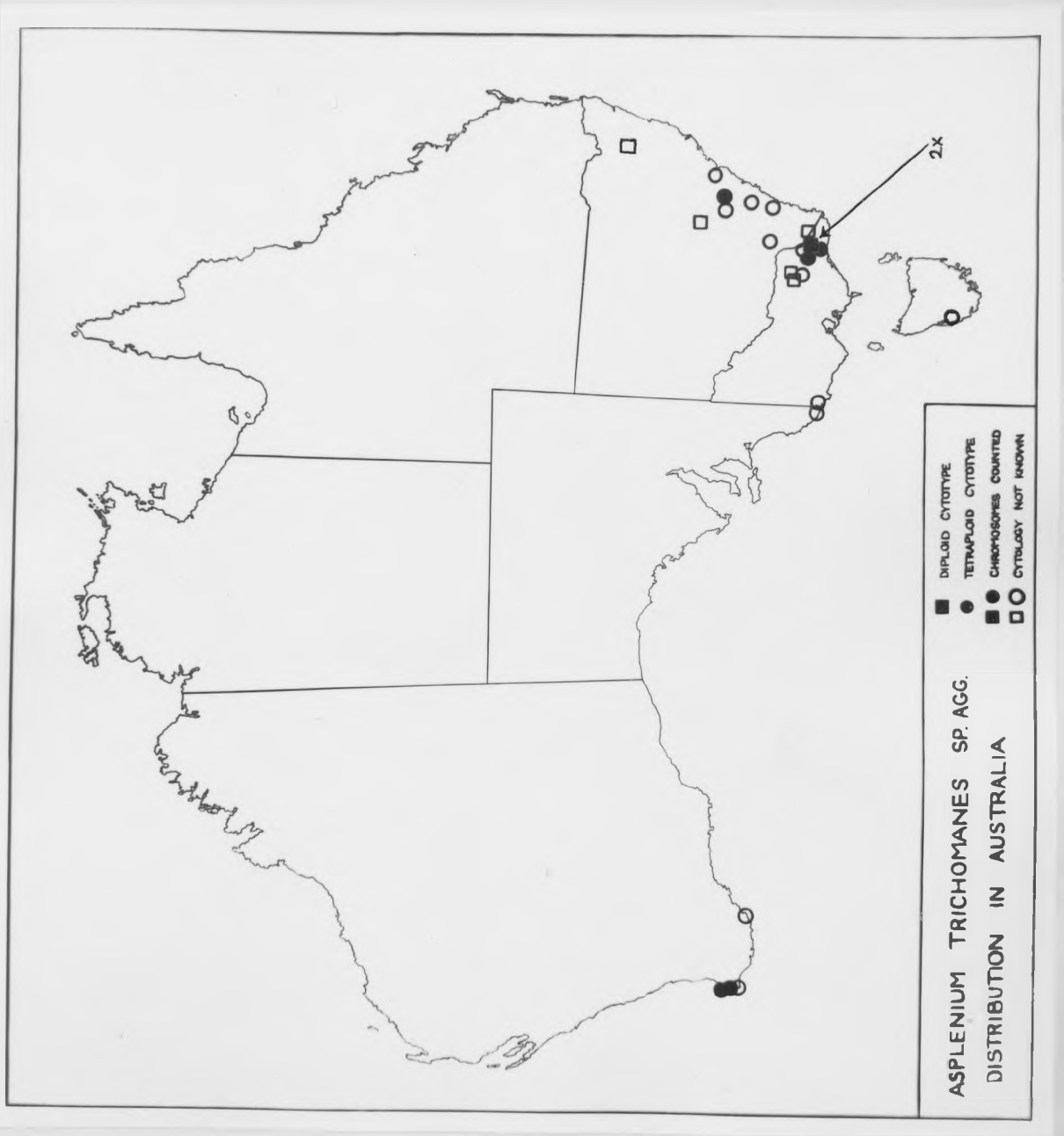
Distribution of the diploid cytotype in the British Isles.

Distribution map. Fig. 5.



Asplenium trichomanes sp. agg.

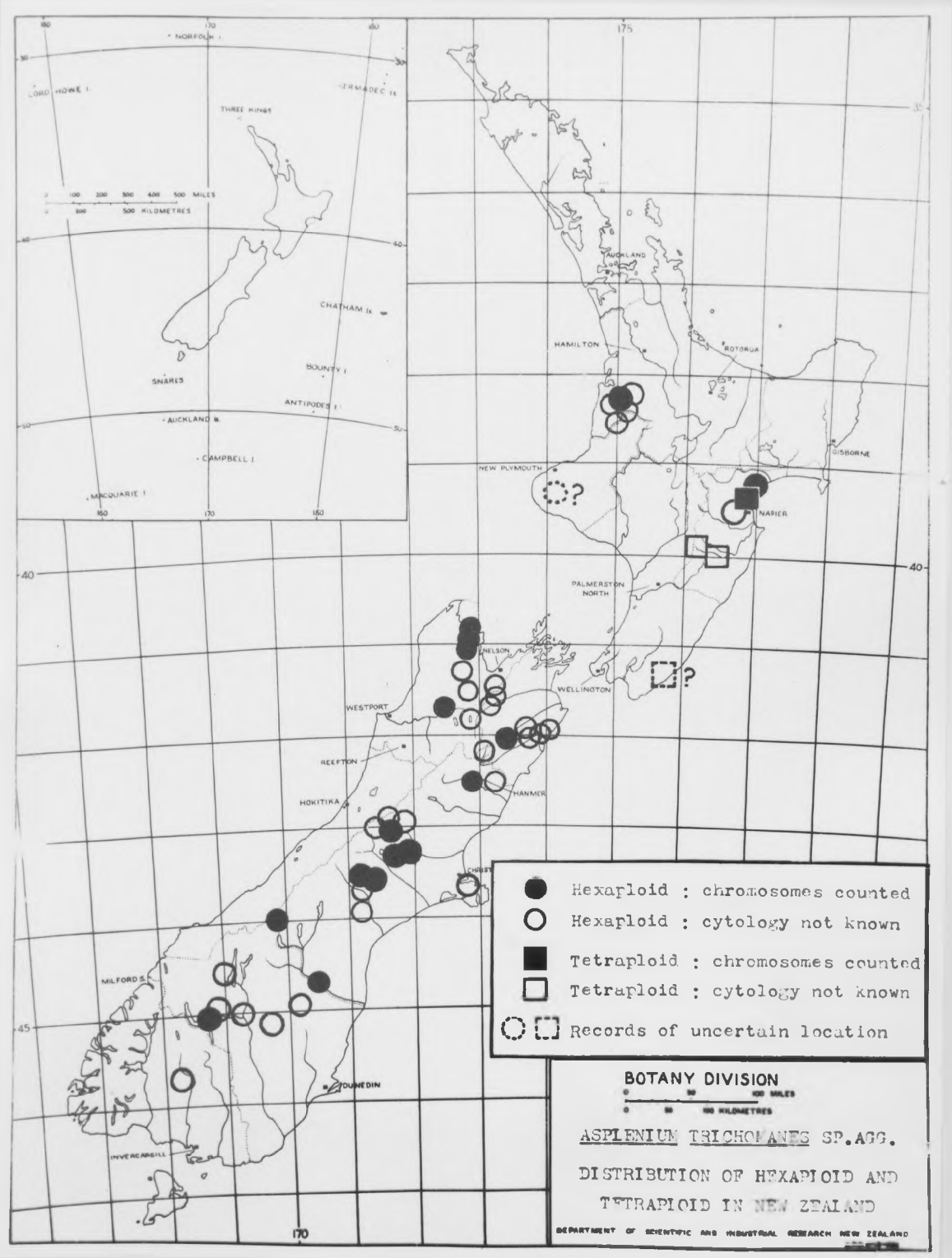
Distribution of the tetraploid cytotype in the British Isles.



Asplenium trichomanes sp. agg.

Distribution of the diploid and tetraploid cytotypes in Australia.

Distribution map. Fig. 7.



Asplenium trichomanes sp. agg.

Distribution of the hexaploid and tetraploid cytotypes in New Zealand.

AUTECOLOGY

HABITAT PHOTOGRAPHS : DIPLOID CYTOTYPE

Figs. 8 - 15.

Fig. 8.

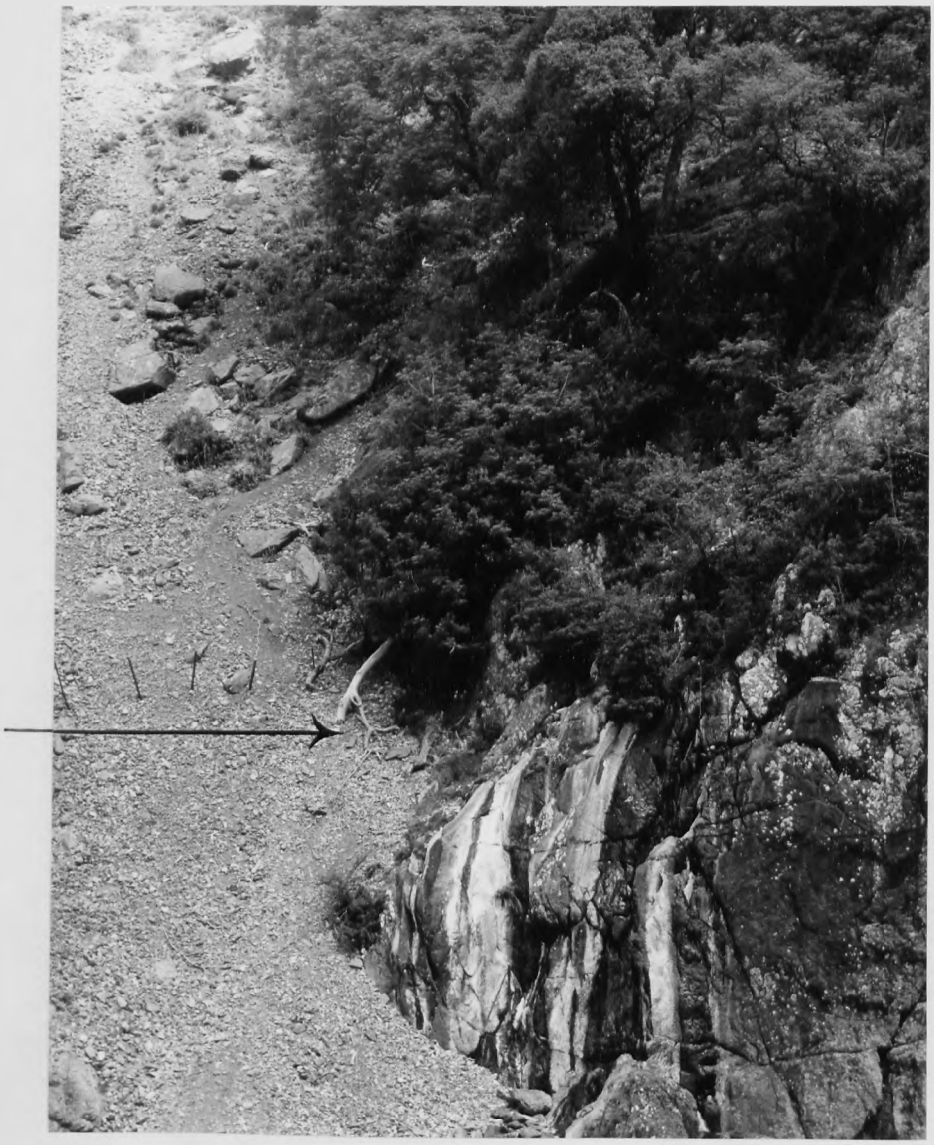
Diploid cytotype.

Aber Falls, Caernarvonshire.

The Falls, which face north, are in flood in this photograph. One colony of the diploid cytotype lies within the influence of the spray visible at the foot of the Falls.

Fig. 9.

Diploid cytotype.



Aber Falls, Caernarvonshire.

Habitat of diploid cytotype on shaded rocks on left of the foot of the Falls.

Fig. 10.

Diploid cytotype.

Pass of Llanberis, Caernarvonshire.

South facing crags above Pass. Habitat of diploid cytotype is in deep shaded clefts and crevices on face of crags.

Fig. 11.

Diploid cytotype.

Stenton Rocks, near Dunkeld, Perthshire.

Plants of the diploid cytotype on mossy rock under shade of light woodland.

Fig. 12.

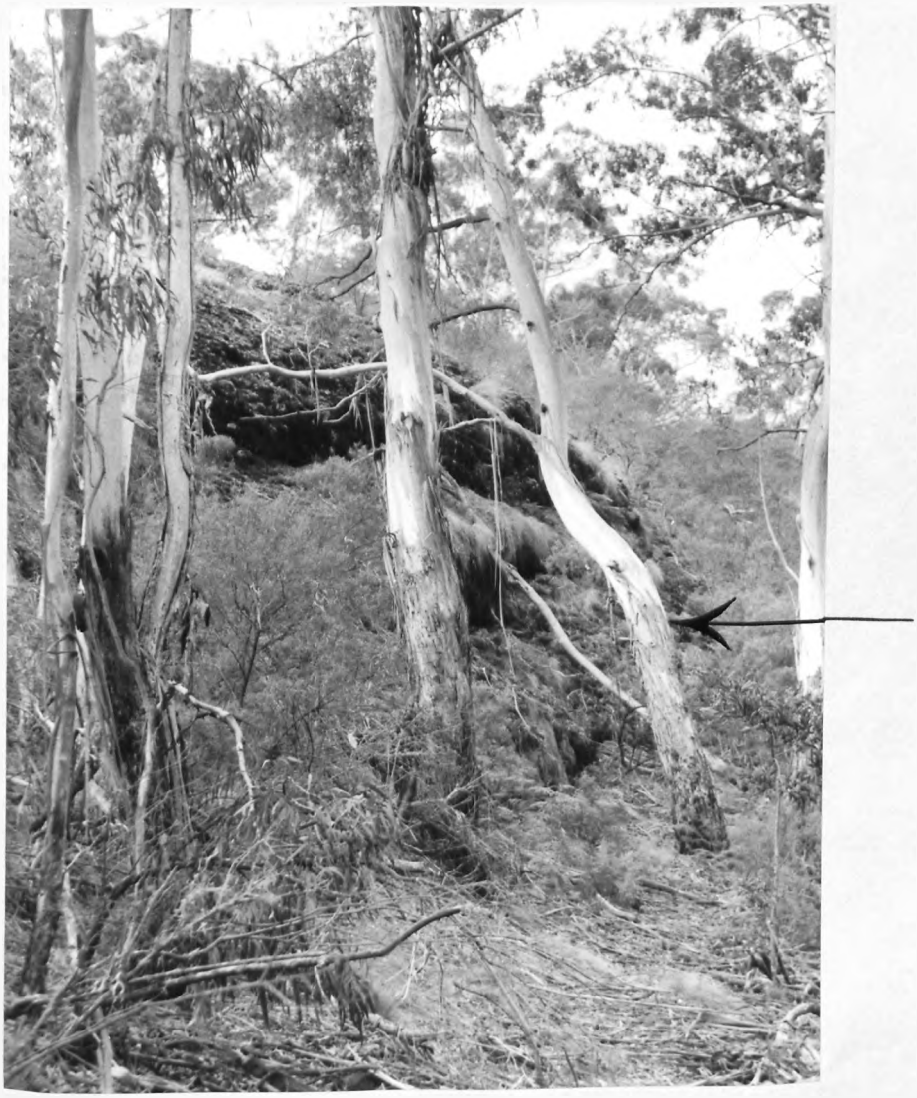
Diploid cytotype.

Craig Dorney, Cabrach, Aberdeenshire.

The diploid cytotype grows in the deep crevices between the tumbled boulders of serpentine rock visible in the fore-ground of the photograph.

Fig. 13.

Diploid cytotype.



Wulgulmerang, Gippsland, Victoria, Australia.

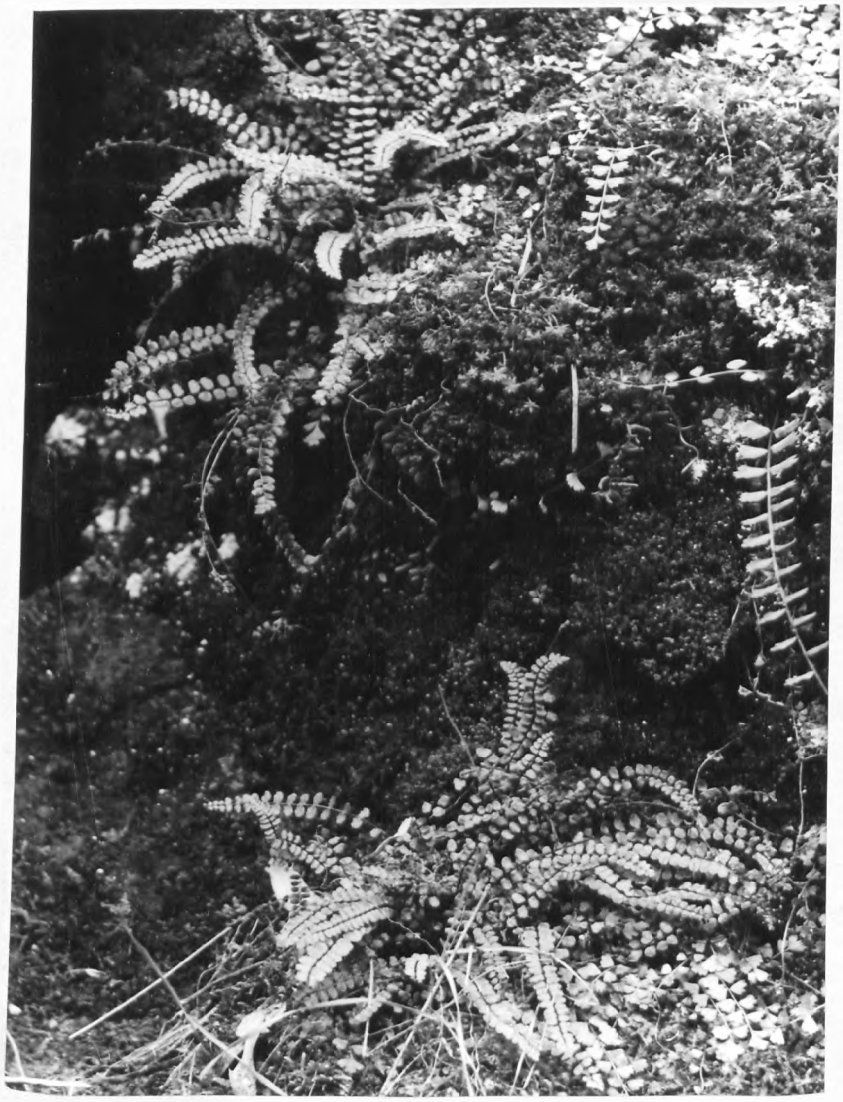
The diploid cytotype grows on the rock exposure in the near distance, under the light shade of Eucalyptus trees.

Diploid cytotype.

Wulgulmerang, Gippsland, Victoria, Australia.

Plants of the diploid cytotype growing on a mossy rock under the shade of light Eucalyptus forest.

Diploid cytotype.



Wulgulmerang, Gippsland, Victoria, Australia.

The diploid cytotype growing intermingled with Asplenium flabellifolium, at base of rock exposure.

AUTECOLOGY

HABITAT PHOTOGRAPHS : TETRAPLOID CYTOTYPE

Figs. 16 - 41.

Fig. 16.

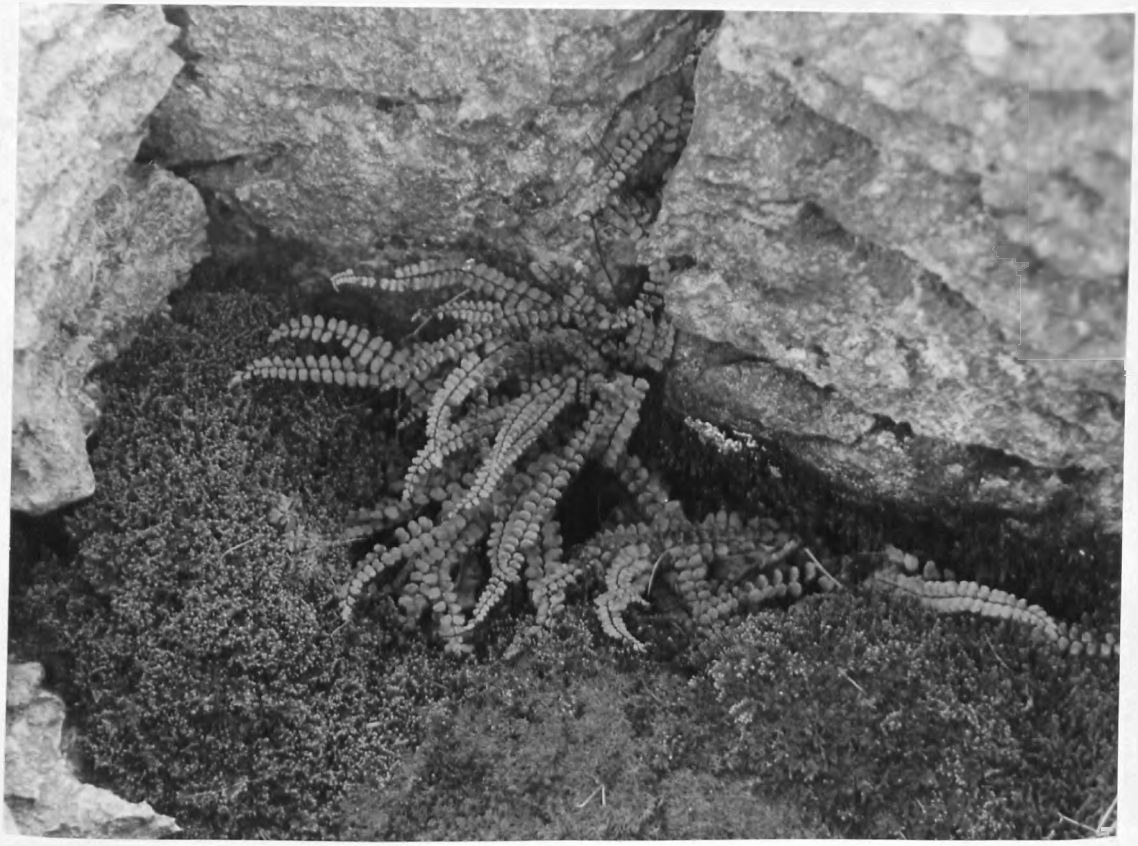
Tetraploid cytotype.

Selside, Ribblesdale, Yorkshire.

The tetraploid cytotype is found in the grikes of the eroded limestone pavement.

Fig. 17.

Tetraploid cytotype.



Selside, Ribblesdale, Yorkshire.

A plant of the tetraploid cytotype growing in a shallow grike in limestone pavement. This photograph, taken in April, shows the persistent character of the fronds under sheltered conditions, even in upland areas.

Fig. 18.

Tetraploid cytotype.

Selside, Ribblesdale, Yorkshire.

A plant of the tetraploid cytotype growing in a grike in limestone pavement near Ingleborough. This photograph, taken in April, demonstrates how the fronds will persist throughout the winter even in upland areas, if the plant is growing in a sheltered situation.

Fig. 19.

Tetraploid cytotype.

Selside, Ribblesdale, Yorkshire.

Plants of the tetraploid cytotype growing on the sides of a deep grike in limestone pavement. See also fig. 20.

Fig. 20.

Tetraploid cytotype.

Selside, Ribblesdale, Yorkshire.

A plant of the tetraploid cytotype growing in the side of a deep grike in limestone pavement. See also fig. 19.

Fig. 21.

Tetraploid cytotype.

Rainscar, Silverdale, N. of Settle, Yorkshire.

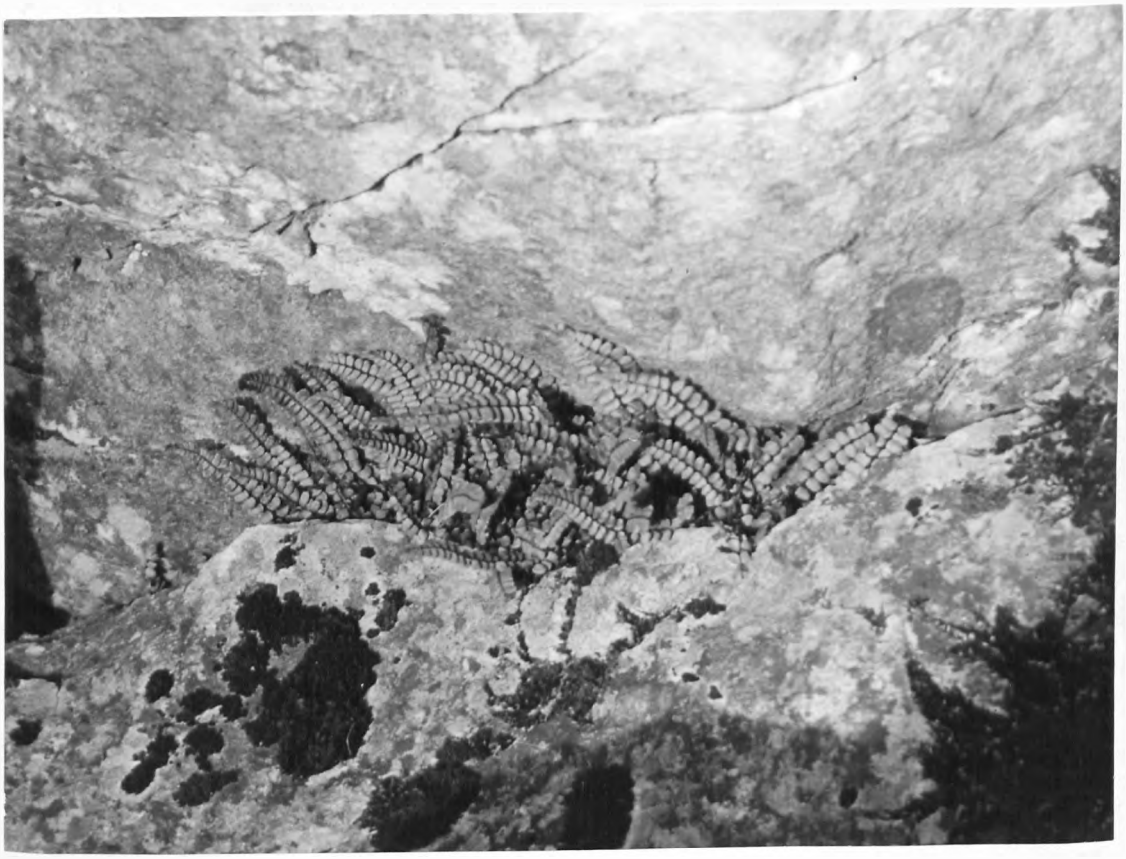
Habitat of the tetraploid cytotype in crevices of limestone crag or scar. Pen-y-Ghent is seen in the background. See also figs. 22 and 23.

Tetraploid cytotype.

Rainscar, Silverdale, N. of Settle, Yorkshire.

A plant of the tetraploid cytotype growing in a crevice on a limestone scar. See also figs. 21 and 23.

Tetraploid cytotype.



Rainscar, Silverdale, N. of Settle, Yorkshire.

A plant of the tetraploid cytotype growing in a crevice on a limestone scar. See also figs. 21 and 22.

Tetraploid cytotype.

Craig Dorney, Cabrach, border of Aberdeen and Banff.

Habitat of the tetraploid cytotype is shaded crevices of serpentine rock in the gully in the photograph.

Fig. 25.

Tetraploid cytotype.

Stenton, near Dunkeld, Perthshire.

Plants of the tetraploid cytotype growing in crevices of a mortared stone wall.

Tetraploid cytotype.

Fountains Abbey, near Ripon, Yorkshire.

Plants of the tetraploid cytotype growing in crevices of a mortared limestone wall near the Abbey, together with Polypodium vulgare s. l.

Fig. 27.

Tetraploid cytotype.

Fountains Abbey, near Ripon, Yorkshire.

Habitat of tetraploid cytotype in crevices between stones of the ruins of the Abbey. See also fig. 28.

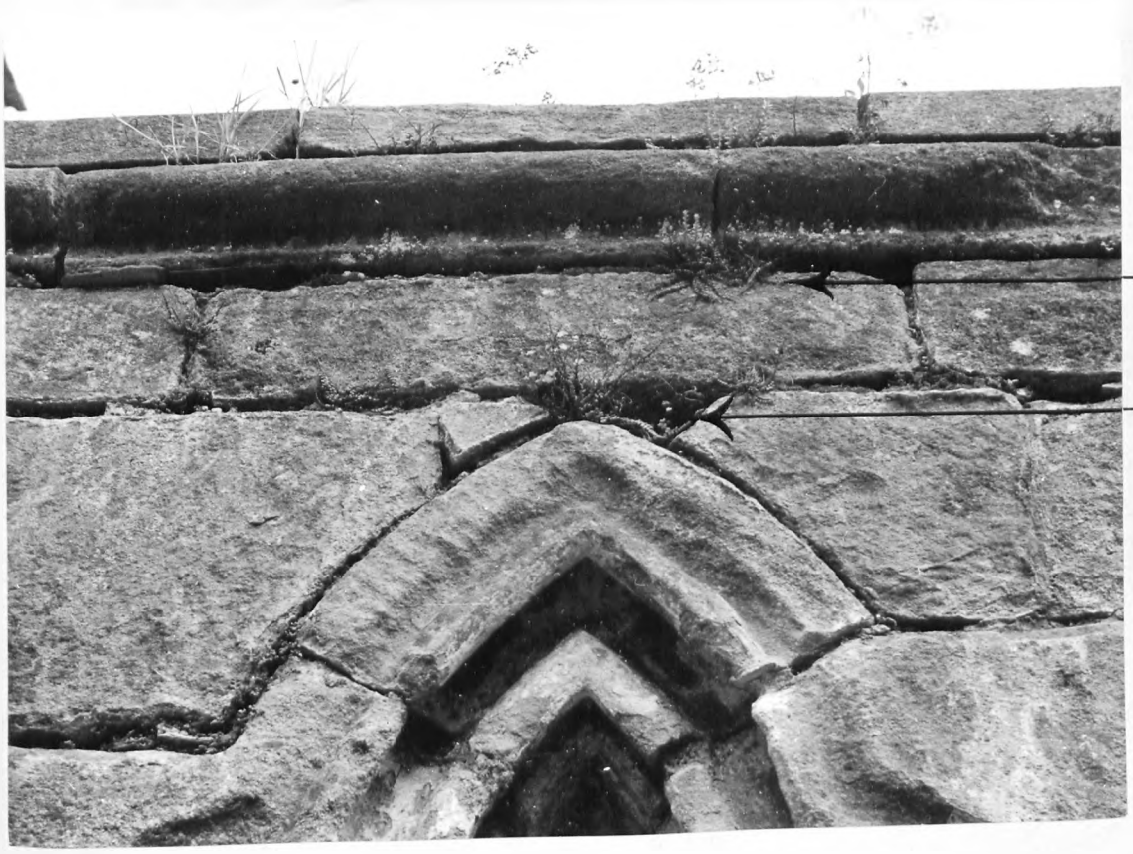
Tetraploid cytotype.

Fountains Abbey, near Ripon, Yorkshire.

Plants of the tetraploid cytotype growing in association with Polypodium vulgare s.l. on a wall of the ruined Abbey. See also fig. 27.

Fig. 29.

Tetraploid cytotype.

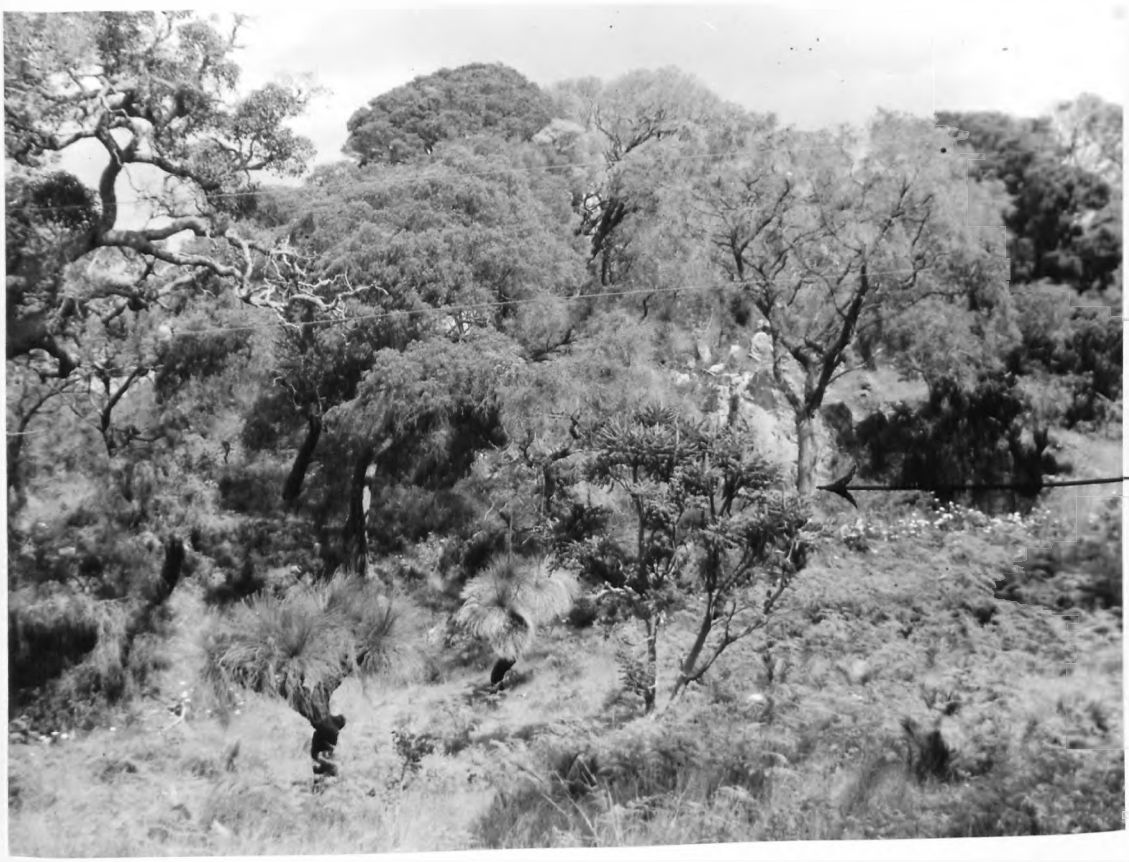


Fountains Abbey, near Ripon, Yorkshire.

Plants of the tetraploid cytotype growing in crevices of a wall of the ruined Abbey. Photograph taken in April.

Fig. 30.

Tetraploid cytotype.



Yallingup, Western Australia.

Habitat of the tetraploid cytotype is in Pleistocene limestone rock under the shade of Eucalyptus gomphocephala (Tuart), visible in middle distance. Banksia grandis and Xanthorrhoea reflexa are seen in the foreground.

Tetraploid cytotype.

Yallingup, Western Australia.

Habitat of the tetraploid cytotype in crevices of Pleistocene limestone rock under shade of Eucalyptus Gomphocephala (Tuart). See also fig. 32.

Tetraploid cytotype.

Yallingup, Western Australia.

Habitat of the tetraploid cytotype in crevices of Pleistocene limestone rock. See also fig. 31.

Fig. 33.

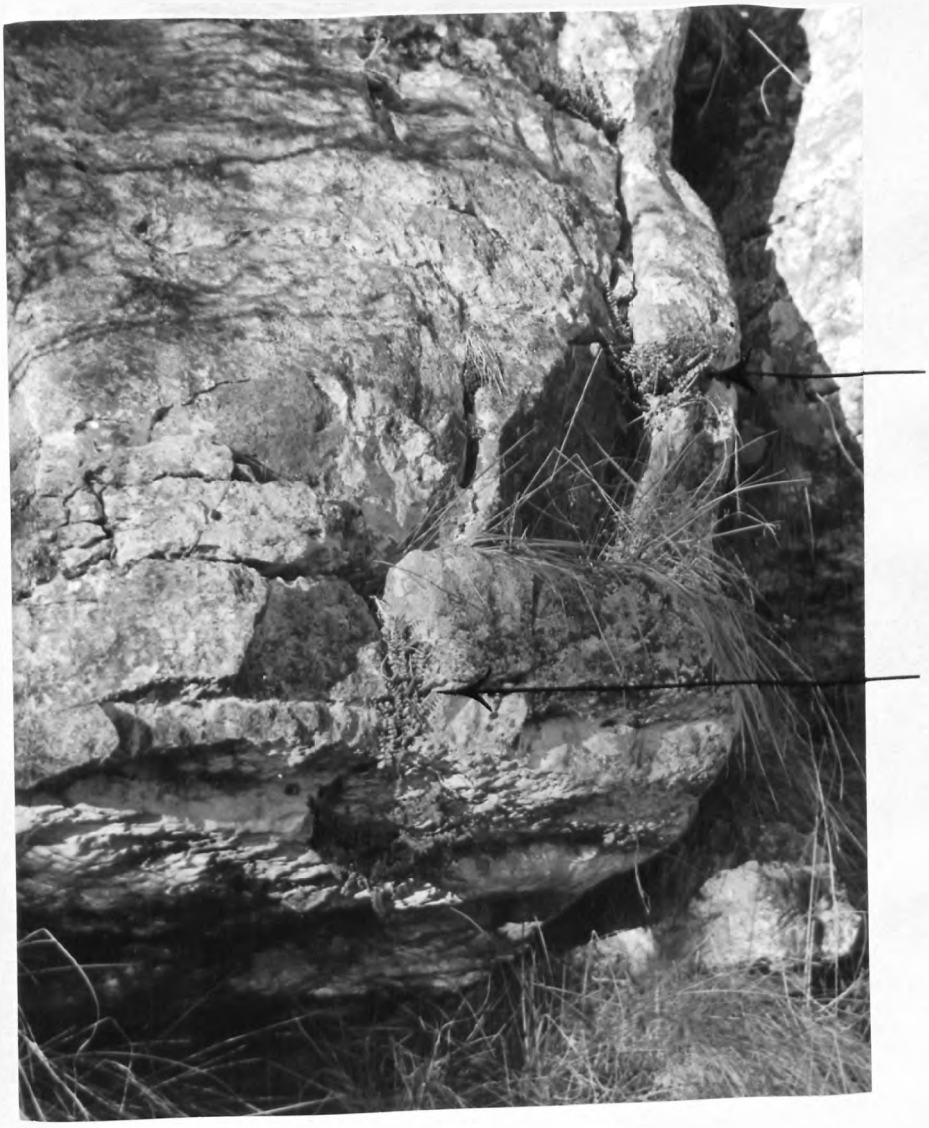
Tetraploid cytotype.

Buchan, Gippsland, Victoria, Australia.

Habitat of the tetraploid cytotype is crevices of limestone rock outcropping on a grassy slope.

Fig. 34.

Tetraploid cytotype.



Buchan, Gippsland, Victoria, Australia.

Plants of the tetraploid cytotype growing in crevices of limestone rock.

Tetraploid cytotype.

Bindi, Gippsland, Victoria, Australia.

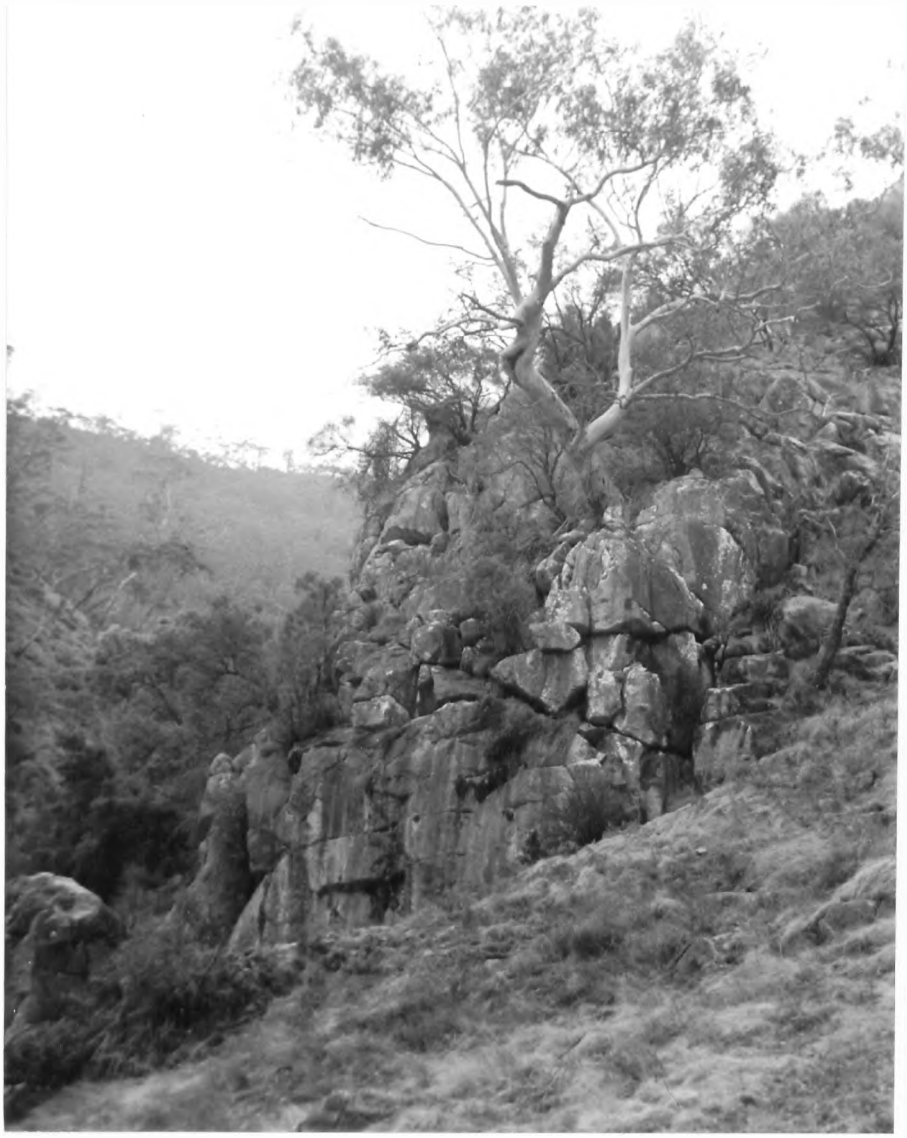
Habitat of the tetraploid cytotype in crevices of limestone rock outcropping on a slope, under the light shade of Eucalyptus forest.

Tetraploid cytotype.

McKeon's Creek, Jenolan Caves, N.S.W., Australia.

Habitat of the tetraploid cytotype in crevices of limestone rock outcropping in light forest in the gully.

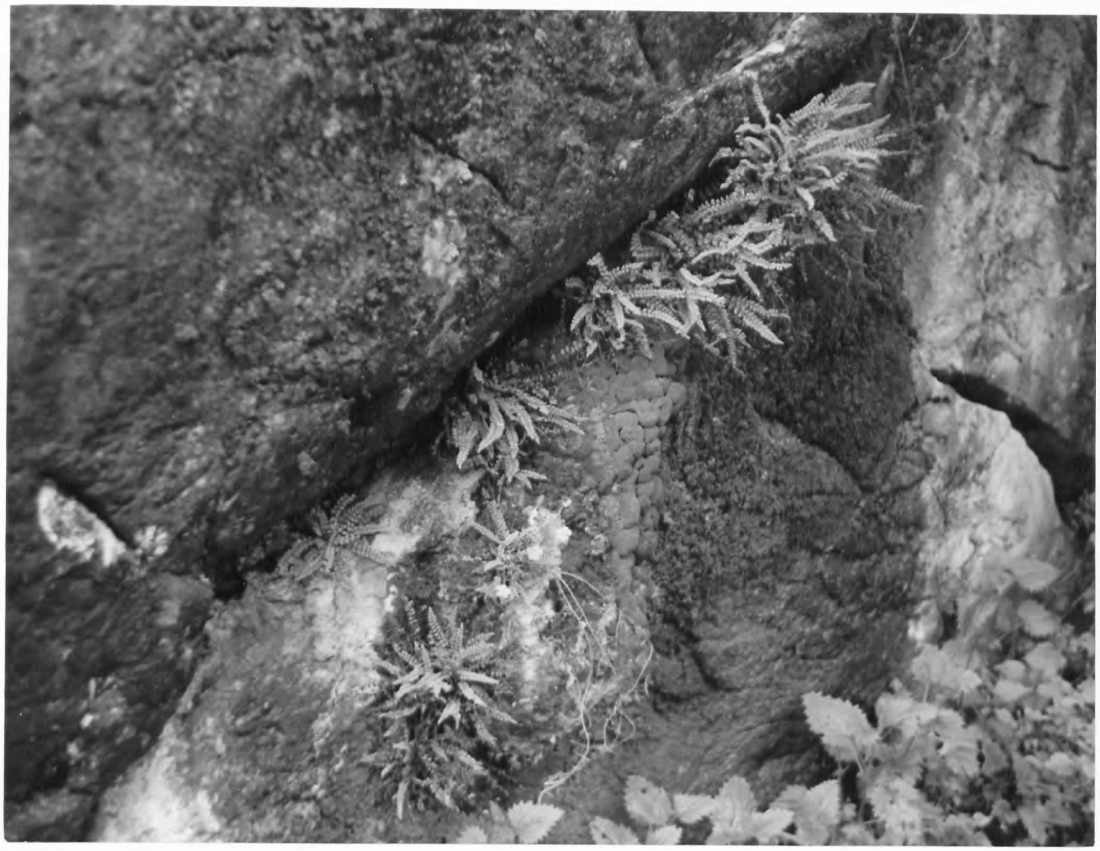
Tetraploid cytotype.



McKeon's Creek, Jenolan Caves, N.S.W., Australia.

Habitat of the tetraploid cytotype in crevices of limestone rocks in sheltered situations. The gully containing McKeon's Creek is visible on left of the photograph.

Tetraploid cytotype.



McKeon's Creek, Jenolan Caves, N.S.W., Australia.

Plants of the tetraploid cytotype growing in crevices of limestone rock. Note formation of tufa in middle of photograph, indicating a constant supply of water.

Tetraploid cytotype.

McKeon's Creek, Jenolan Caves, N.S.W., Australia.

Plants of the tetraploid cytotype growing in crevices of limestone rock.

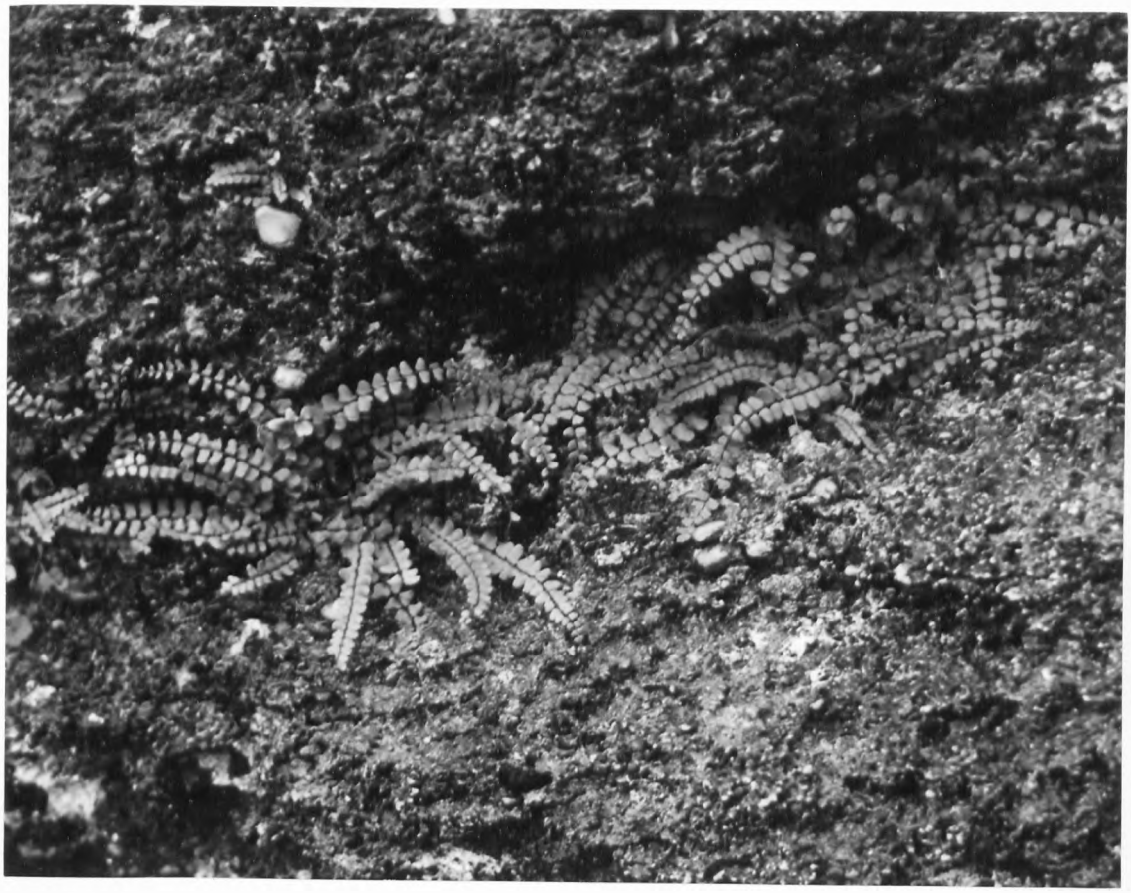
Tetraploid cytotype.

Tangoio, N. of Petane, Hawke's Bay, New Zealand.

Habitat of the tetraploid cytotype, in crevices of limestone rock outcropping on grassy slopes.

Fig. 41.

Tetraploid cytotype.



Tangoio, N. of Petane, Hawke's Bay, New Zealand.

Plants of the tetraploid cytotype, growing in a crevice of limestone rock.

AUTECOLOGY

HABITAT PHOTOGRAPHS : HEXAPLOID CYTOTYPE

Figs. 42 - 66.

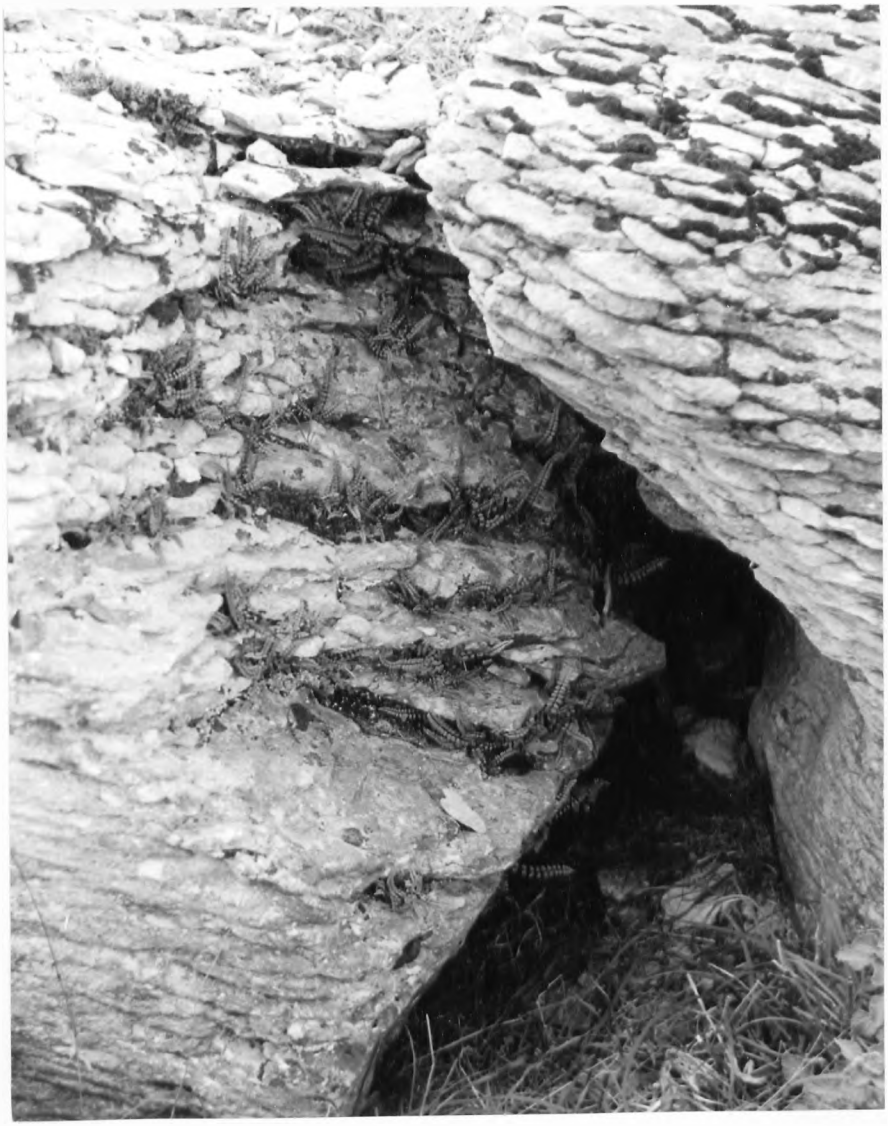
Hexaploid cytotype.

Mt.Somers, Canterbury, New Zealand.

Habitat of hexaploid cytotype in crevices of eroded "pancake" limestone.

Fig. 43.

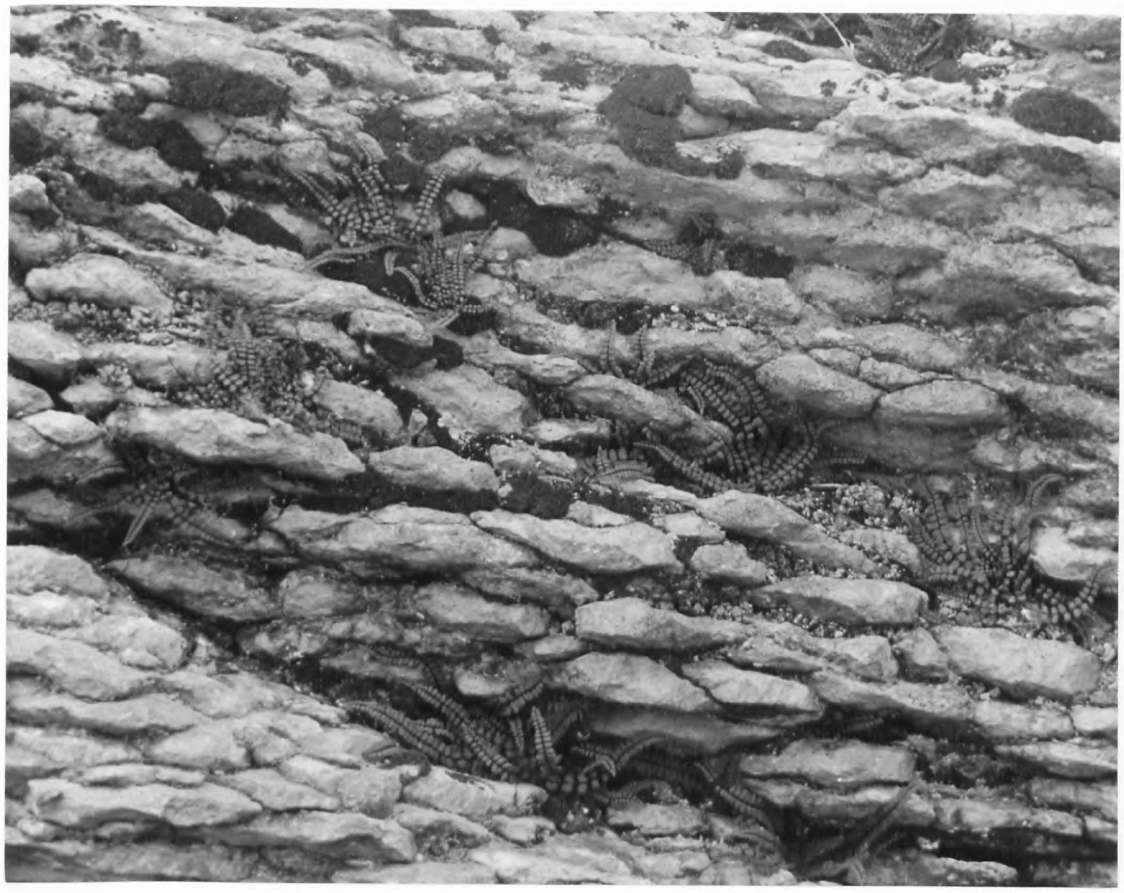
Hexaploid cytotype.



Mt. Somers, Canterbury, New Zealand.

Plants of the hexaploid cytotype growing on the side of a grike in crevices of "pancake" limestone. Note the more distant pinnae of plants growing in shade in comparison with those of plants growing on the outside of the grike.

Hexaploid cytotype.

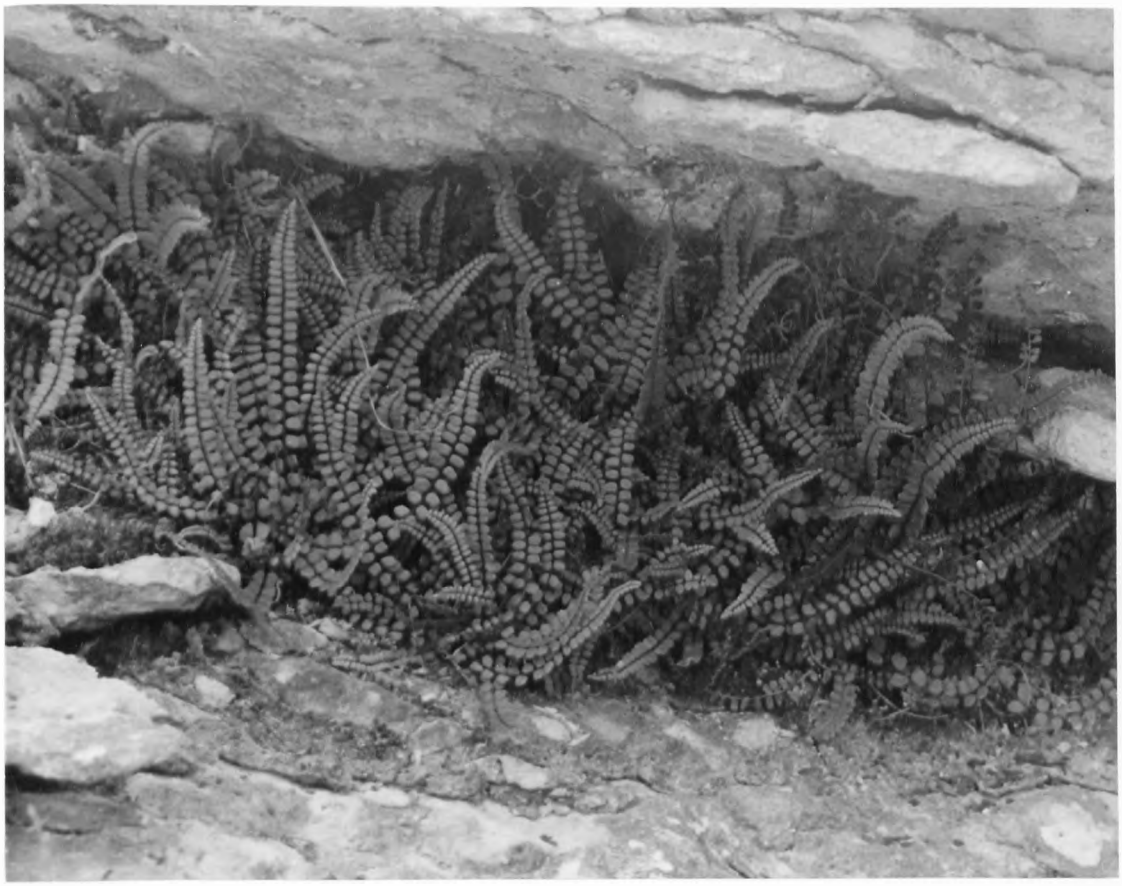


Mt.Somers, Canterbury, New Zealand.

Plants of the hexaploid cytotype growing in crevices of "pancake" limestone.

Fig. 45.

Hexaploid cytotype.



Mt. Somers, Canterbury, New Zealand.

Plants of the hexaploid cytotype growing in a crevice in "pancake" limestone.

Fig. 46.

Hexaploid cytotype.

Waiau, Canterbury, New Zealand.

Habitat of the hexaploid cytotype, in crevices of limestone rock.

Hexaploid cytotype.

Canaan Track, Pikikiruna Range, Nelson, New Zealand.

Habitat of the hexaploid cytotype, in crevices of exposed outcrops of metamorphic limestone, in an area relatively recently de-forested.

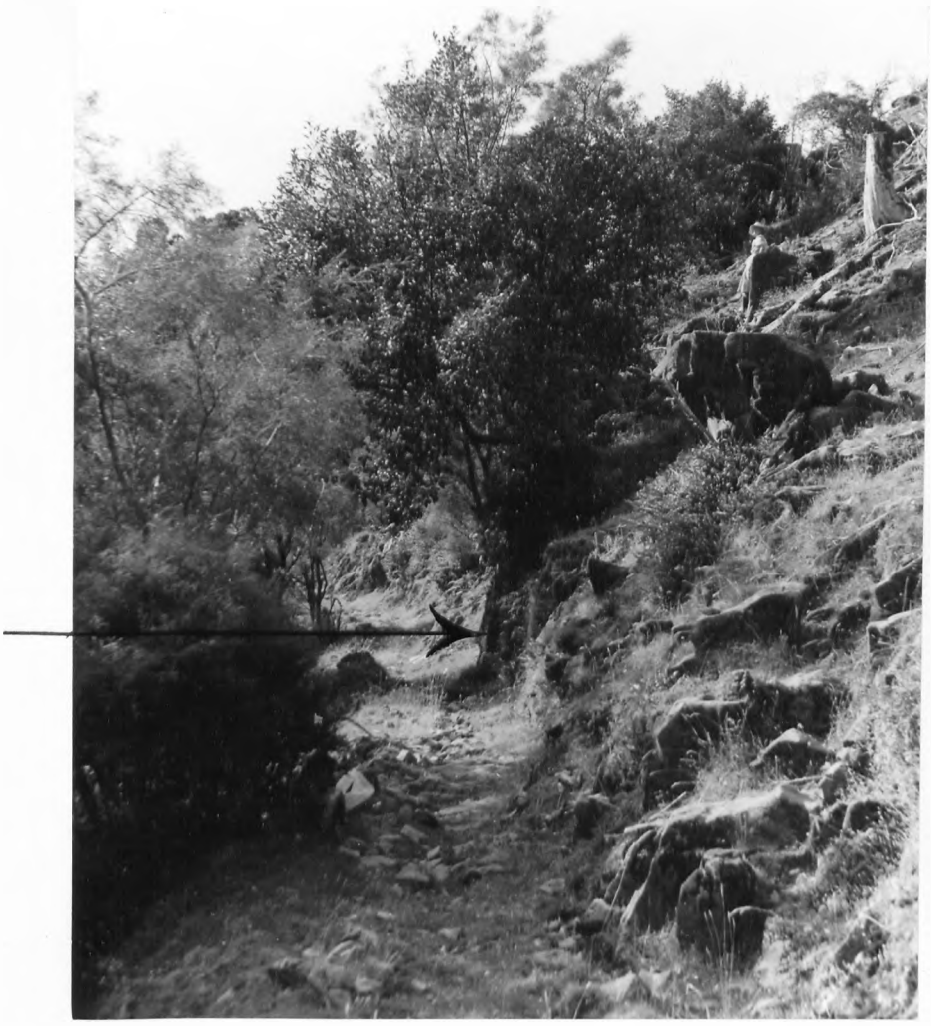
Fig. 48.

Hexaploid cytotype.

Canaan Track, Pikikiruna Range, Nelson, New Zealand.

Plants of the hexaploid cytotype growing in crevices of metamorphic limestone rock in an exposed situation.

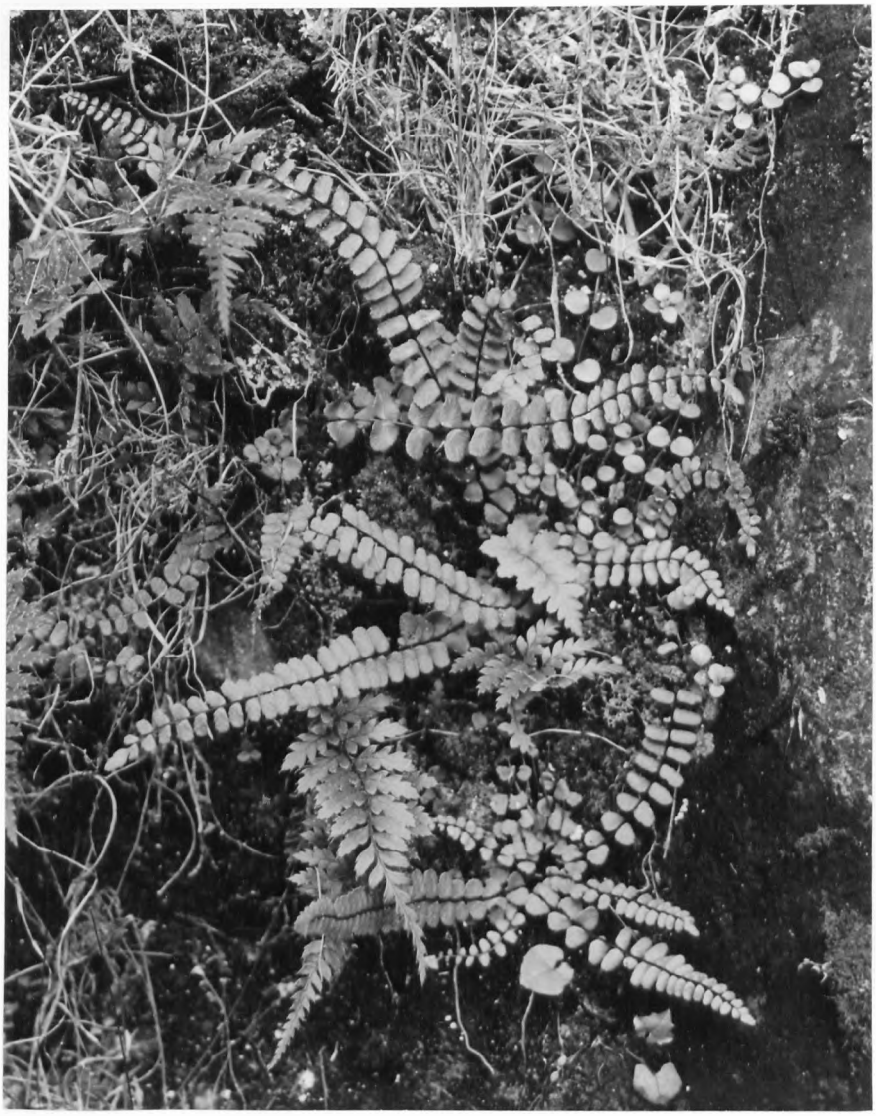
Hexaploid cytotype.



Canaan Track, Pikikiruna Range, Nelson, New Zealand.

Habitat of the hexaploid cytotype in crevices of metamorphic limestone rock on the edge of mixed Podocarpus forest. See also fig. 50.

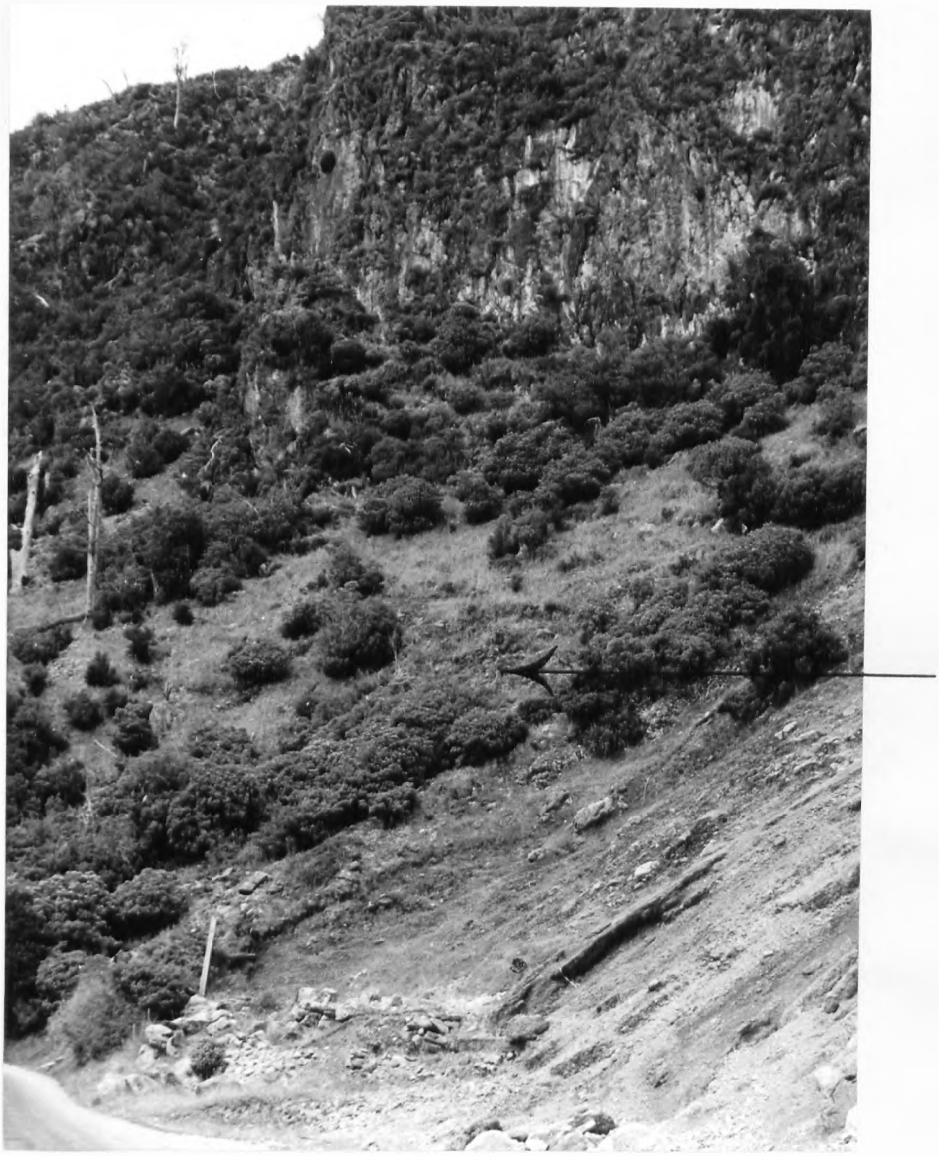
Hexaploid cytotype.



Canaan Track, Pikikiruna Range, Nelson, New Zealand.

Plants of the hexaploid cytotype growing on shaded limestone rock in association with Polystichum richardii, in the station shown on fig. 49.

Hexaploid cytotype.



Takaka Hill, Pikikiruna Range, Nelson, New Zealand.

Habitat of the hexaploid cytotype on metamorphic limestone rock outcropping on a grassy slope, only relatively recently de-forested. See also figs. 52 and 53.

Hexaploid cytotype.

Takaka Hill, Pikikiruna Range, Nelson, New Zealand.

Habitat of the hexaploid cytotype on limestone rock outcropping on a grassy slope, only relatively recently de-forested. See also figs. 51 and 53.

Fig. 53.

Hexaploid cytotype.

Takaka Hill, Pikikiruna Range, Nelson, New Zealand.

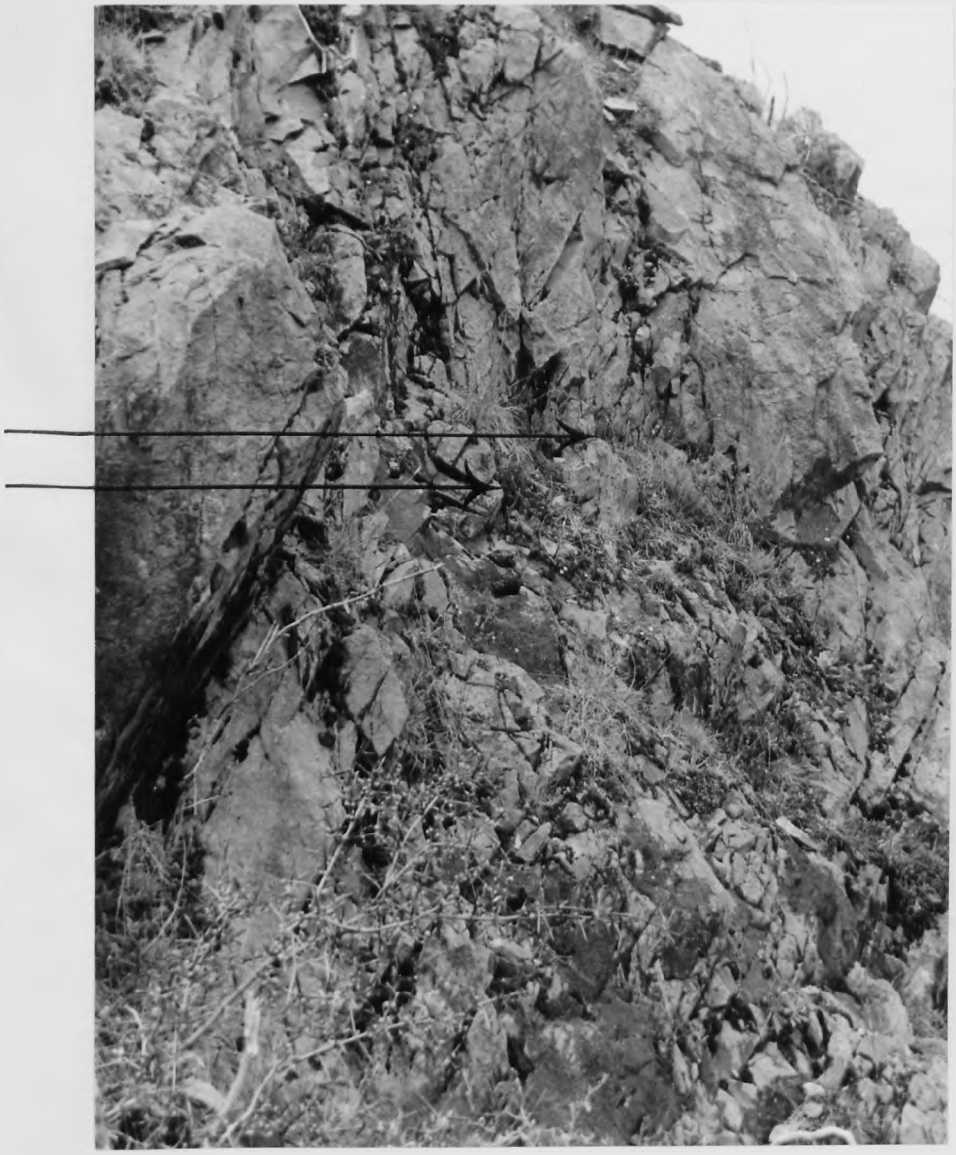
Plants of the hexaploid cytotype growing in association with Verbascum thapsus and Arenaria serpyllifolia in crevices of limestone rock outcropping on a relatively recently de-forested slope. See also figs. 51 and 52.

Hexaploid cytotype.

Cass, Canterbury, New Zealand.

Habitat of the hexaploid cytotype in crevices of Greywacke rock outcropping on a slope covered by Discaria scrub and tussock grassland. See also figs. 55 and 56.

Hexaploid cytotype.



Cass, Canterbury, New Zealand.

Habitat of the hexaploid cytotype, in crevices of Greywacke rock. See also figs. 54 and 56.

Hexaploid cytotype.

Cass, Canterbury, New Zealand.

Plants of the hexaploid cytotype growing in a crevice of Greywacke rock. See also figs. 54 and 55.

Fig. 57.

Hexaploid cytotype.

Monument, Hooker valley, Canterbury, New Zealand.

Habitat of the hexaploid cytotype, in crevices between tumbled Greywacke rock in terminal moraine. See also figs. 58 and 59.

Fig. 58.

Hexaploid cytotype.

Monument, Hooker valley, Canterbury, New Zealand.

Habitat of the hexaploid cytotype, in crevices between tumbled Greywacke rock in terminal moraine. See also figs. 57 and 59.

Hexaploid cytotype.

Monument, Hooker valley, Canterbury, New Zealand.

Plants of the hexaploid cytotype growing in crevices between tumbled Greywacke rocks in terminal moraine. See also figs. 57 and 58.

Hexaploid cytotype.

Glencoe Creek, Hooker valley, Canterbury, New Zealand.

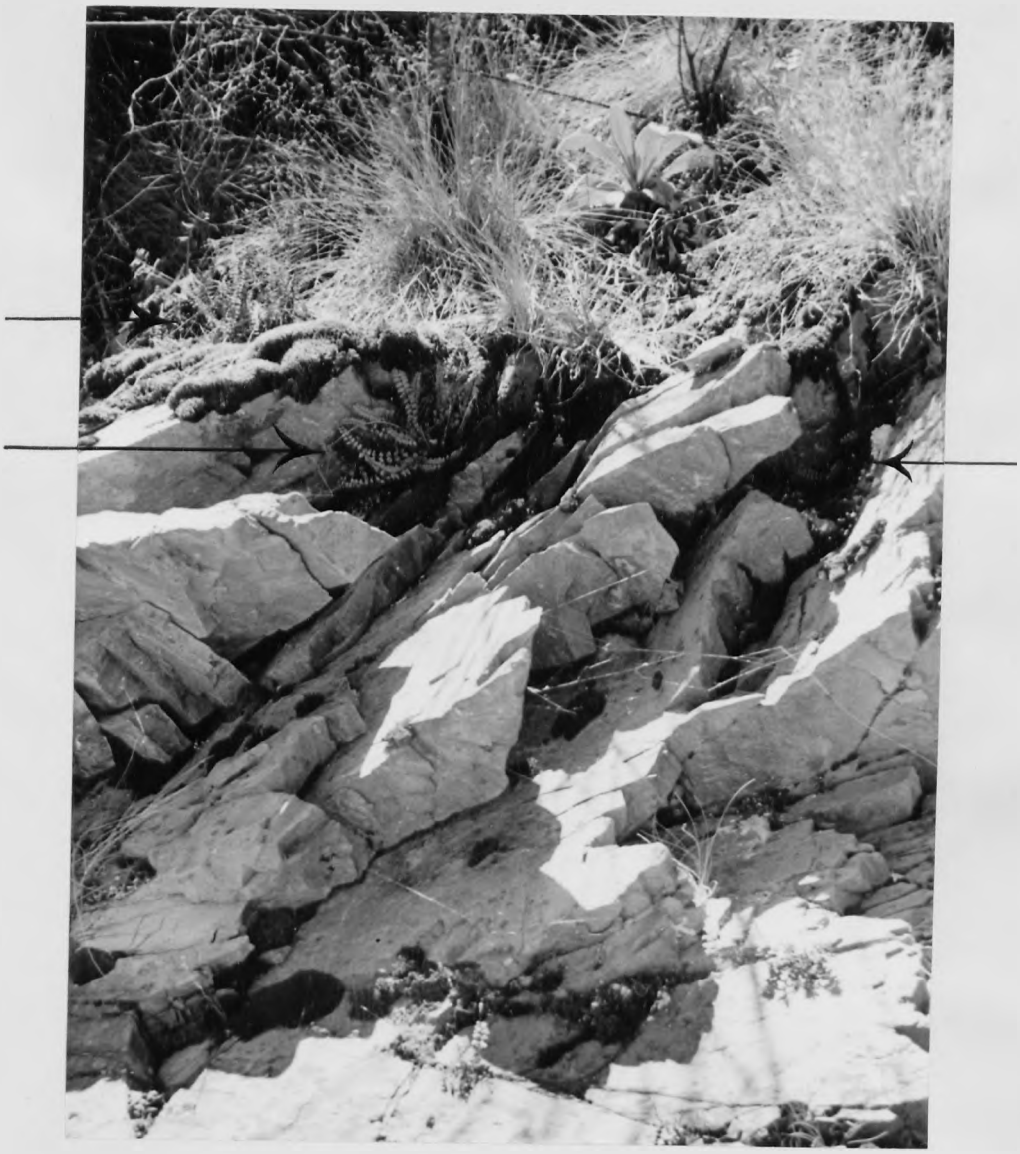
Habitat of the hexaploid cytotype, in crevices of Greywacke rock exposed along the sides of the creek, in sub-alpine scrub. See also figs. 61 and 62.

Hexaploid cytotype.

Glencoe Creek, Hooker valley, Canterbury, New Zealand.

Habitat of the hexaploid cytotype, in crevices of Greywacke rock exposed along sides of the creek, in sub-alpine scrub. See also figs. 60 and 62.

Hexaploid cytotype.



Glencoe Creek, Hooker valley, Canterbury, New Zealand.

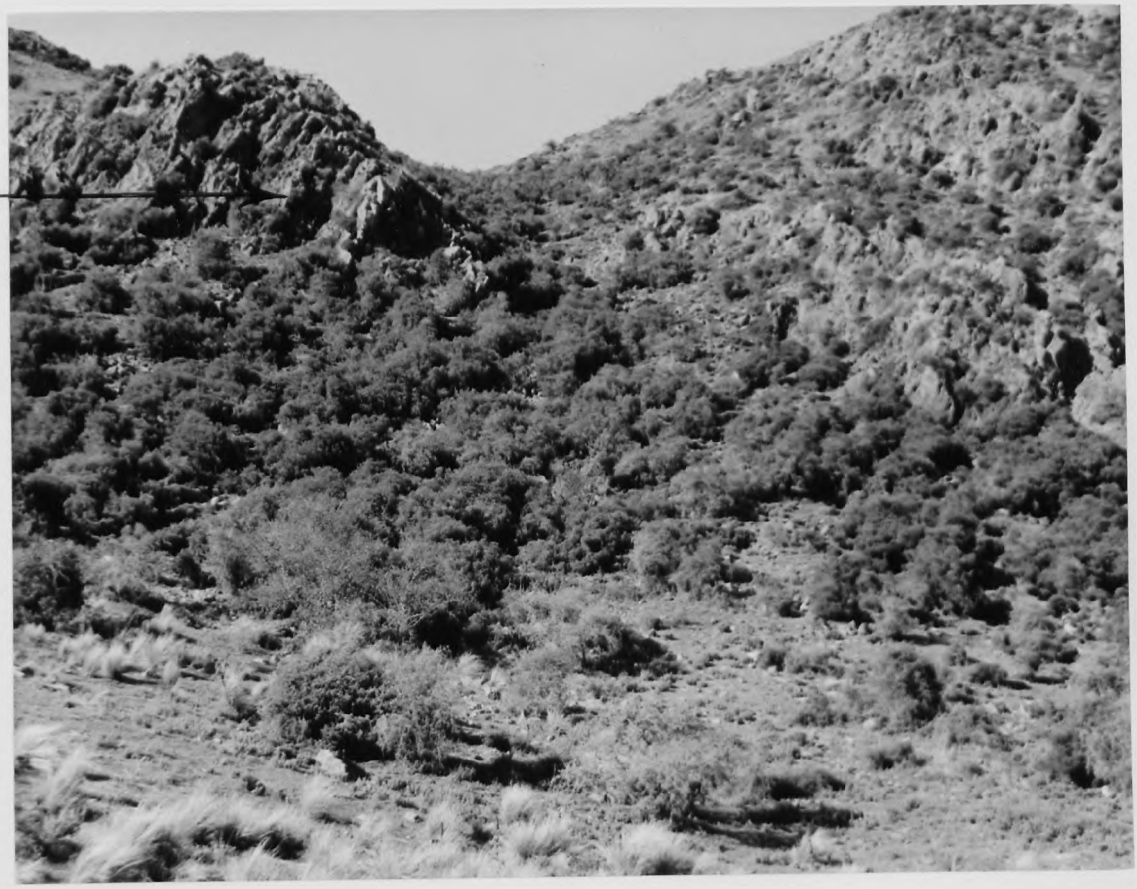
Plants of the hexaploid cytotype growing in crevices of Greywacke rock exposed along the sides of the creek. See figs. 60 and 61.

Hexaploid cytotype.

Waitaki, Canterbury, New Zealand.

Habitat of the hexaploid cytotype, in crevices of dry Greywacke rock outcropping on slopes covered with Discaria scrub and tussock grassland. See also fig. 64.

Hexaploid cytotype.



Waitaki, Canterbury, New Zealand.

Habitat of the hexaploid cytotype, in crevices of dry Greywacke rock outcropping on slope covered with Discaria scrub and tussock grassland. See also fig. 63.

Fig. 65.

Hexaploid cytotype.

Ben Lomond massif, Queenstown, Otago, New Zealand.

Habitat of the hexaploid cytotype, in shaded crevices of mica-schist rock.

Hexaploid cytotype.

Ben Lomond massif, Queenstown, Otago, New Zealand.

Plants of the hexaploid cytotype growing in shaded crevices of mica-schist rock.

RHIZOME SCALES

Figs. 67 - 71.

Rhizome scales.

A. B. C.

Rhizome scales of the diploid cytotype : X 25.

A. Aber Falls, Caernarvonshire.

B. Parry Sound, Ontario, Canada.

C. Gyadzong, S.E. Tibet.

Rhizome scales.

Rhizome scales of the diploid cytotype. X 25 mag.

Wulgulmerang, Victoria, Australia.

Rhizome scales.

Rhizome scales of the tetraploid cytotype : X 25.

A. Buchan, Victoria, Australia.

B. Tangoio, Hawke's Bay, New Zealand.

C. Mauna Loa, Hawaii.

D. Buckden, Wharfedale, Yorkshire.

Fig. 70.

Rhizome scales.

Rhizome scales of the tetraploid cytotype : X 25 magn.

Honsyu, Japan.

Rhizome scales.

A. B. C. D.

Rhizome scales of the hexaploid cytotype : X 25 magn.

A. Queenstown, Otago, N.Z.

B. Hooker valley, Canterbury, N.Z.

C. Ashburton River, Canterbury, N.Z.

D. Otorohanga, South Auckland, N.Z.

Rhizome scales.

A. B.

Rhizome scales of the hexaploid cytotype : X 25.

A. Cass, Canterbury, N.Z.

B. Rangihaieta, Nelson, N.Z.

SPORES

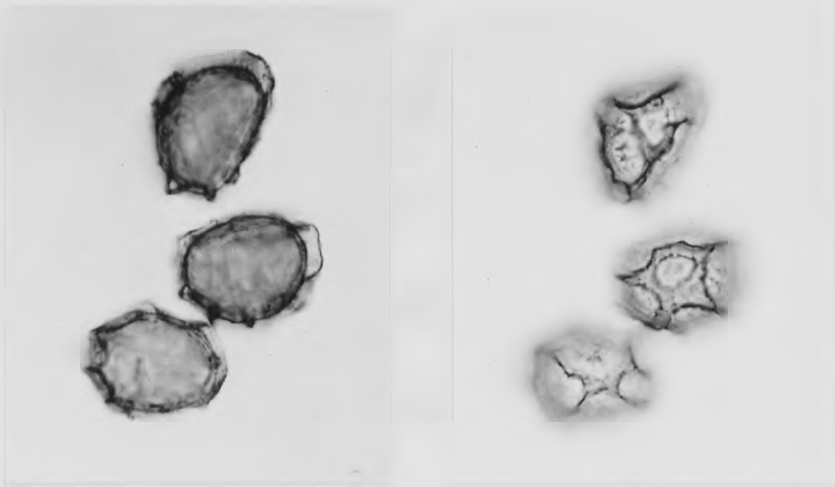
Figs. 73 - 84.

Two planes of focus have been photographed for each group of spores. The left photograph of each pair is in median plane of focus, the photograph on the right is focussed on the upper surface of the spore. All photographs are at the same magnification, 500 diameters.

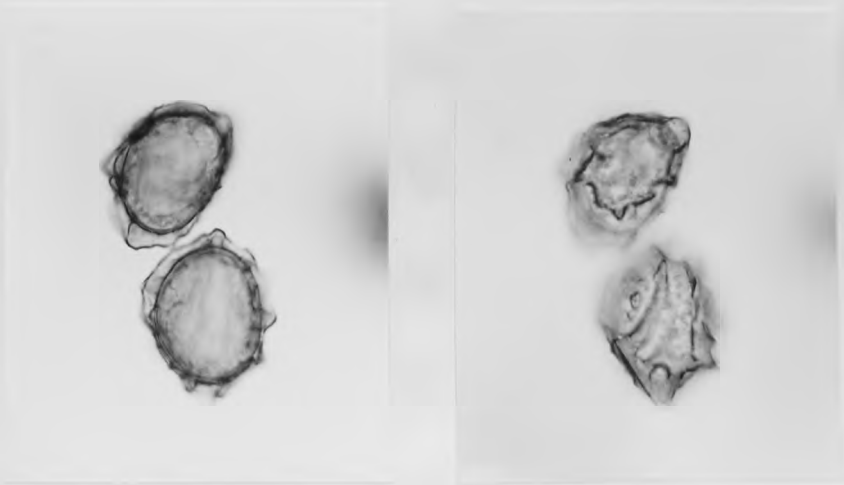
Objective : Cooke, Troughton & Simms 3.75mm X 45 Fluorite Oil Immersion. Filter : Green monochromatic. Negative : Ilford Special Rapid Panchromatic Plates. Magnification : X 500. Print : Contact.

Spores : X 500.

Asplenium trichomanes : diploid cytotype.



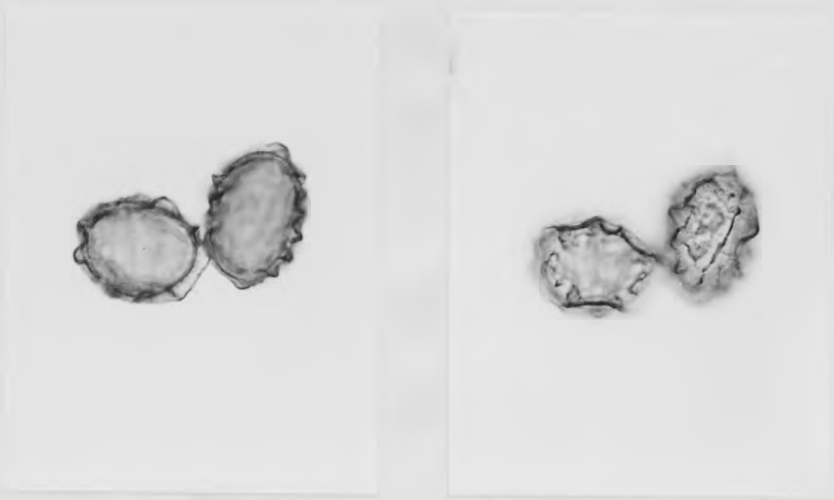
A. Tyn-y-Groes, Merioneth.



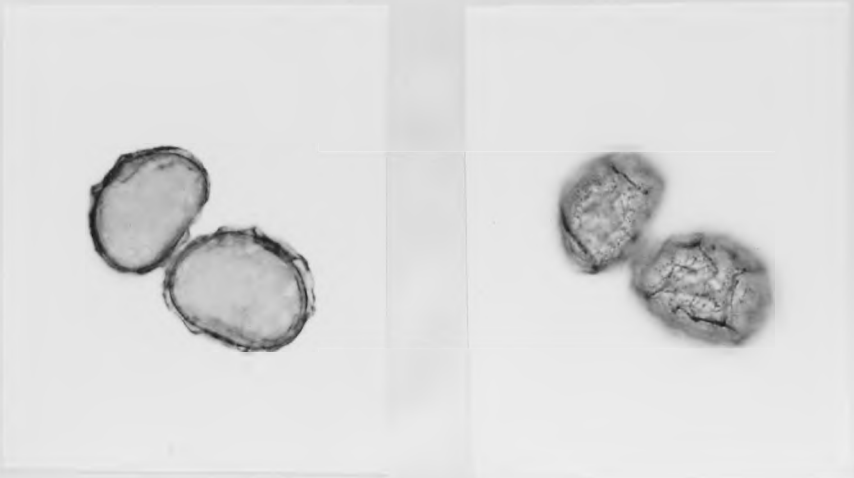
B. Craigs of Succouth, Aberdeen.

Spores : X 500.

Asplenium trichomanes : diploid cytotype.



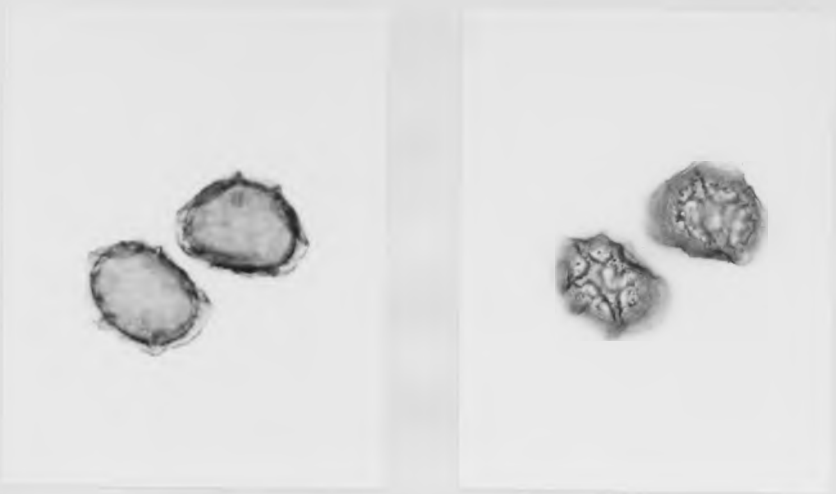
A. Kangra Himalaya, Punjab.



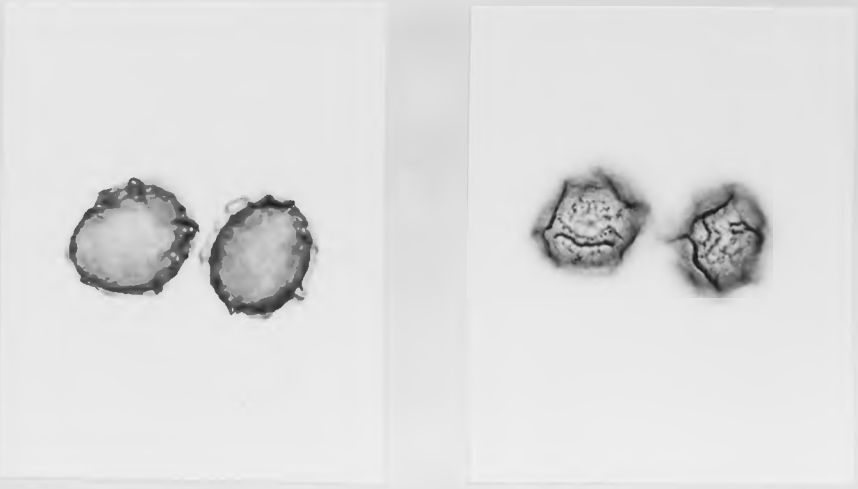
B. Gyadzong, S.E. Tibet.

Spores : X 500.

Asplenium trichomanes : diploid cytotype.



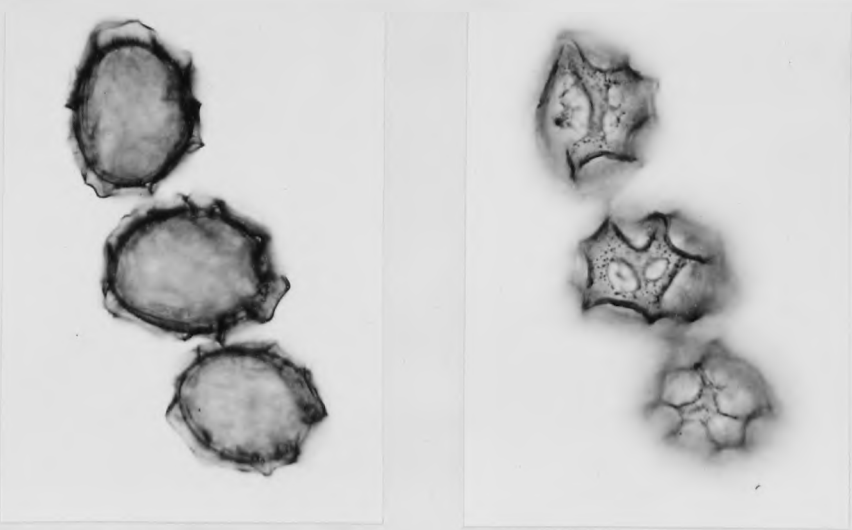
A. Wulgulmerang, Victoria, Australia.



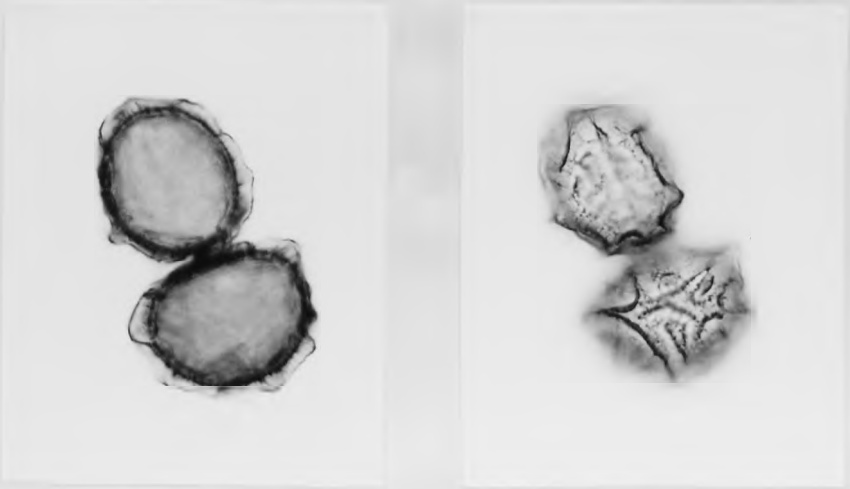
B. Wulgulmerang, Victoria, Australia.

Spores : X 500.

Asplenium trichomanes : tetraploid cytotype.



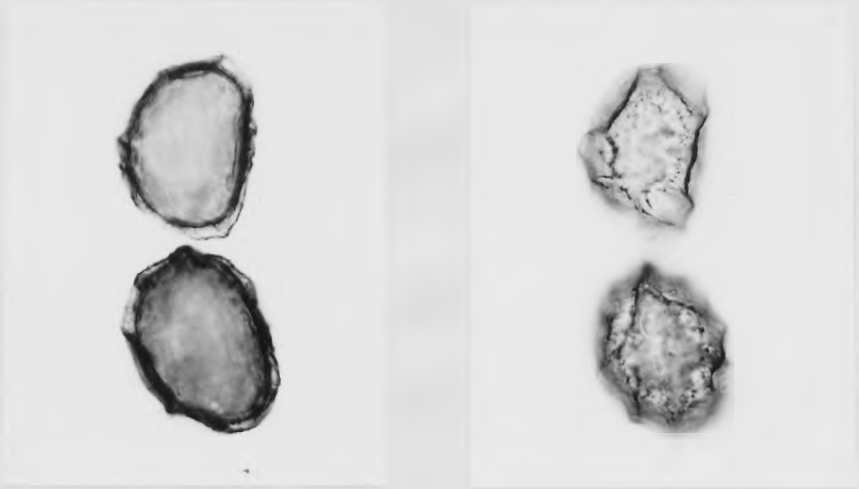
A. Aber Falls, Caernarvonshire.



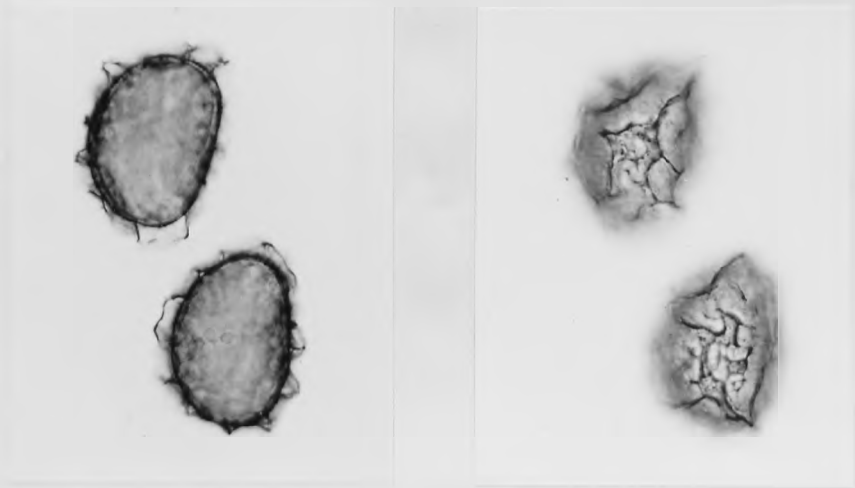
B. Craig Dorney, Aberdeenshire.

Spores : X 500.

Asplenium trichomanes : tetraploid cytotype.



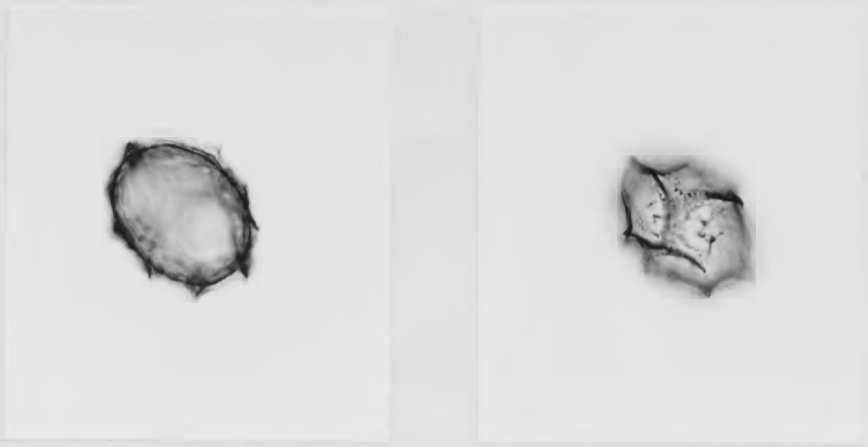
A. Rattlesnake Point, Ontario, Canada.



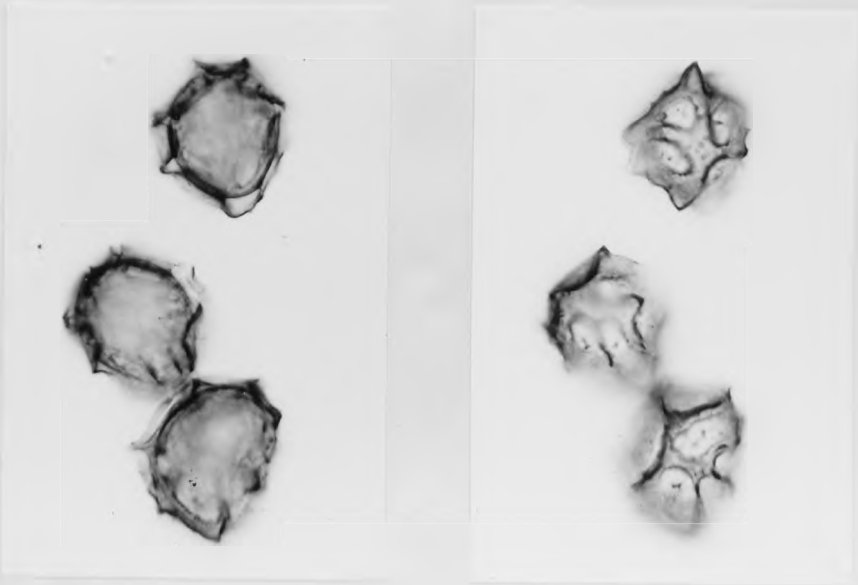
B. Haleakala, Maui, Hawaii.

Spores : X 500.

Asplenium trichomanes : tetraploid cytotype.



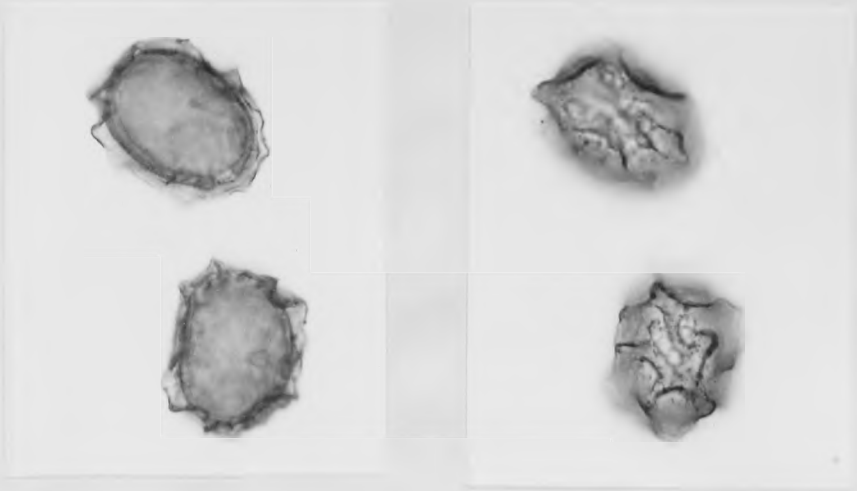
A. Tsanatalana, Basutoland, South Africa.



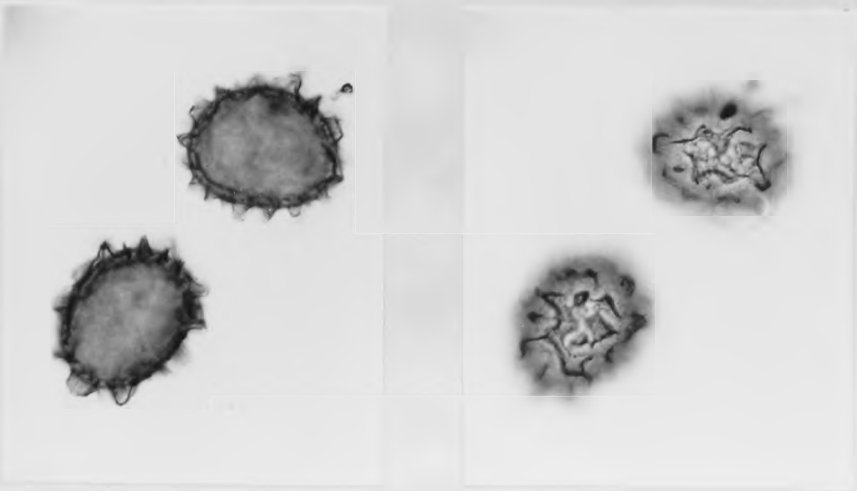
B. Jenolan Caves, New South Wales, Australia.

Spores : X 500.

Asplenium trichomanes : tetraploid cytotype.



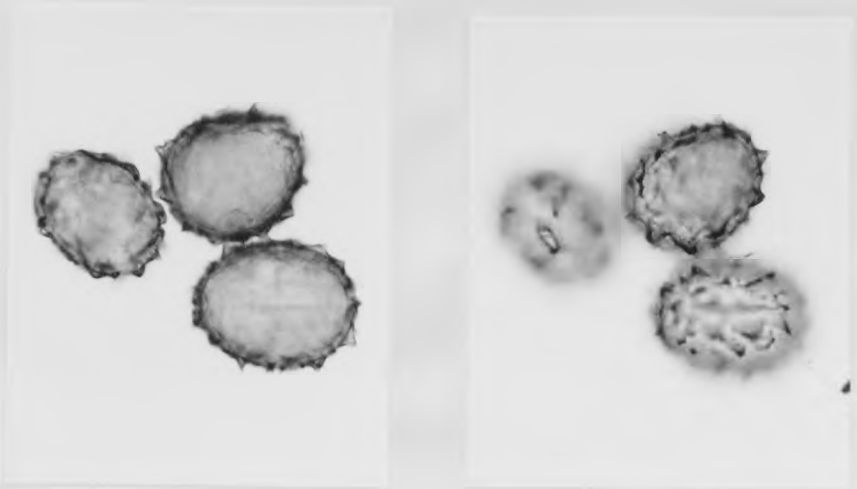
A. Tangoio, Hawke's Bay, New Zealand.



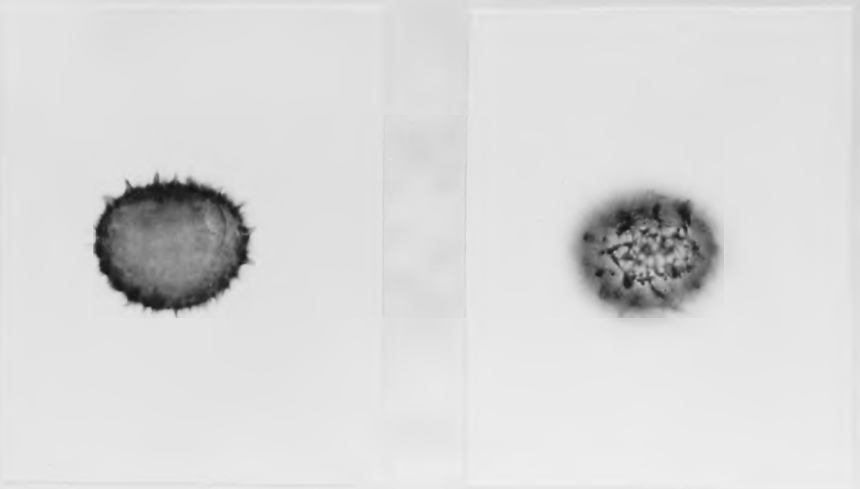
B. Tangoio, Hawke's Bay, New Zealand.

Spores : X 500.

Asplenium trichomanes : tetraploid cytotype.



A. Tangoio, Hawke's Bay, New Zealand.

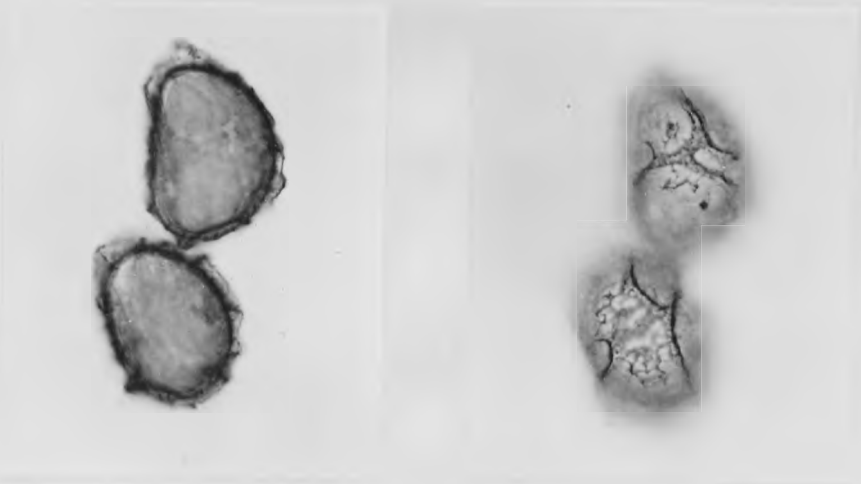


B. Pukeora, Hawke's Bay, New Zealand.

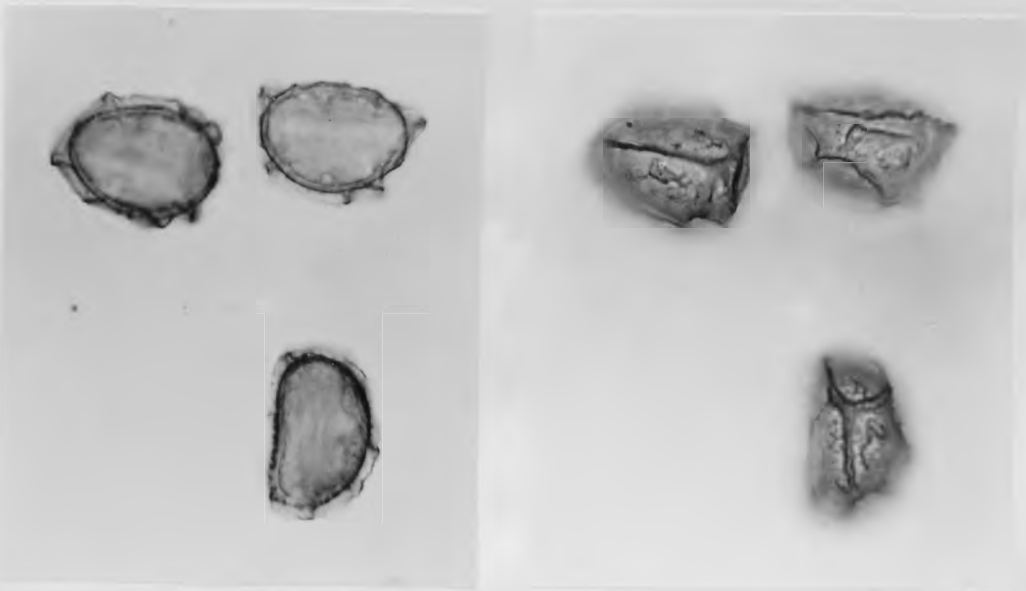
Fig. 81.

Spores : X 500.

Asplenium trichomanes : tetraploid cytotype.



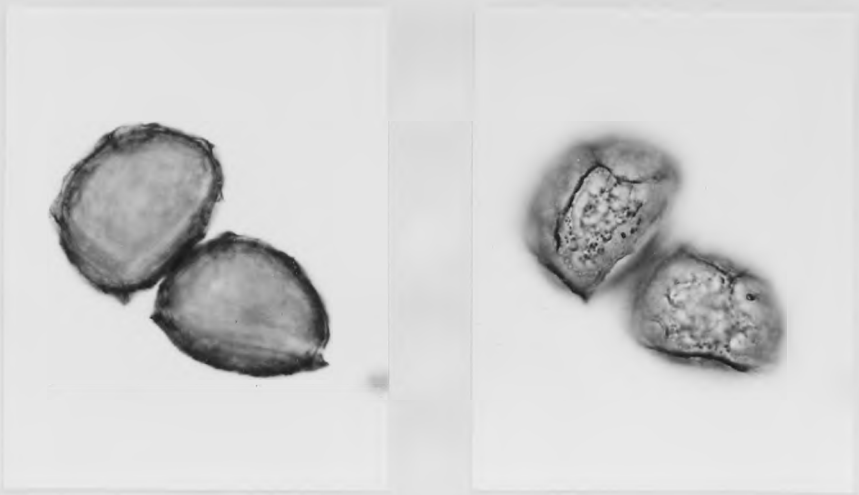
A. Mt. Sefuri, Kyushu, Japan.



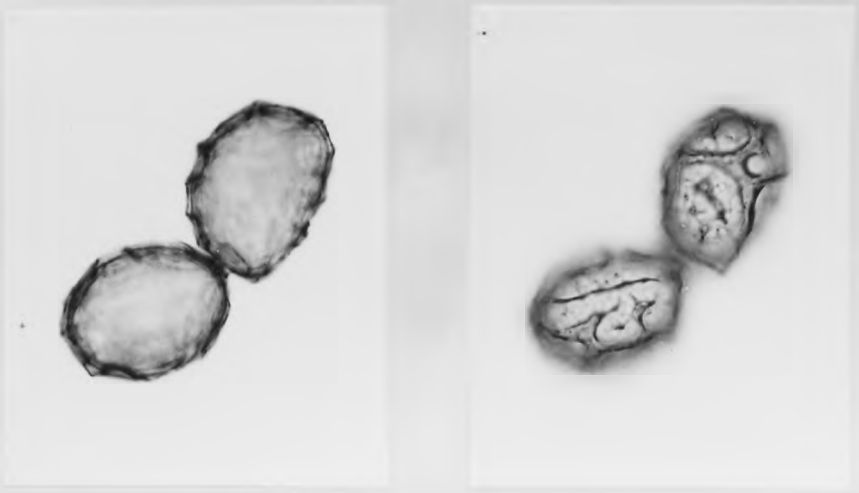
B. Honsyu, Japan.

Spores : X 500.

Asplenium trichomanes : hexaploid cytotype.



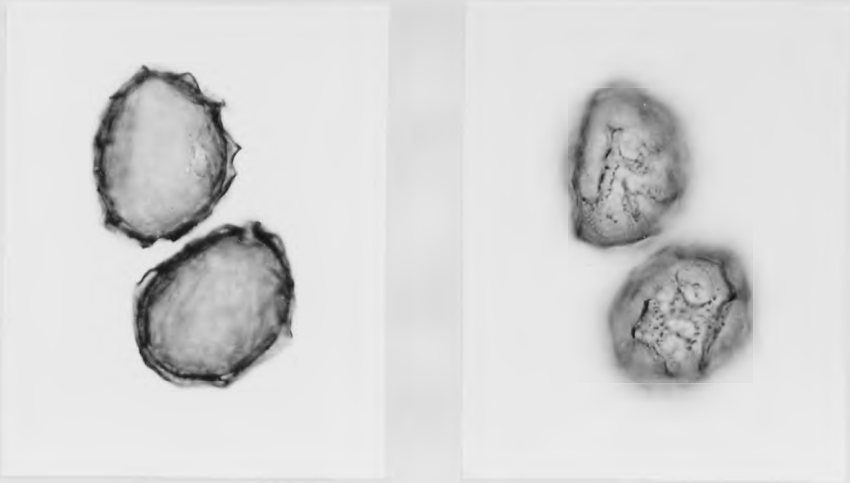
A. Waitomo, South Auckland L.D., New Zealand.



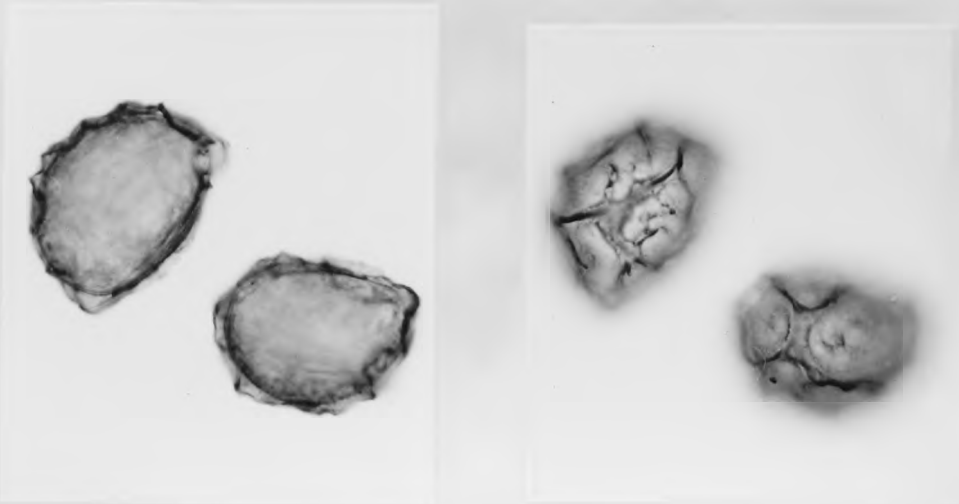
B. Napier, Hawke's Bay, New Zealand.

Spores : X 500.

Asplenium trichomanes : hexaploid cytotype.



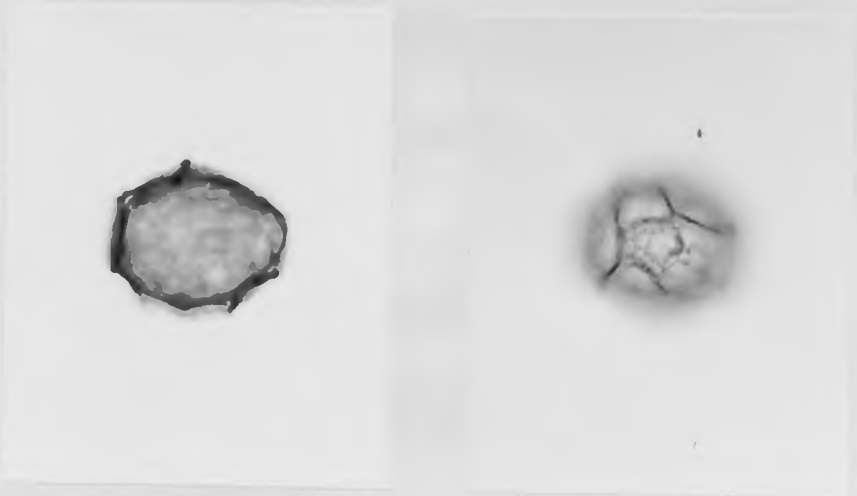
A. Cass, Canterbury, New Zealand.



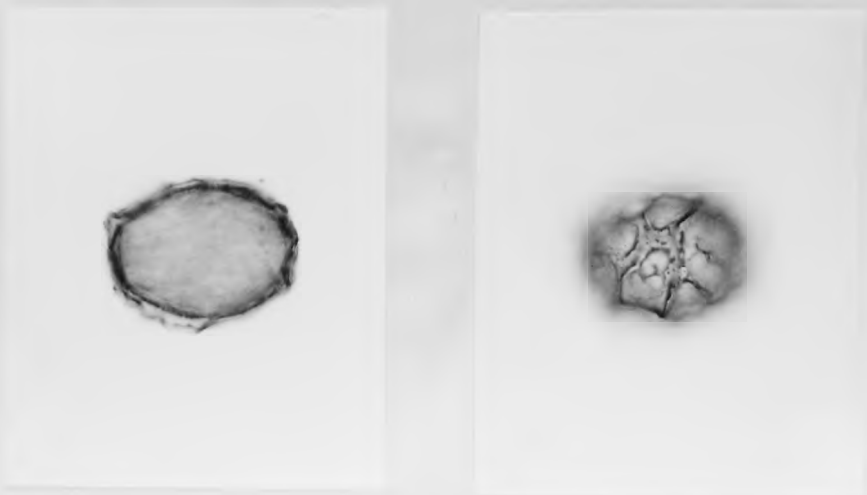
B. Queenstown, Otago, New Zealand.

Spores : X 500.

Asplenium trichomanes : hexaploid cytotype.



A. Waitaki, Canterbury, New Zealand.



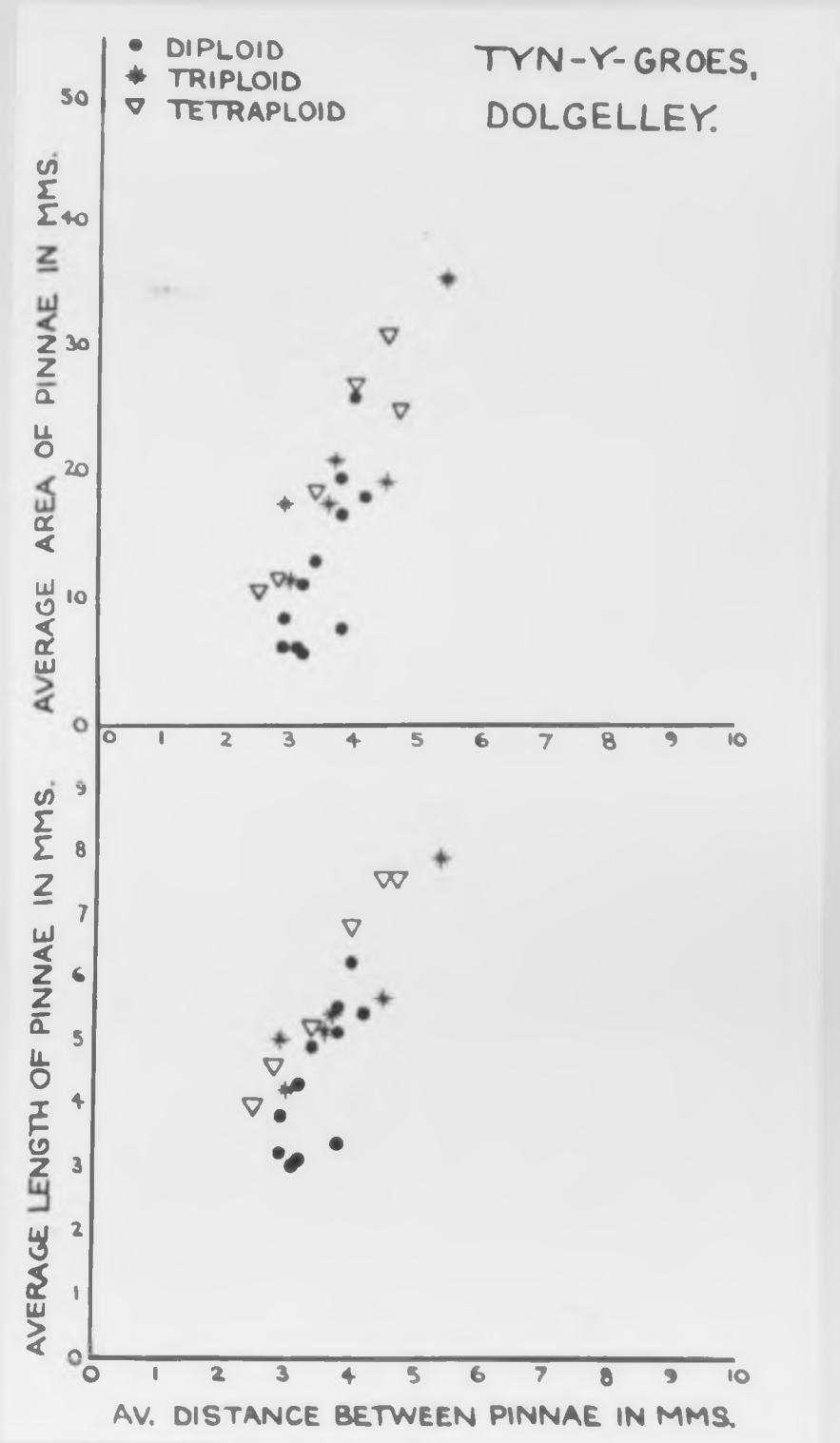
B. Holotype of Asplenium melanolepis Colenso,
Hawke's Bay, New Zealand.

FROND CHARACTERISTICS

SCATTER DIAGRAMS

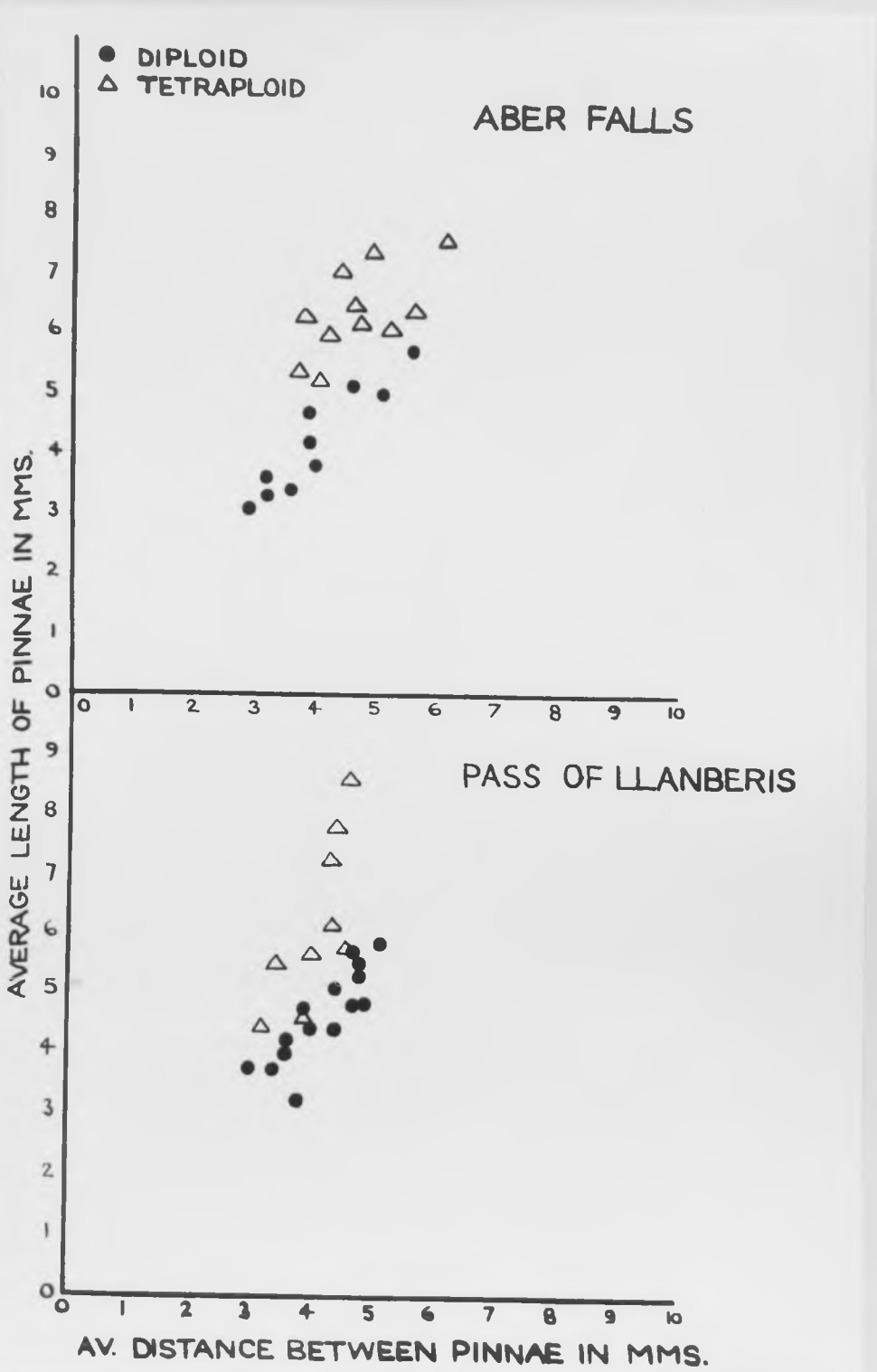
Figs. 85 -88.

The manner in which the points on these scatter diagrams have been obtained is described on Page 68 of the text.

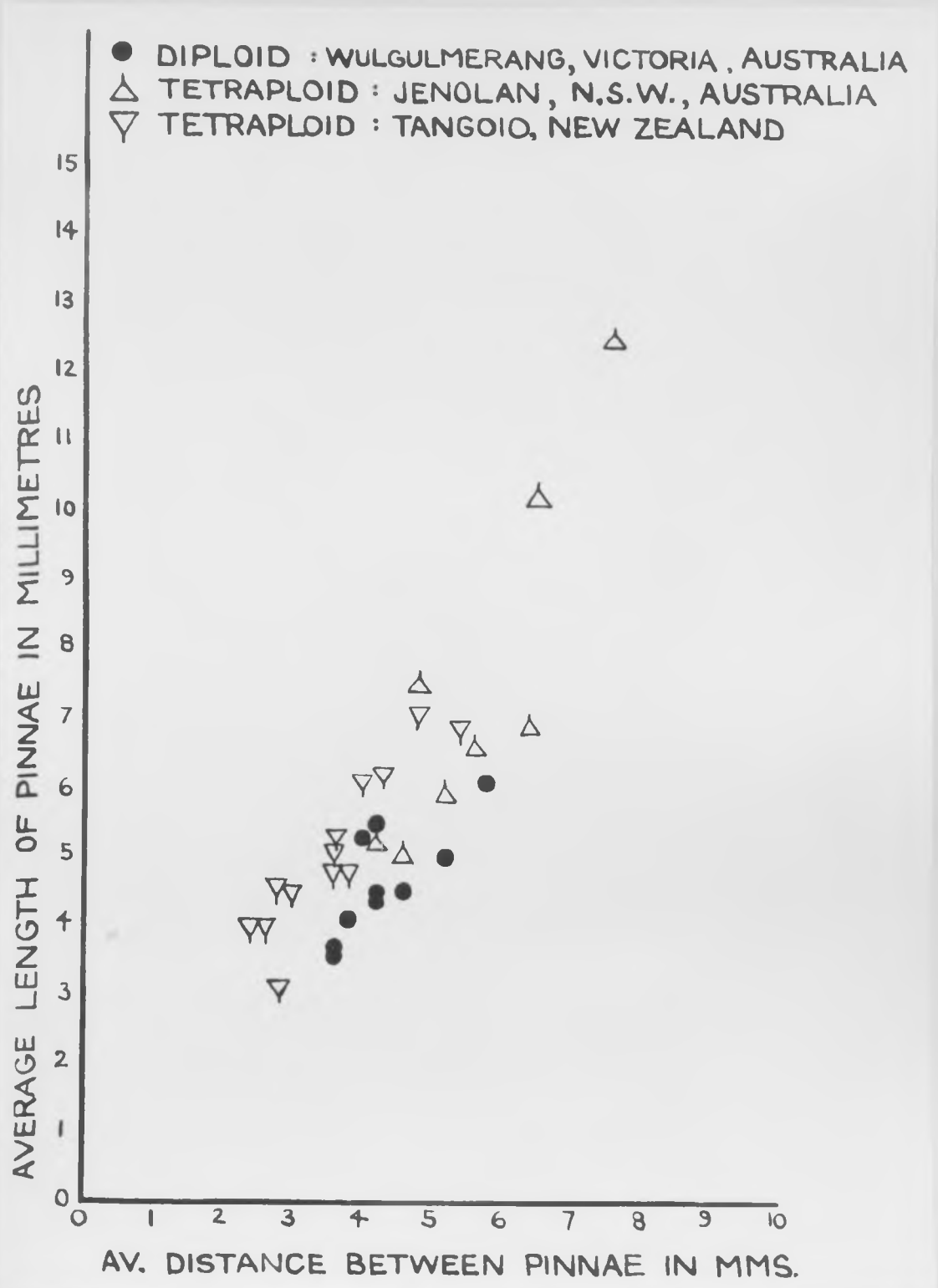


Scatter diagrams showing relationship between pinna size and pinna separation in fronds of diploid, triploid, and tetraploid plants from Tyn-y-Groes, Dolgelley, Caernarvon.

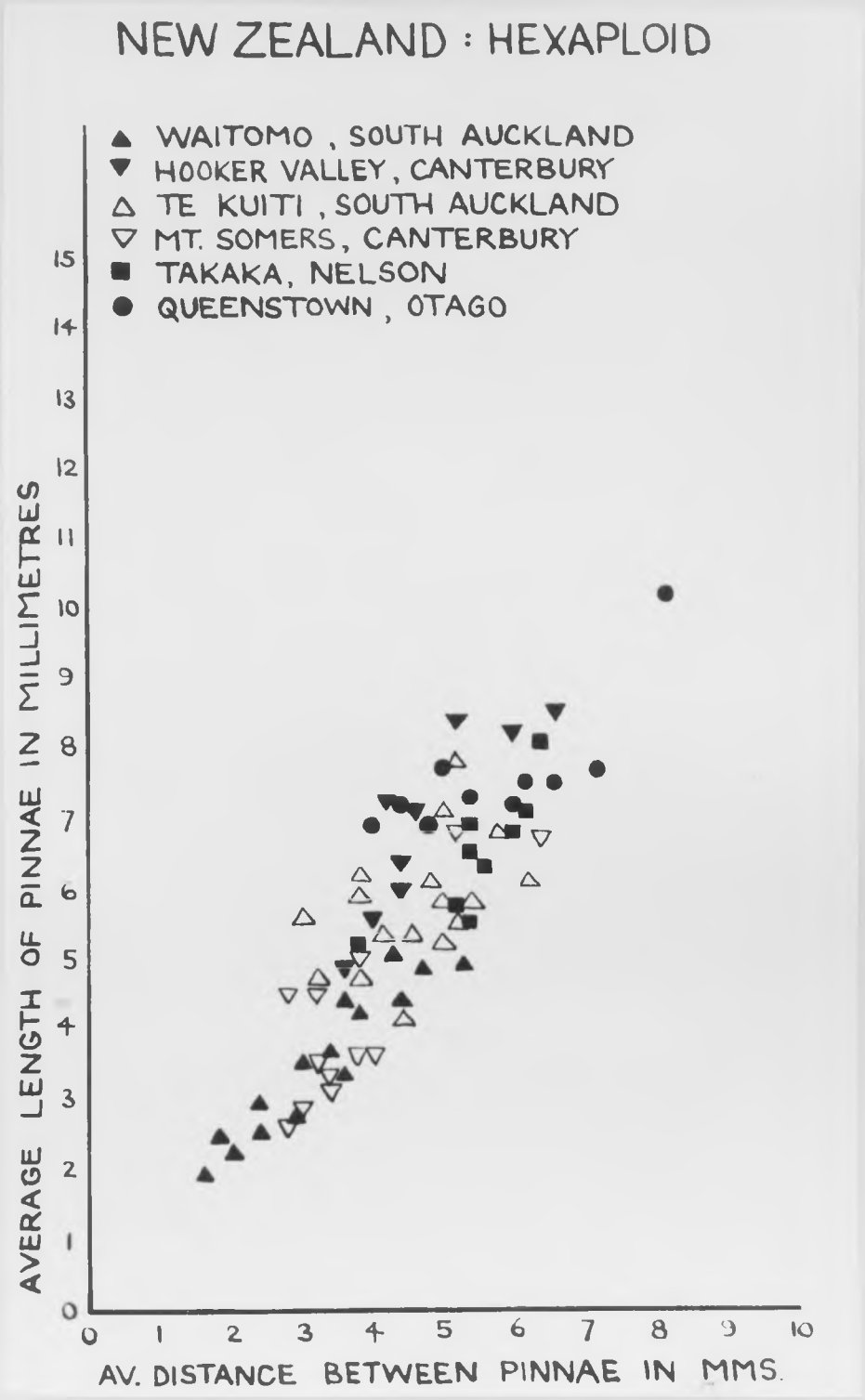
Scatter diagram. Fig. 86.



Scatter diagrams showing relationship between pinna length and pinna separation in fronds of diploid and tetraploid plants from Aber Falls, Caernarvonshire, and Pass of Llanberis, Caernarvonshire.



Scatter diagram showing relationship between pinna length and pinna separation in fronds of diploid plants from Wulgulmerang, Victoria, Australia, and in fronds of tetraploid plants from Jenolan Caves, N.S.W., Australia, and Tangoio, Hawke's Bay, New Zealand.

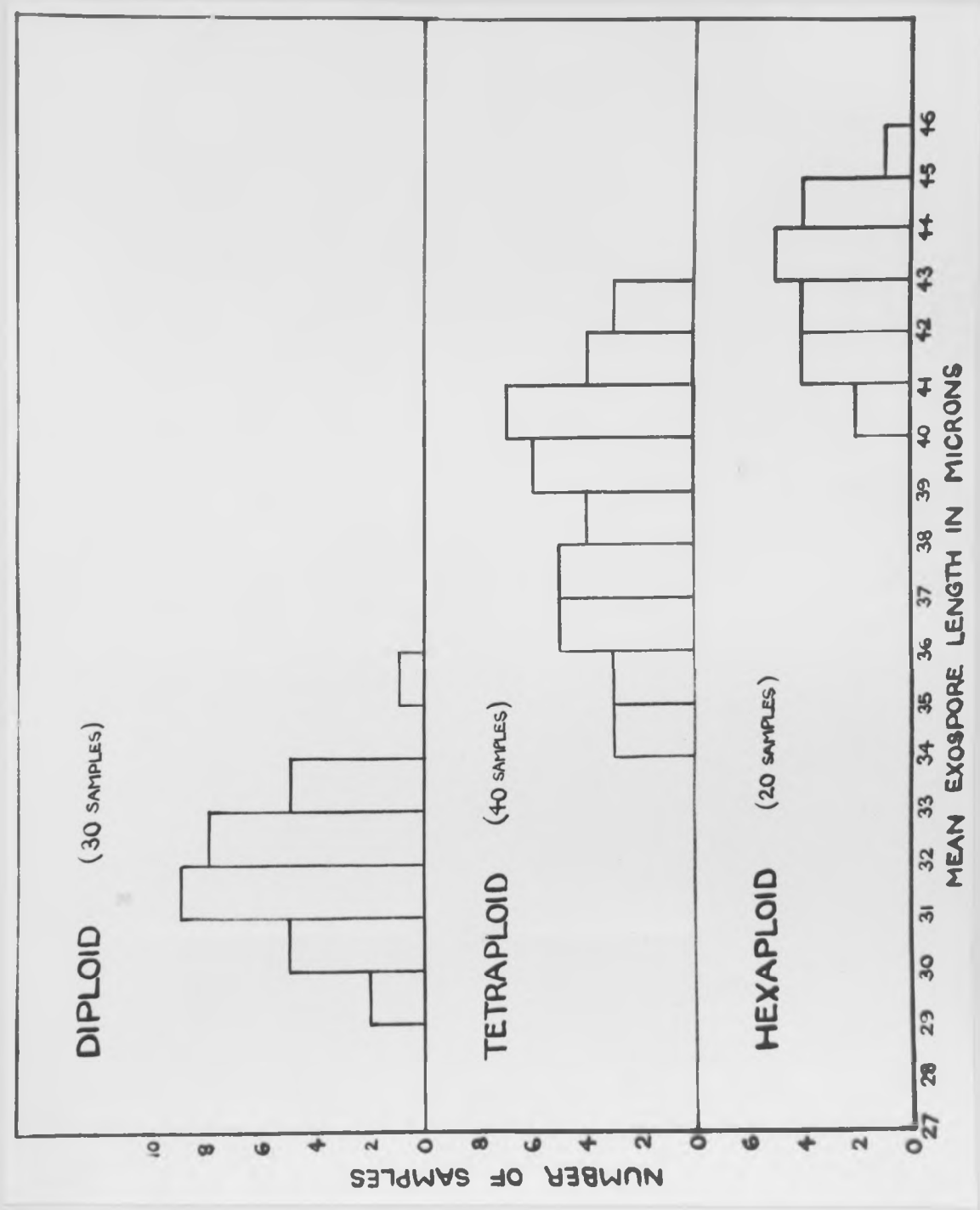


Scatter diagram showing relationship between pinna length and pinna size in fronds of plants of the hexaploid cytotype from various localities.

SPORE SIZE

HISTOGRAMS

Fig. 89.



Histograms for all three cytotypes of mean exospore measurements in samples taken from different collections of known chromosome number.

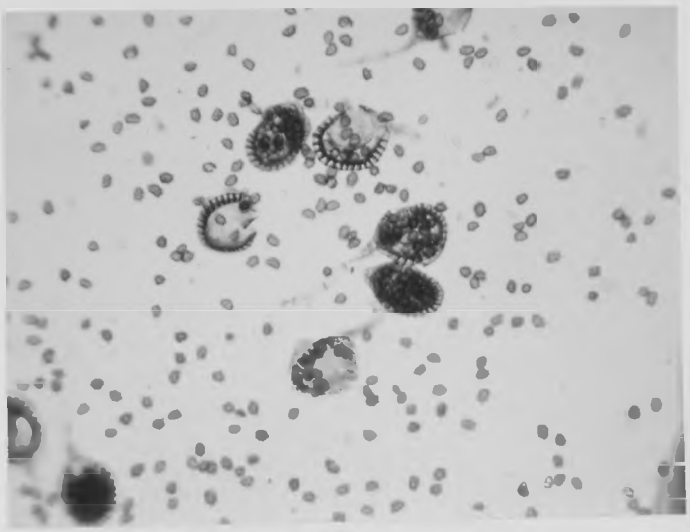
SPORANGIA & SPORES

PHOTOGRAPHS

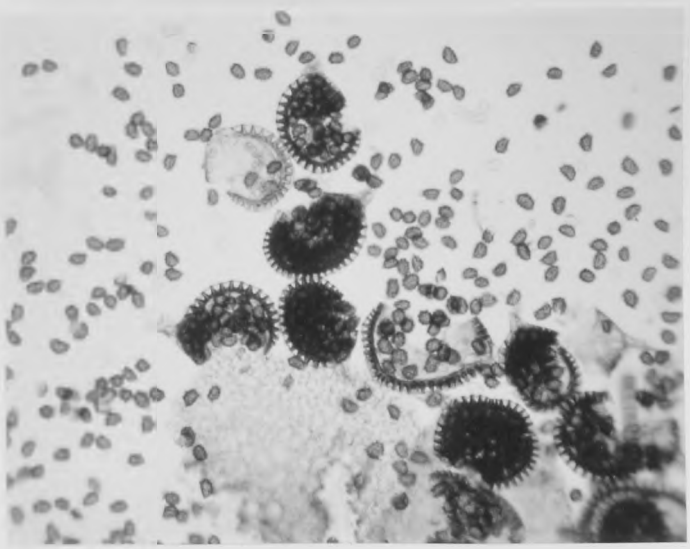
Figs. 90 - 93.

Sporangia : X 50.

A. Asplenium trichomanes : diploid cytotype
Tyn-y-Groes, Merioneth.

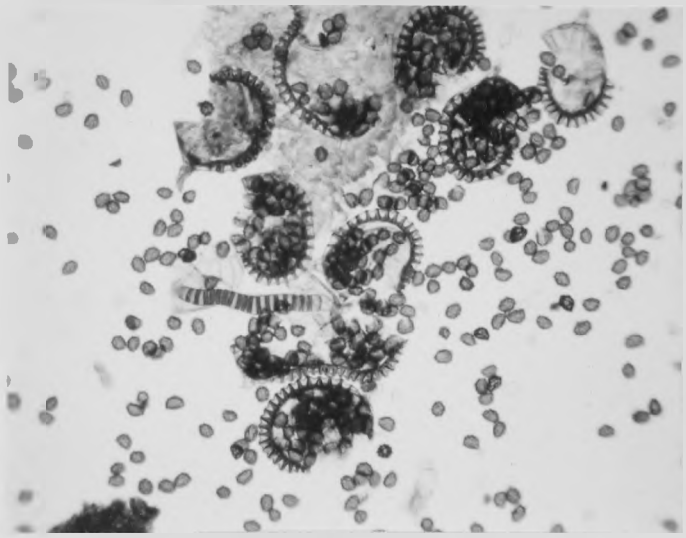


B. Asplenium trichomanes : tetraploid cytotype
Plymouth, Devon.



Sporangia : X 50.

A. Asplenium trichomanes : hexaploid cytotype
Canterbury, N.Z.



B. Asplenium trichomanes : artificial triploid hybrid.
4x N.America x 2x Europe

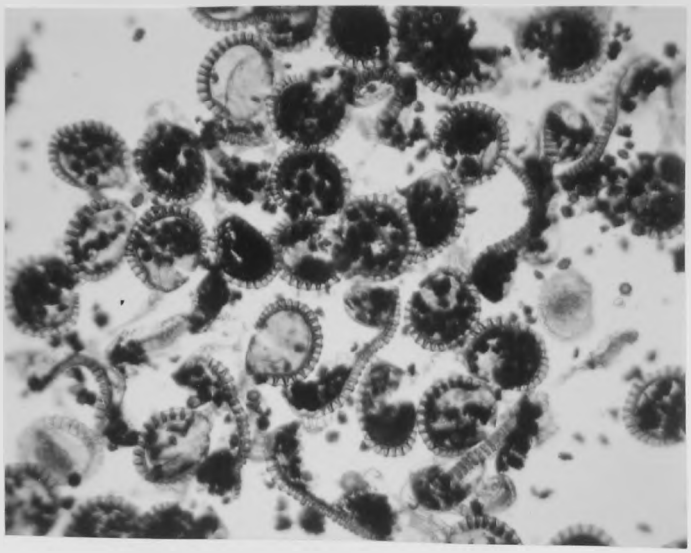
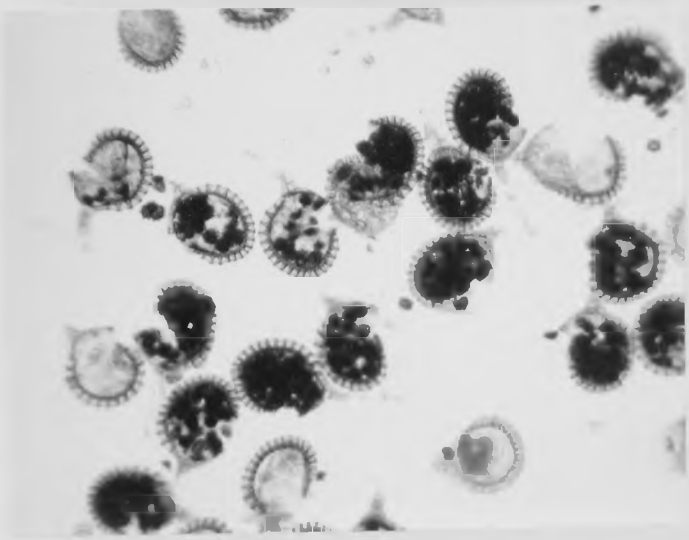


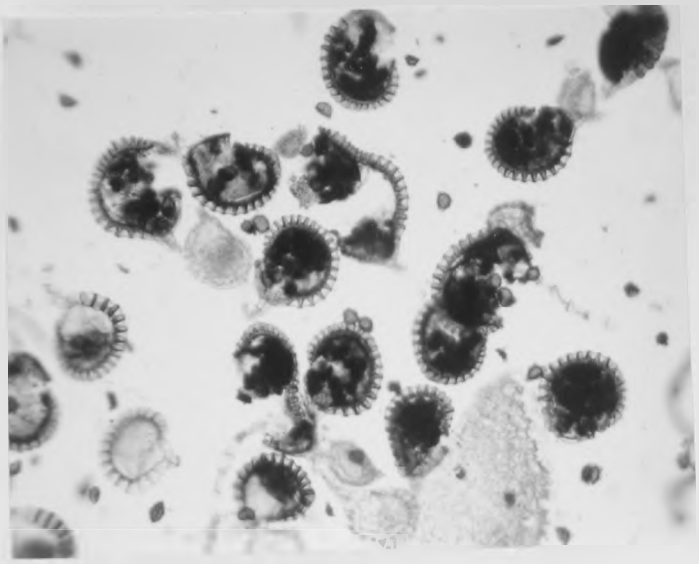
Fig. 92.

Sporangia : X 50.

A. Asplenium trichomanes : artificial tetraploid hybrid.
6x New Zealand x 2x Europe

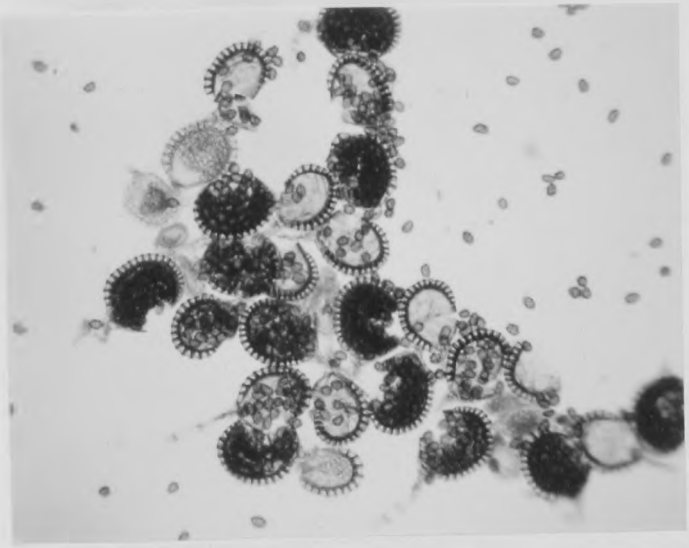


B. Asplenium trichomanes : artificial pentaploid hybrid.
6x New Zealand x 4x Europe

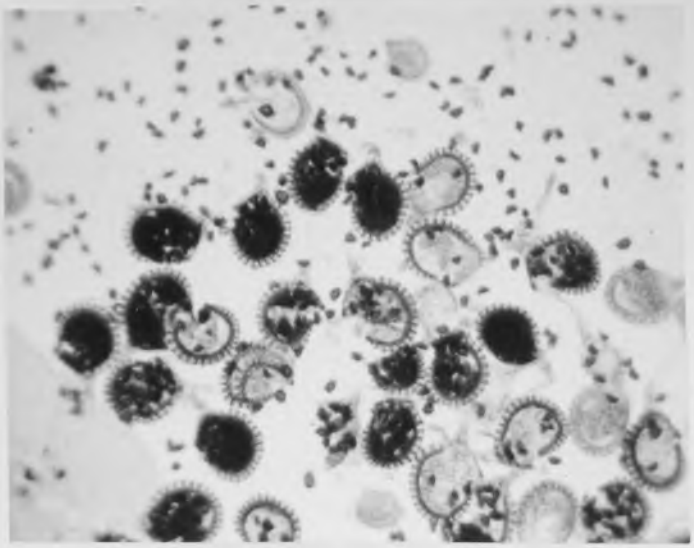


Sporangia : X 50.

A. Asplenium trichomanes : diploid cytotype
Tyn-y-Groes, Merioneth.



B. Asplenium trichomanes : artificial diploid hybrid.
2x Australia x 2x Europe.



CYTOLOGY

DIPLOID, TETRAPLOID, & HEXAPLOID CYTOTYPES

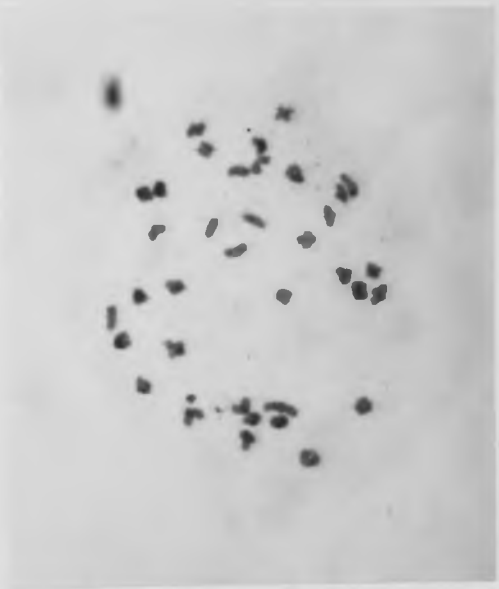
PHOTOGRAPHS

Figs. 94 - 104.

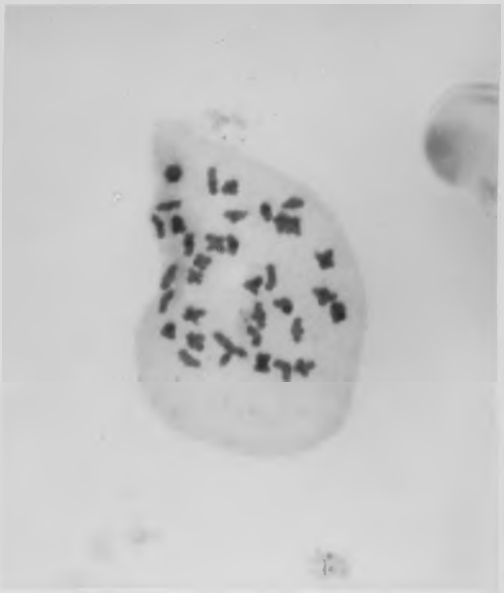
Objective : Watson 2mm NA 1.28 X 1000 Parachromatic Oil Immersion. Filter : Monochromatic Green. Magnification : X 1000. Negative : Ilford Special Rapid Panchromatic & Thin Film Half Tone Plates. Print : Contact.

Asplenium trichomanes : diploid cytotype

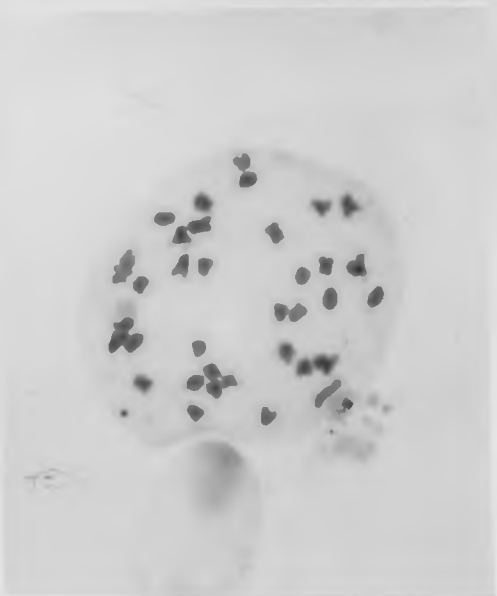
n = 36



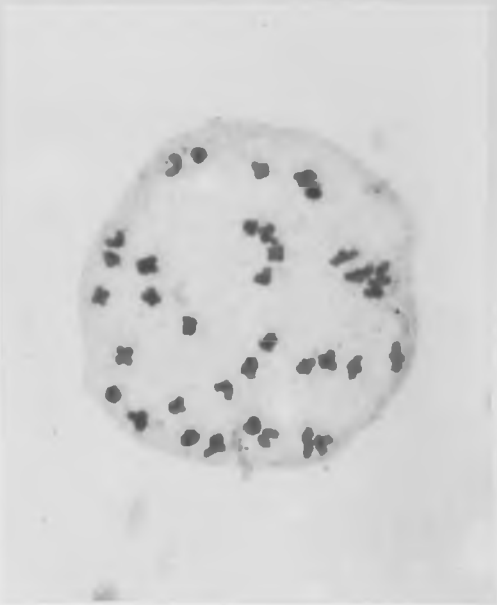
A. Pass of Llanberis, Caernarvon.



B. Castle Crag, Borrowdale, Cumberland.



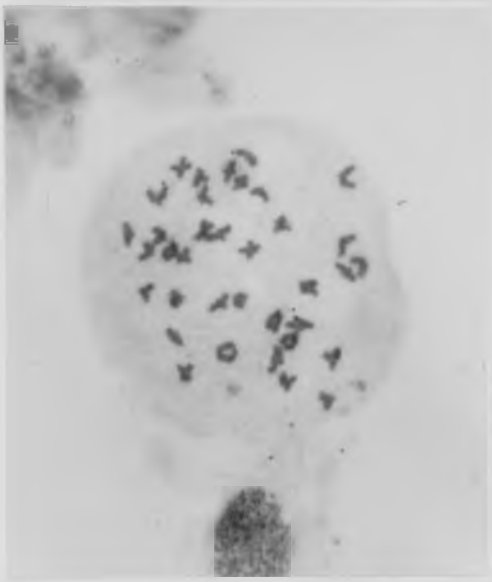
C. Stenton Rocks, Perthshire.



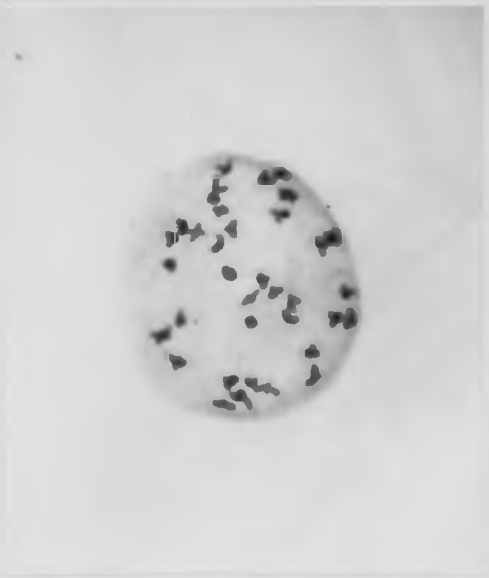
D. Craigs of Succouth, Aberdeen.

Asplenium trichomanes : diploid cytotype

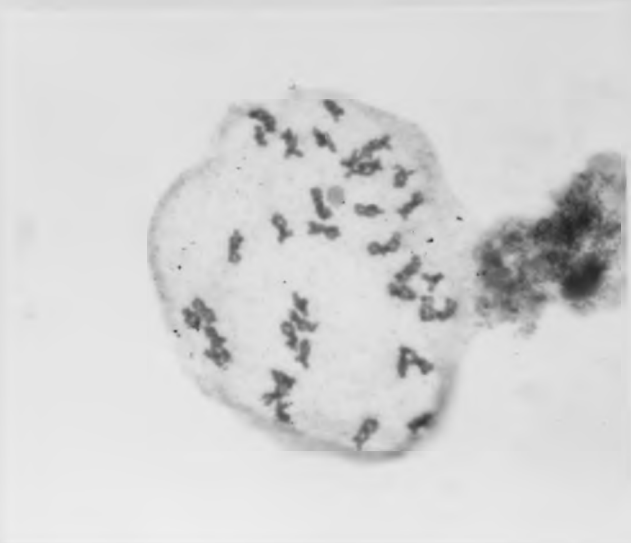
n = 36



A. St. Nectaire, Auvergne, France.

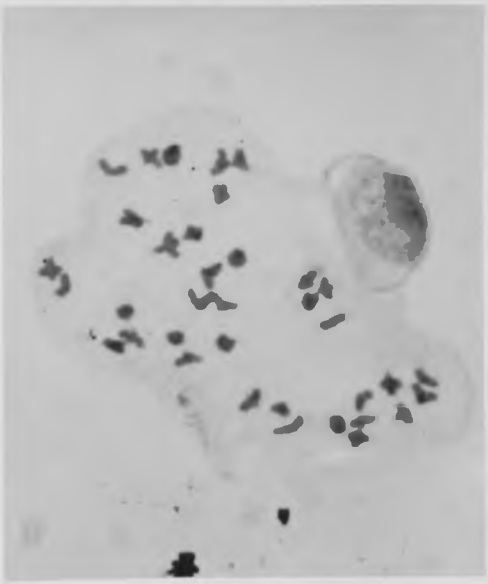


B. Mundheim, Hardanger, Norway.

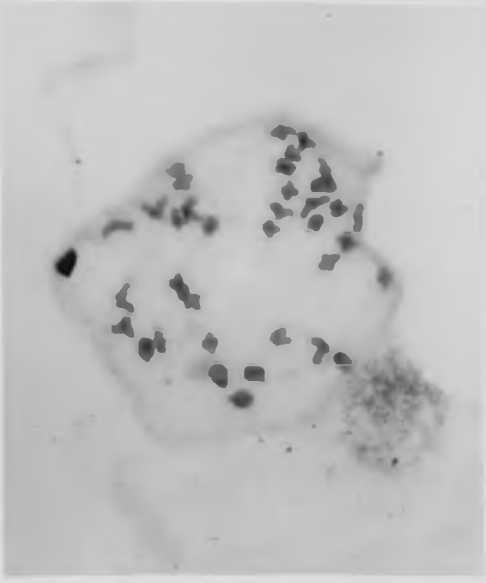


C. Wulgulmerang, Victoria, Australia.

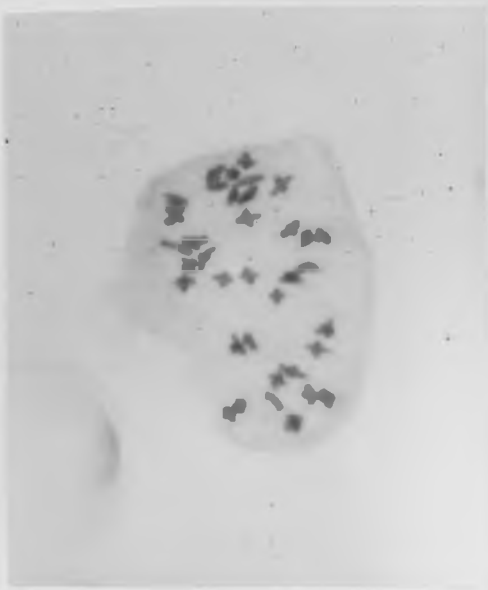
Asplenium trichomanes : diploid cytotype
n = 36



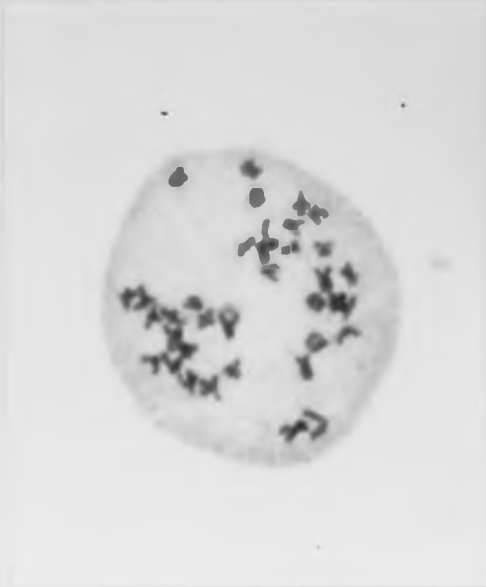
A. Kangra Himalaya, Punjab.



B. Gyadzong, S.E. Tibet.



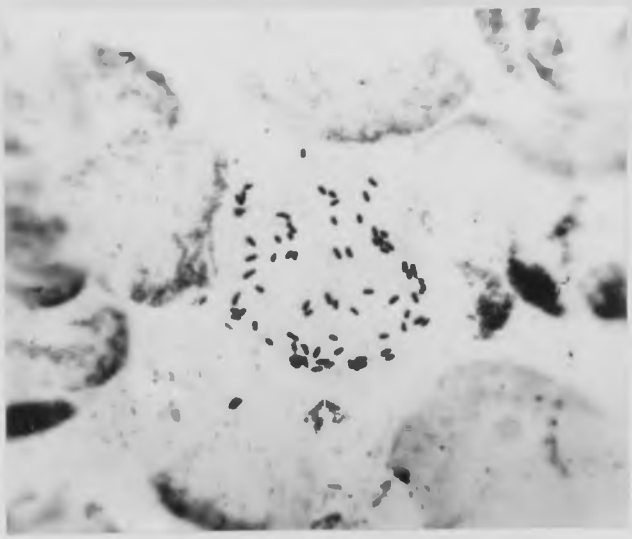
C. Walong, Lohit, Assam.



D. Parry Sound, Ontario, Canada.

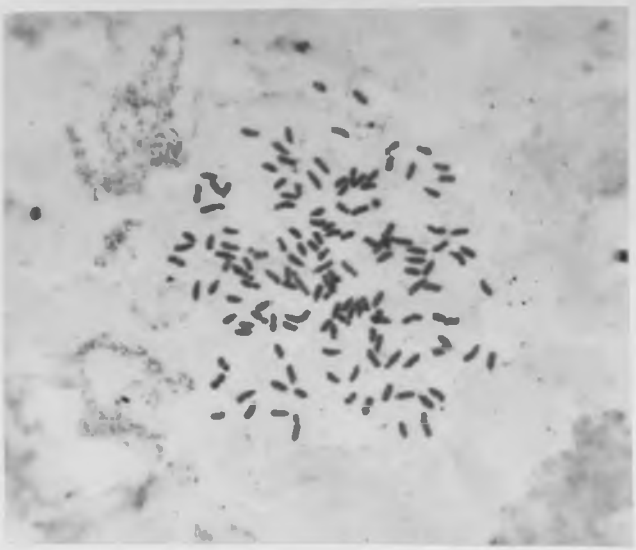
A. Asplenium trichomanes : diploid cytotype
2n = 72

Mont d'Ottan, Valais, Switzerland.



B. Asplenium trichomanes : tetraploid cytotype
2n = 144

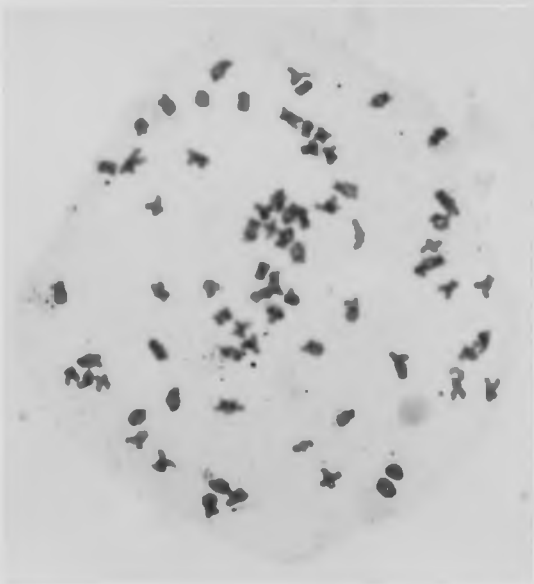
Buchan, Victoria, Australia.



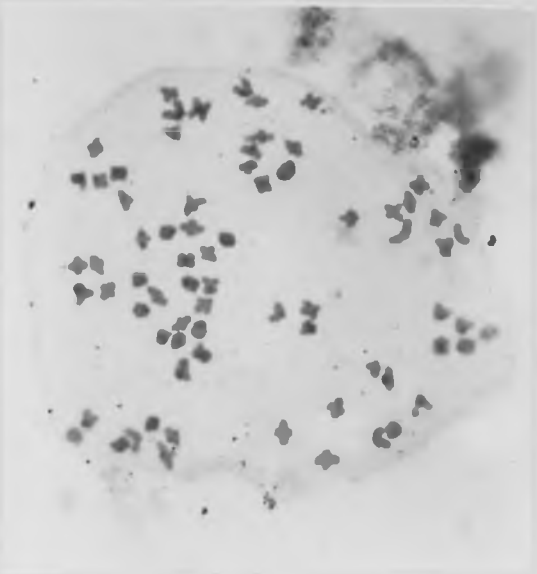
Cytology : meiosis, X 1000. Fig. 98.

Asplenium trichomanes : tetraploid cytotype

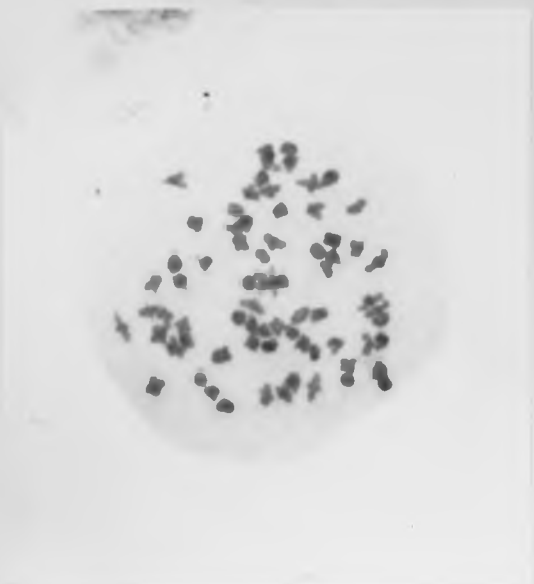
n = 72



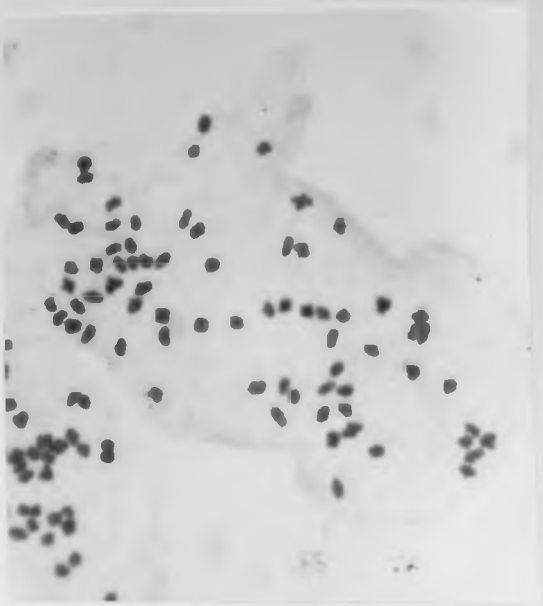
A. Tyn-y-Groes, Dolgelley, Merioneth.



B. Hayes, Kent.



C. Craig Dorney, Aberdeen.

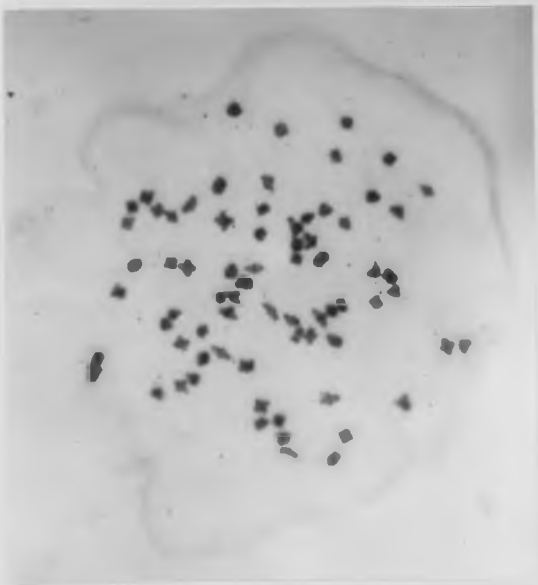


D. Aysgarth, Wensleydale, Yorkshire.

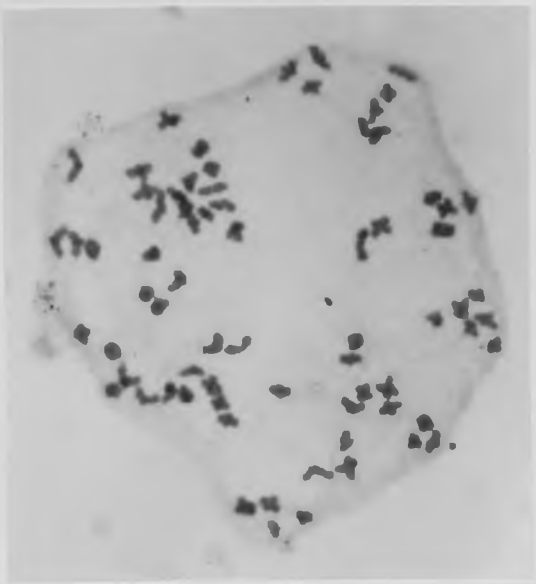
Cytology : meiosis, X 1000. Fig. 99.

Asplenium trichomanes : tetraploid cytotype

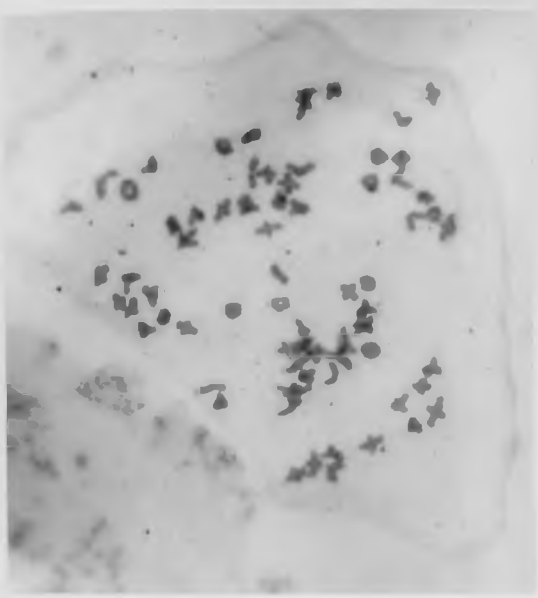
n = 72



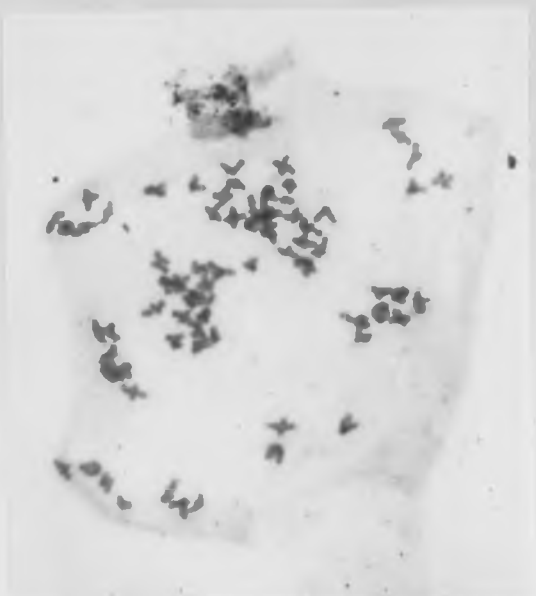
A. Rocky Saugeen River, Ontario, Canada.



B. Honsyu, Japan.



C. Soda, S.W. Saudi-Arabia.



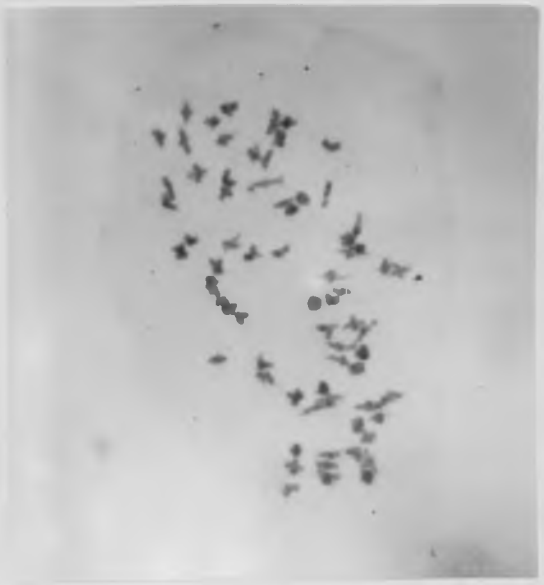
D. Tsanatalana, Basutoland, South Africa.

Cytology : meiosis, X 1000. Fig. 100.

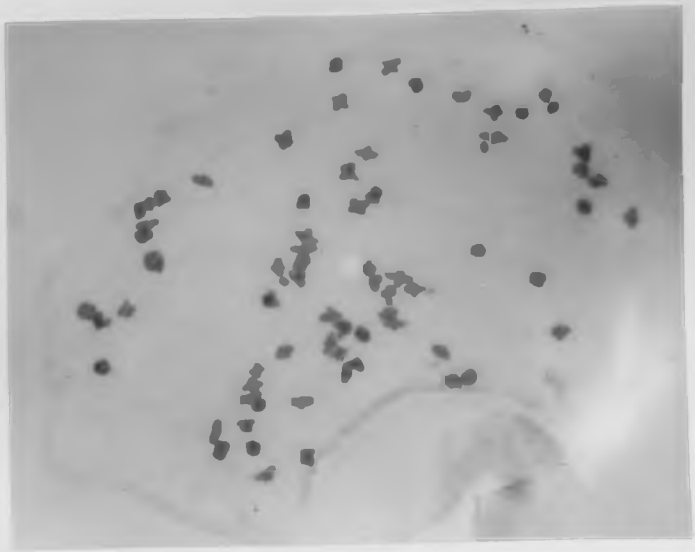
Asplenium trichomanes : tetraploid cytotype

n = 72

A. Tangoio, Hawke's Bay, New Zealand.



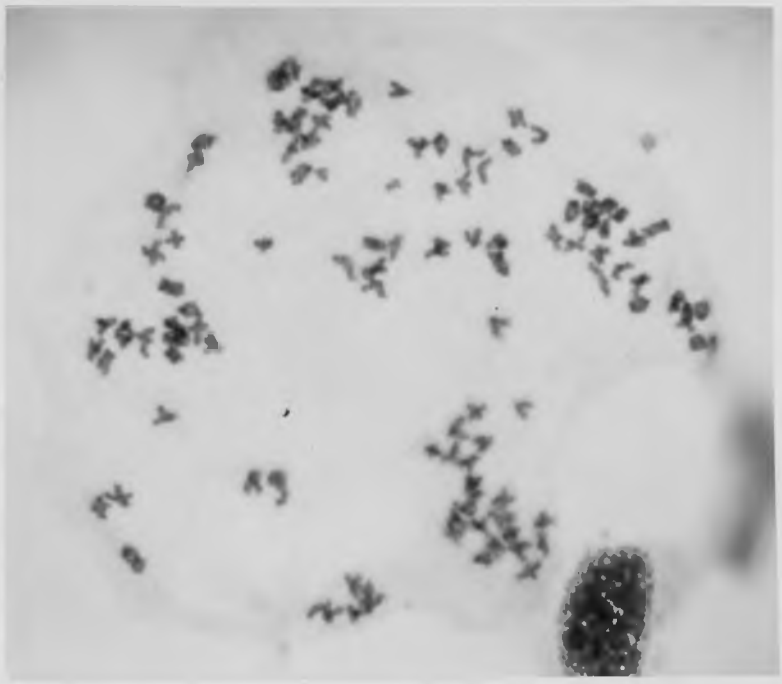
B. Buchan, Victoria, Australia.



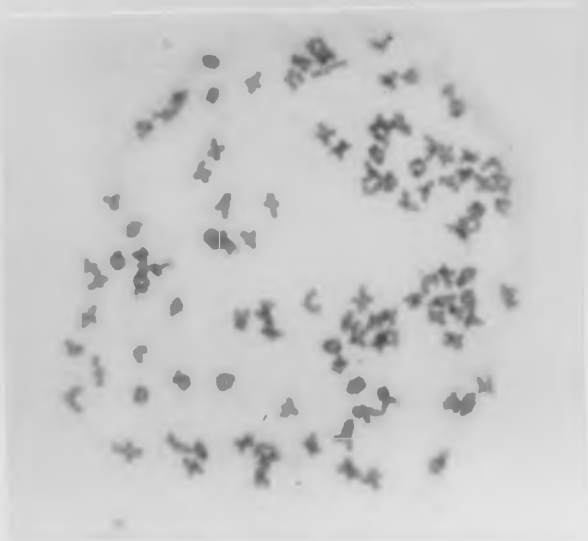
C. Mauna Loa, Hawaii.

Cytology : meiosis, X 1000. Fig. 101.

Asplenium trichomanes : hexaploid cytotype
n = 108



A. Waitomo, South Auckland L.D.,
New Zealand.

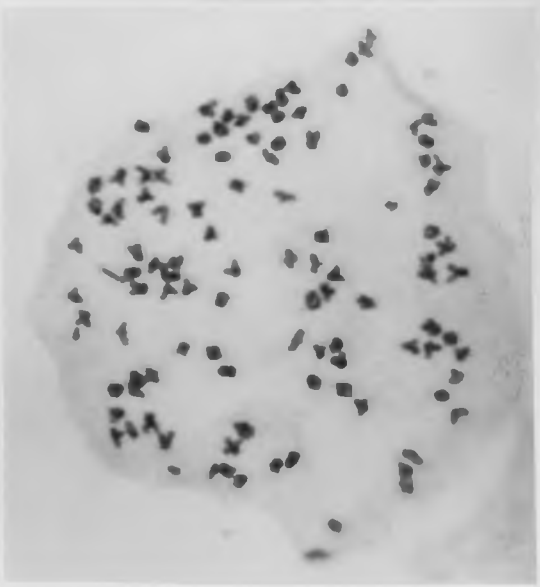


B. Takaka Hill, Nelson, New Zealand.

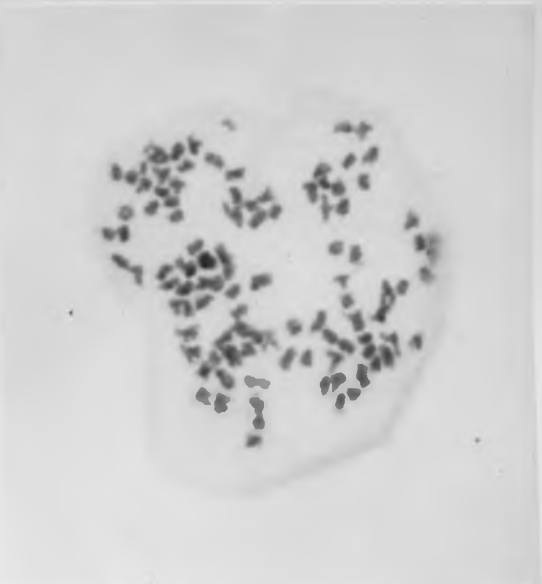
Asplenium trichomanes : hexaploid cytotype

n = 108

A. Castle Hill, Canterbury, N.Z.



B. Avoca, Canterbury, N.Z.



C. Molesworth, Marlborough, N.Z.

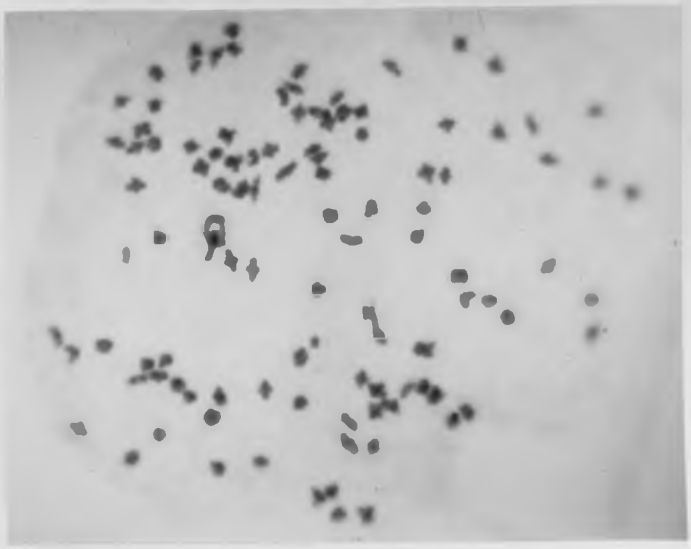
Cytology : meiosis, X 1000. Fig. 103.

Asplenium trichomanes : hexaploid cytotype

n = 108

A. Monument, Hooker valley, Canterbury, N.Z.

Regular meiosis, 108 bivalents.



B. Monument, Hooker valley, Canterbury, N.Z.

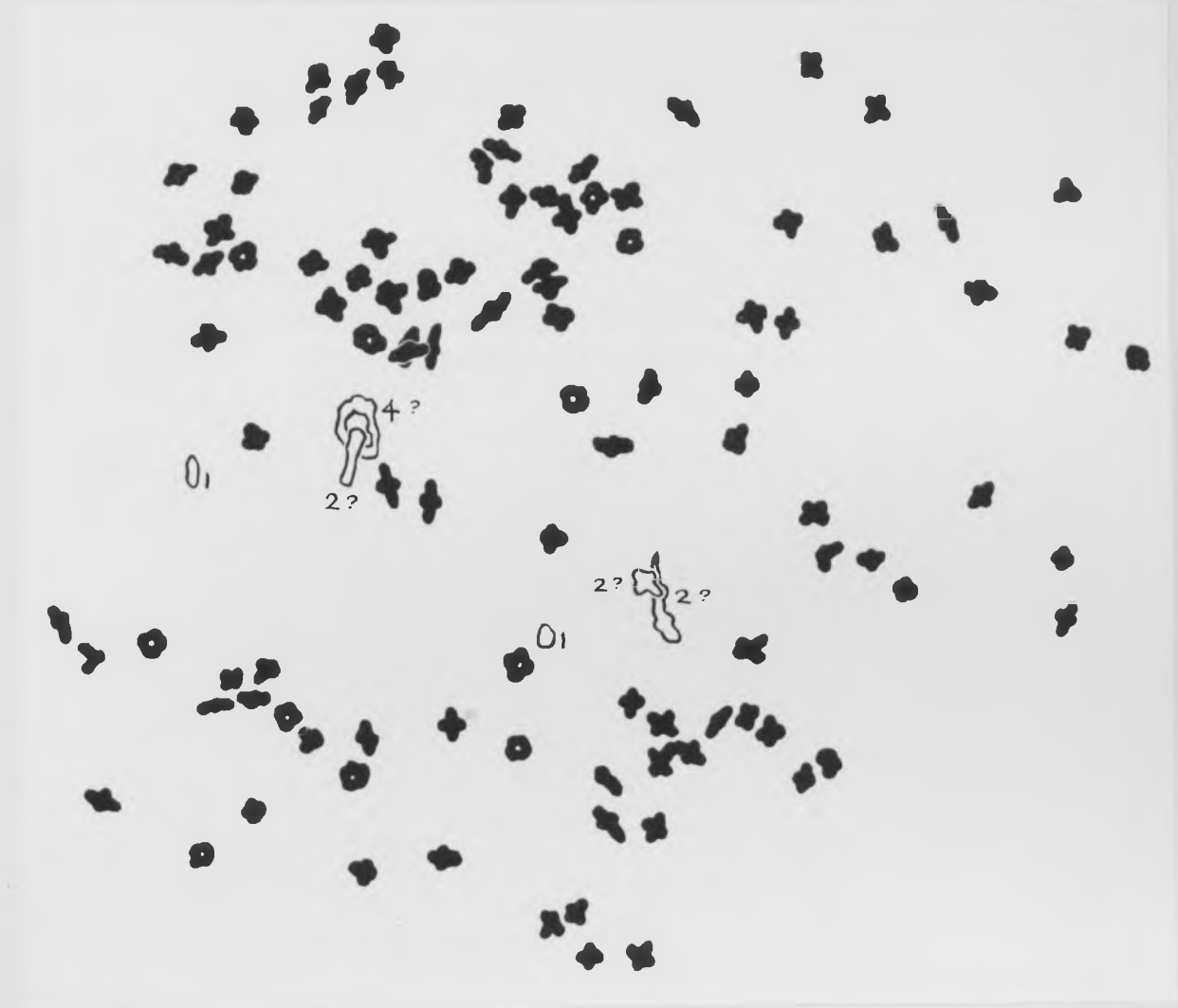
Slightly irregular meiosis, one or two multivalents.

See also fig. 104.

Cytology : meiosis. Fig. 104.

Asplenium trichomanes : hexaploid cytotype
n = 108

Meiosis X 2000. See also fig. 103 b.



Monument, Hooker valley, Canterbury, N.Z.

Two univalents and apparent multivalent associations are shown in white. Undoubted bivalents are in black.

CYTOLOGY

HYBRIDS

Figs. 105 - 130.

Explanatory diagrams are given for all photographs. In these, bivalents are shown in black, and univalents in outline. Trivalents are indicated by a small figure 3. Wherever possible these diagrams are placed on the same sheet as the photograph they accompany, but this has not always been possible. See, for example, figs. 110a & b.

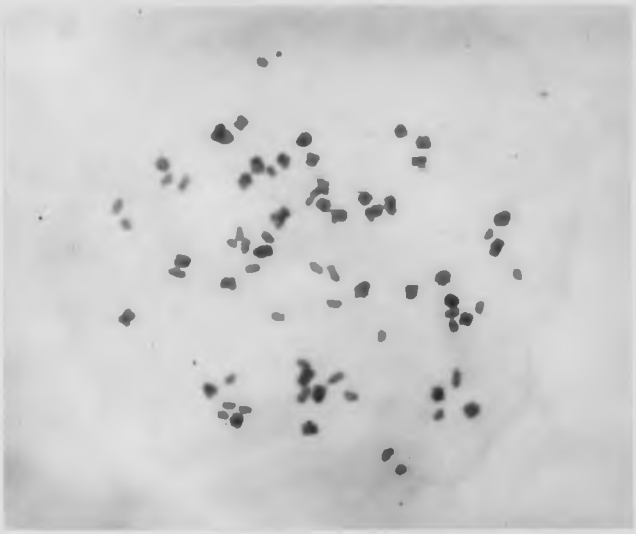
Technical data are the same as for figs. 94 - 104.

Asplenium trichomanes 2x Europe x A. adulterinum.
Caernarvon Austria

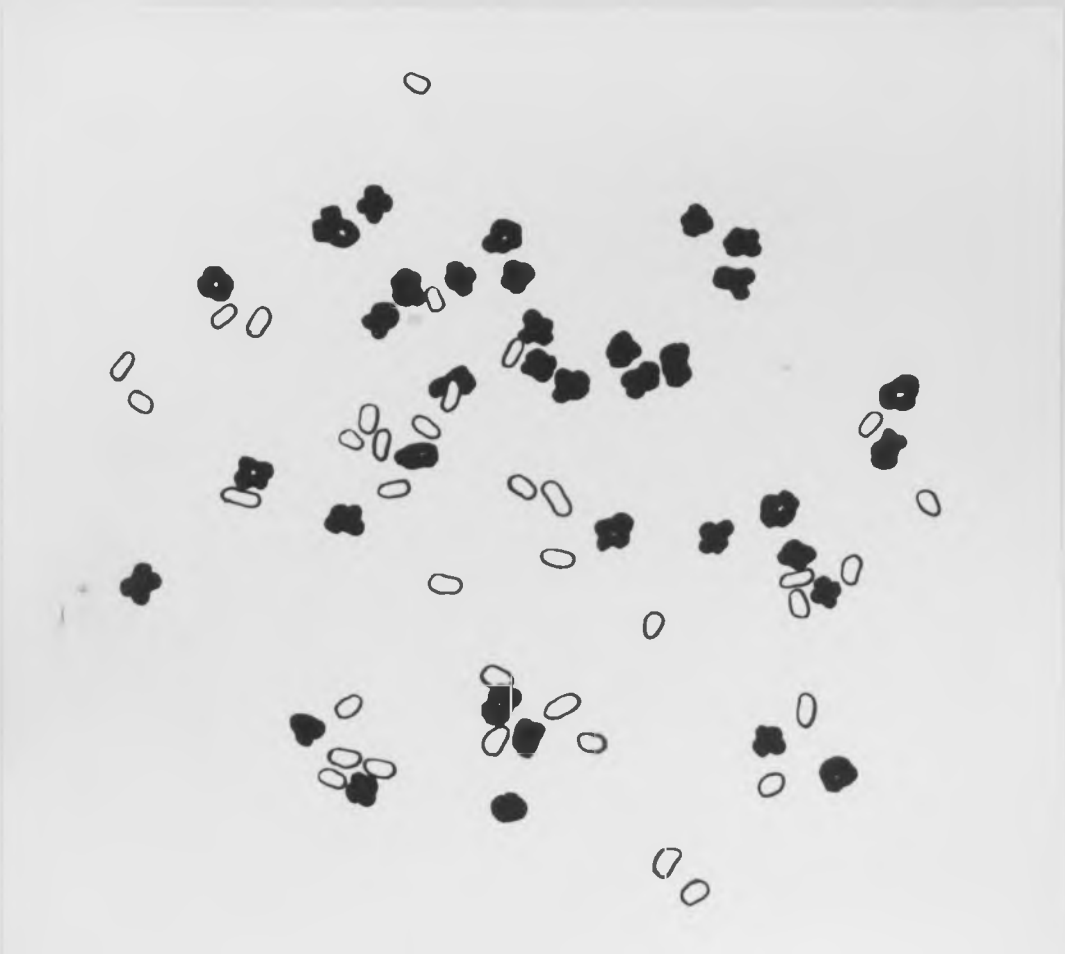
Analysis of meiotic pairing : 36 bivalents & 36 univalents.

Meiosis

X 1000



X 2000



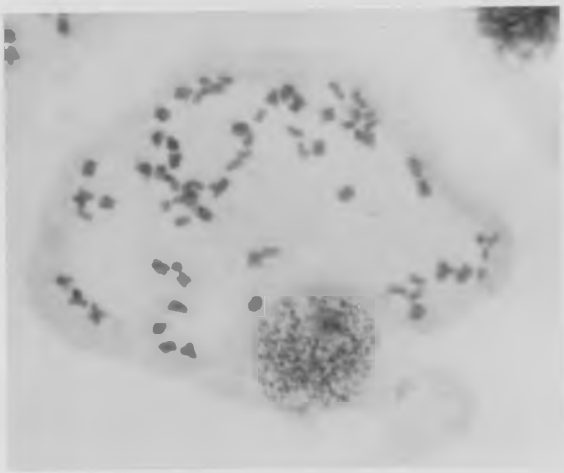
Cytology : artificial triploid hybrid. Fig. 106.

Asplenium trichomanes 2x Asia x A. adulterinum.
Tibet Austria

Analysis of meiotic pairing : 36 bivalents & 36 univalents.

Meiosis

X 1000



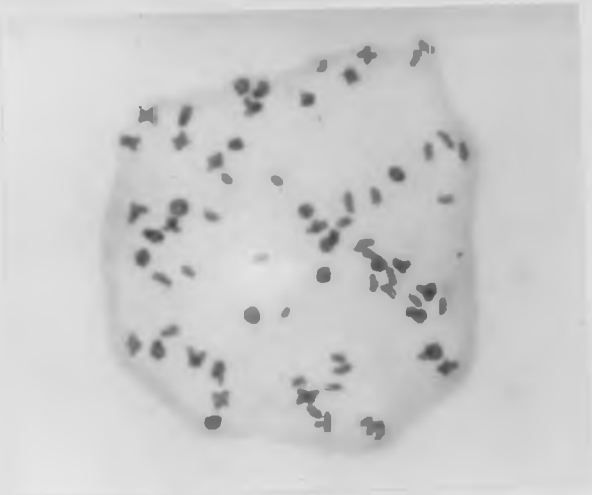
X 2000

Asplenium trichomanes 2x N.America x A. adulterinum
Ontario Austria

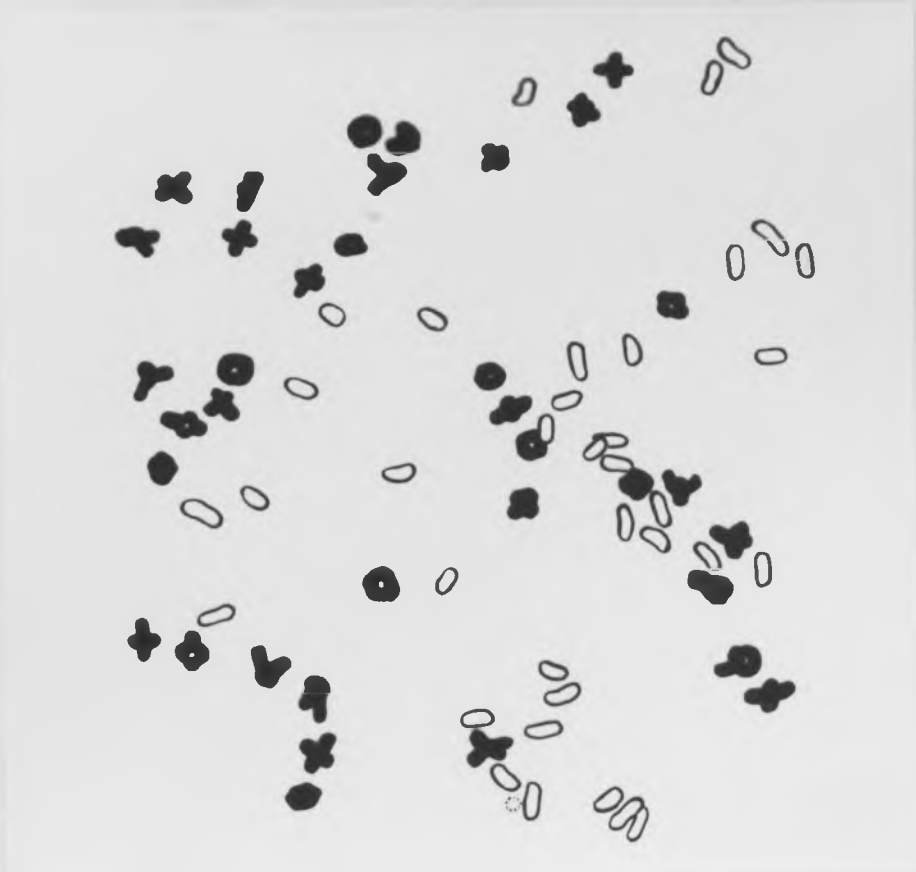
Analysis of meiotic pairing : 36 bivalents & 36 univalents.

Meiosis

X 1000



X 2000

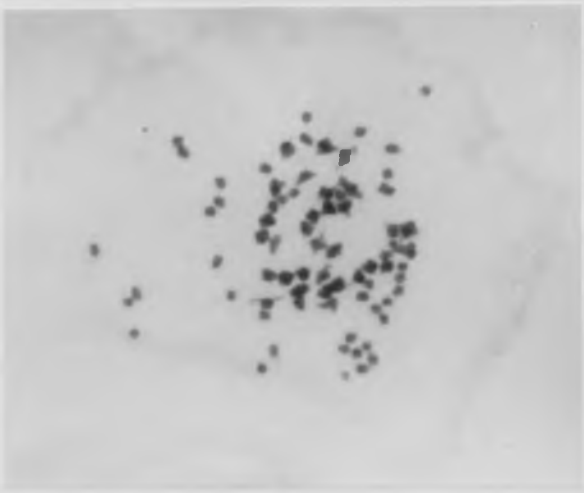


Asplenium trichomanes 2x Australia x A. adulterinum.
Victoria

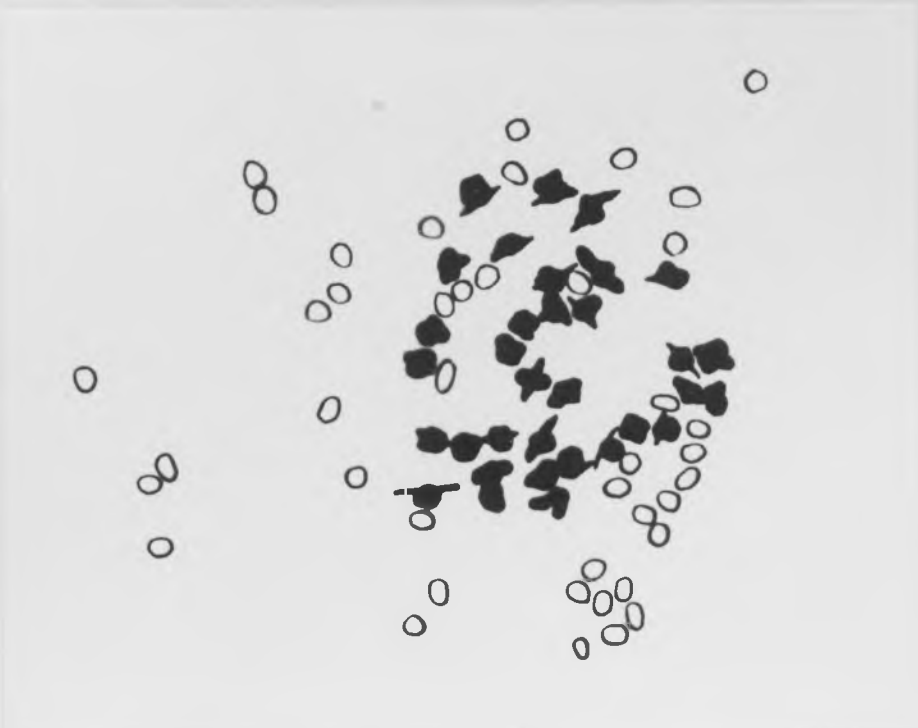
Analysis of meiotic pairing : 33 bivalents & 42 univalents.

Meiosis

X 1000



X 2000



Cytology : artificial tetraploid hybrid. Fig. 109.

Asplenium adulterinum x A. trichomanes 4x Europe.
Austria Devon

Analysis of meiotic pairing : 3 triv., 33 biv., & 69 univ.

Meiosis

X 1000

X 2000

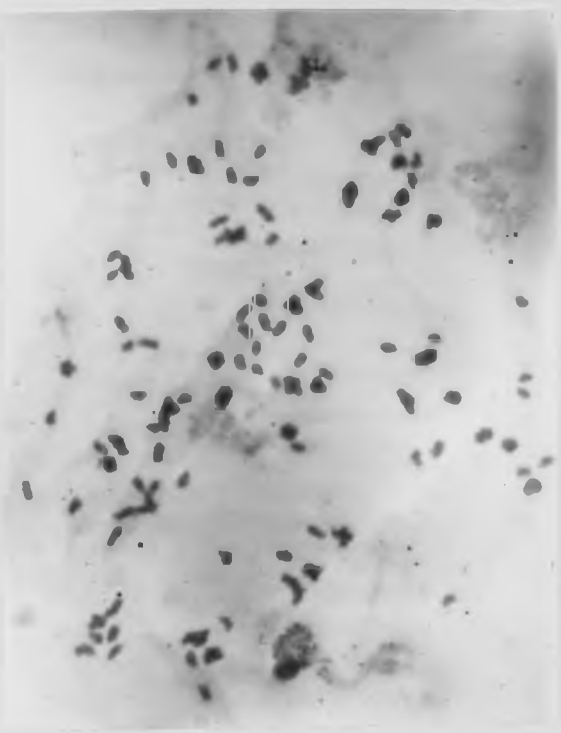
Cytology : artificial tetraploid hybrid. Fig. 110 a.

Asplenium trichomanes 4x N.America x A. adulterinum.
Vermont Austria

Analysis of meiotic pairing : 2 triv., 33 biv., & 72 univ.

Meiosis

X 1000



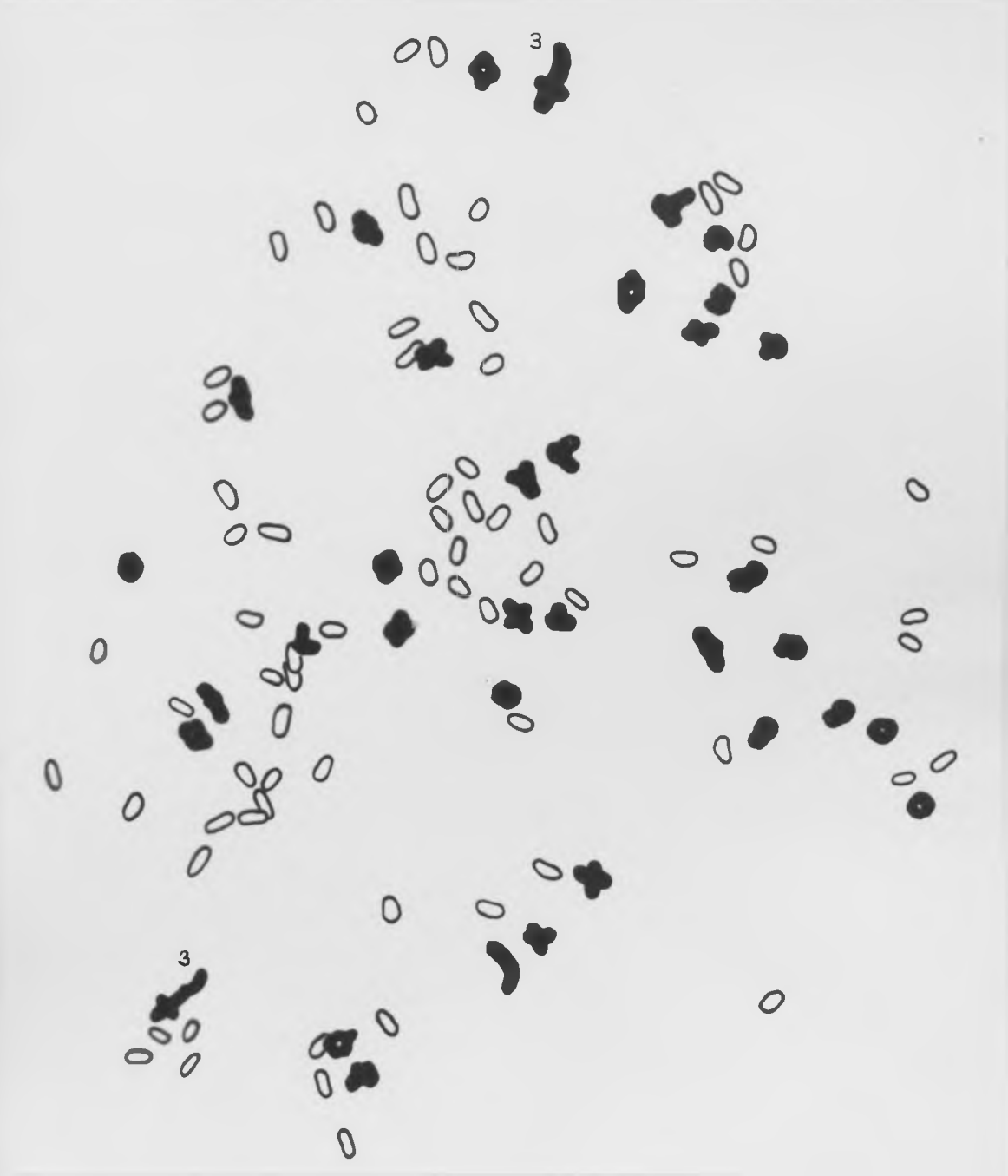
See also fig. 110 b.

Cytology : artificial tetraploid hybrid. Fig. 110 b.

Asplenium trichomanes 4x N.America x A. adulterinum.
Vermont Austria

Analysis of meiotic pairing : 2 triv., 33 biv., & 72 univ.

X 2000 See also fig. 110 a.

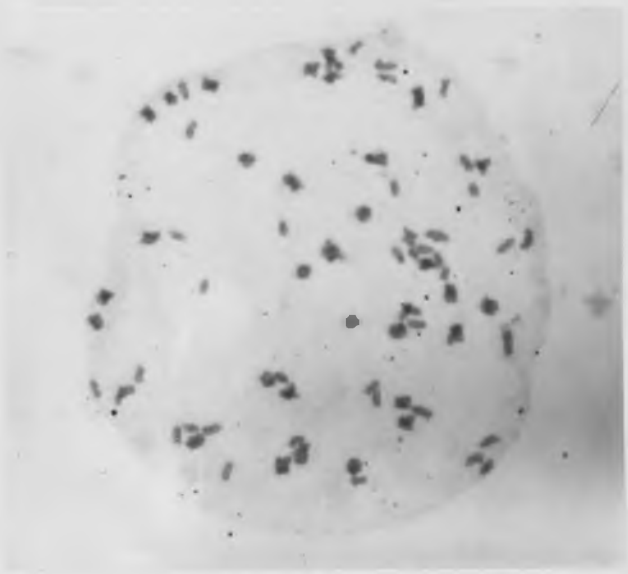


Asplenium trichomanes 3x : Tyn-y-Groes, Caernarvon.

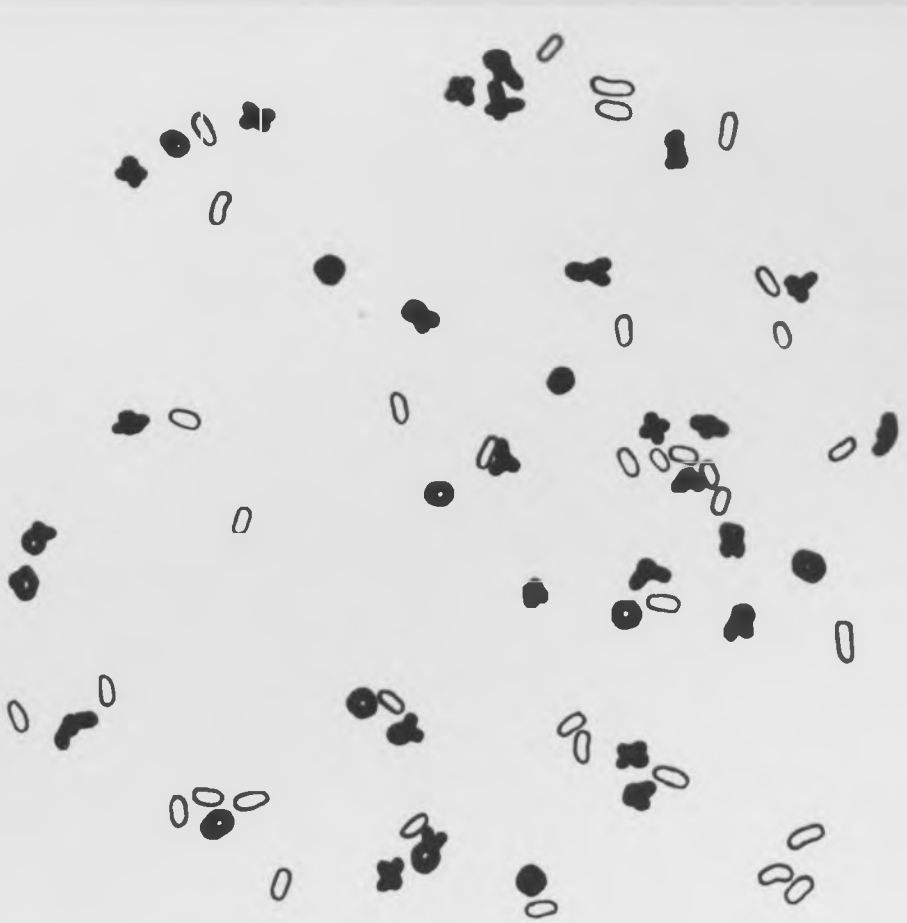
Analysis of meiotic pairing : 36 bivalents & 36 univalents.

Meiosis

X 1000



X 2000

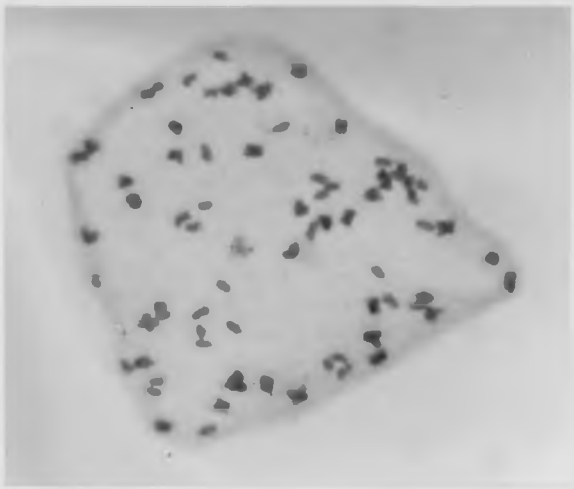


Asplenium trichomanes 3x : Tyn-y-Groes, Caernarvon.

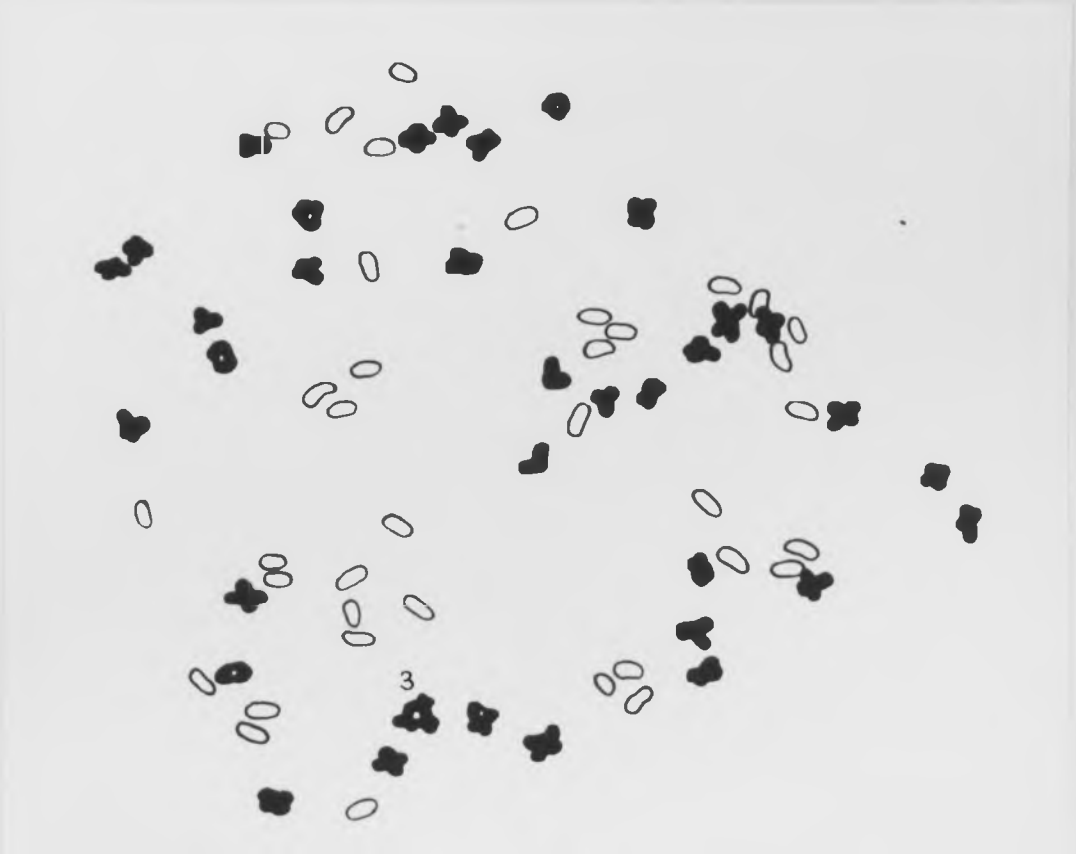
Analysis of meiotic pairing : 1 triv., 34 biv., & 37 univ.

Meiosis

X 1000



X 2000

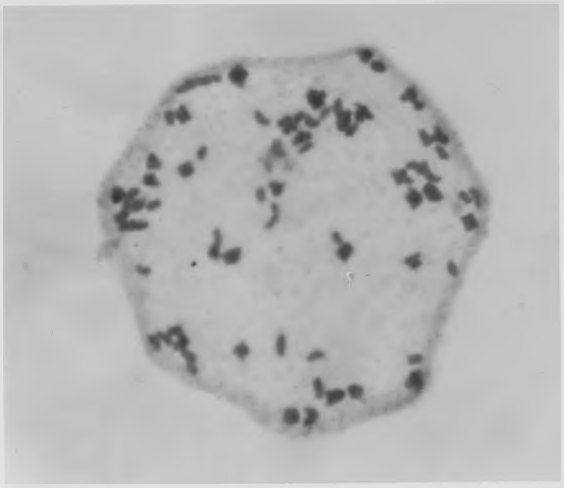


Asplenium trichomanes 3x : Tyn-y-Groes, Caernarvon.

Analysis of meiotic pairing : 4 triv., 31 biv., & 34 univ.

Meiosis

X 1000



X 2000

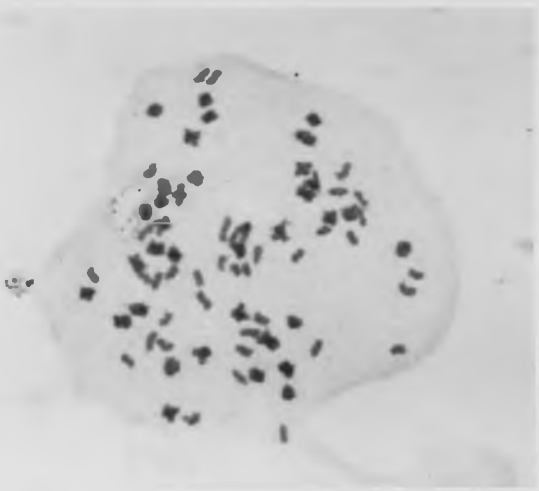
Cytology : natural triploid hybrid. Fig. 114.

Asplenium trichomanes 3x : Tyn-y-Groes, Caernarvon.

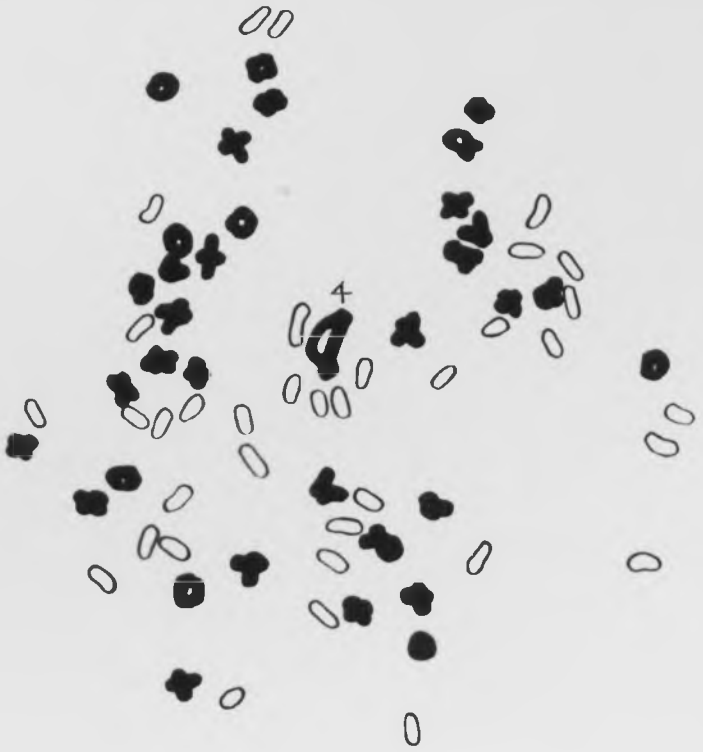
Analysis of meiotic pairing : 1 quadriv., 34 biv., & 36 univ.

Meiosis

X 1000



X 2000



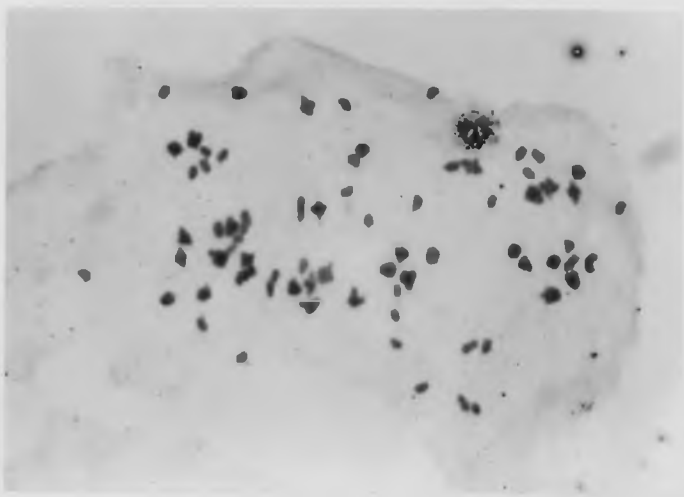
Cytology : natural triploid hybrid. Fig. 115.

Asplenium trichomanes 3x : Tyn-y-Groes, Caernarvon.

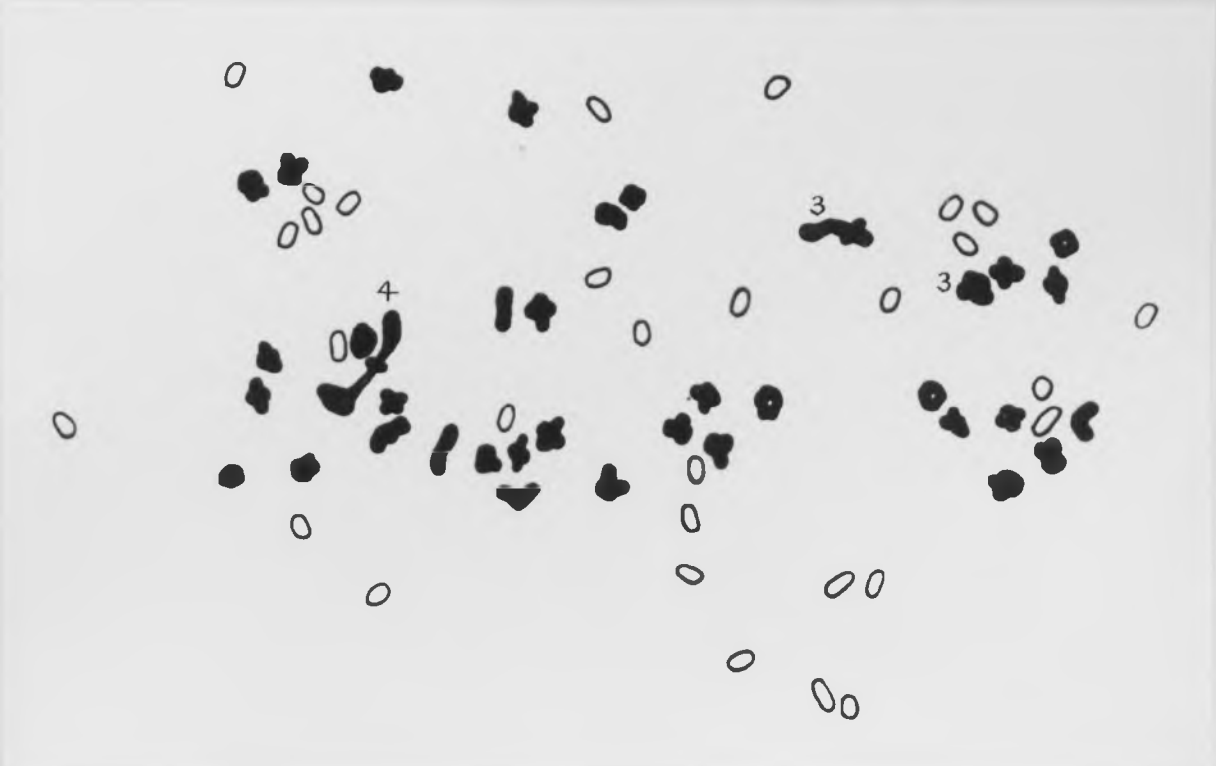
Analysis of meiotic pairing : 1 quadriv., 2 triv., 34 biv., & 30 univ.

Meiosis

X 1000



X 2000

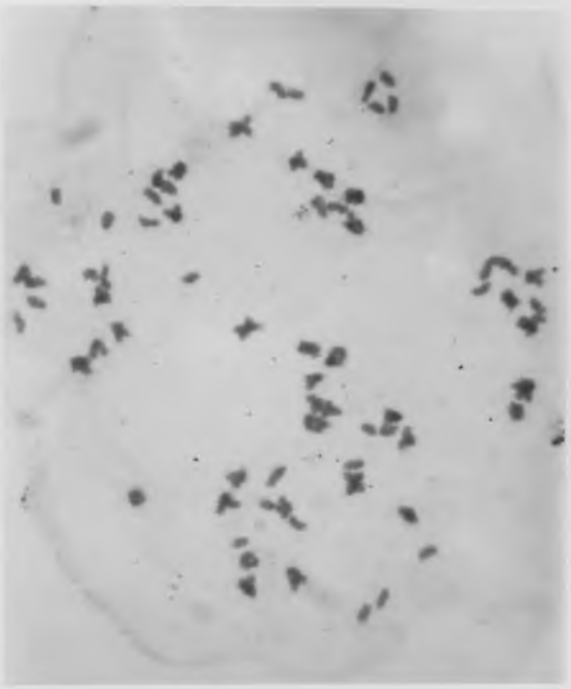


Cytology : natural triploid hybrid. Fig. 116 a.

Asplenium trichomanes 3x : Mundheim, Norway.

Analysis of meiotic pairing : 1 triv., 36 biv., & 33 univ.

Meiosis X 1000. See also fig. 116 b.

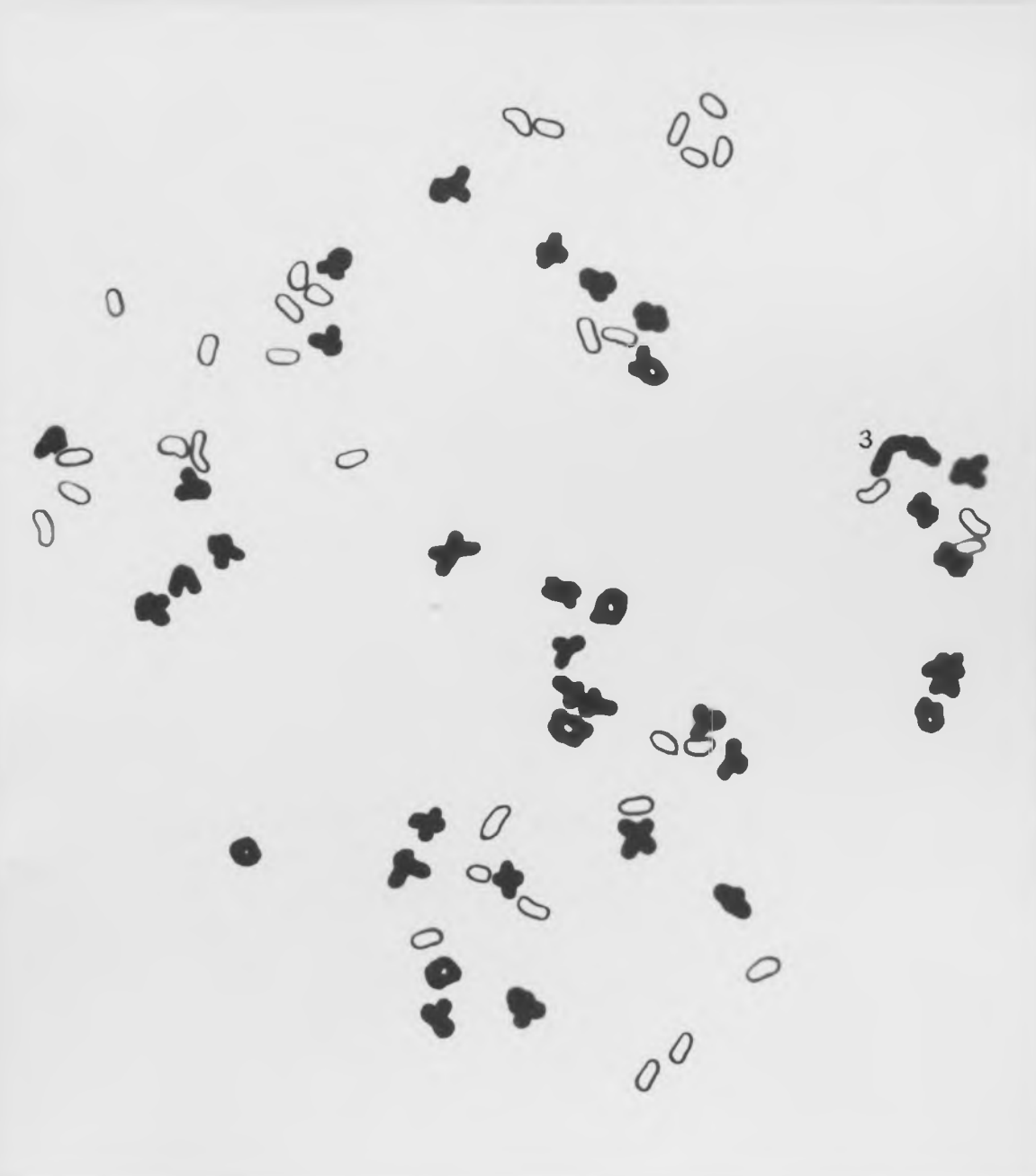


Cytology : natural triploid hybrid. Fig. 116 b.

Asplenium trichomanes 3x : Mundheim, Norway.

Analysis of meiotic pairing : 1 triv., 36 biv., & 33 univ.

Meiosis X 2000. See also fig. 116 a.



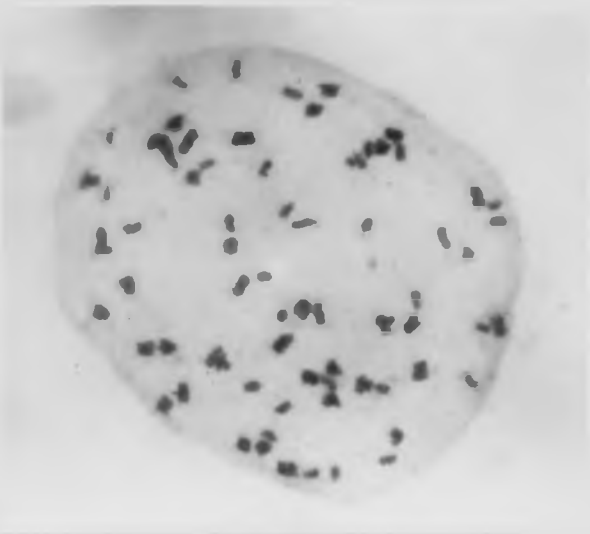
Cytology : artificial triploid hybrid. Fig. 117.

Asplenium trichomanes 2x Asia Nepal x A. trichomanes 4x Europe Devon

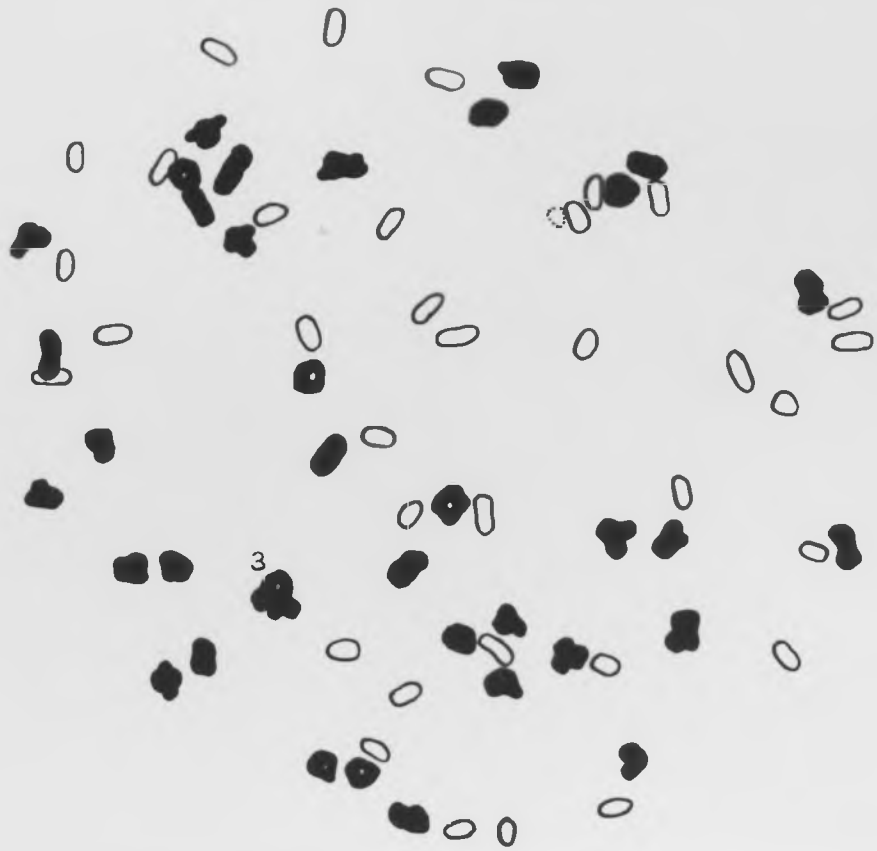
Analysis of meiotic pairing : 1 triv., 35 biv., & 35 univ.

Meiosis

X 1000



X 2000



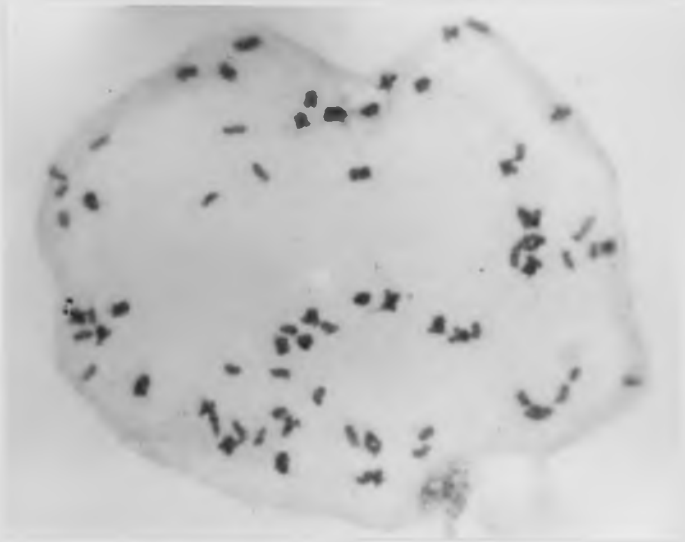
Cytology : artificial triploid hybrid. Fig. 118.

Asplenium trichomanes 2x N.America Ontario x A.trichomanes 4x Europe Devon

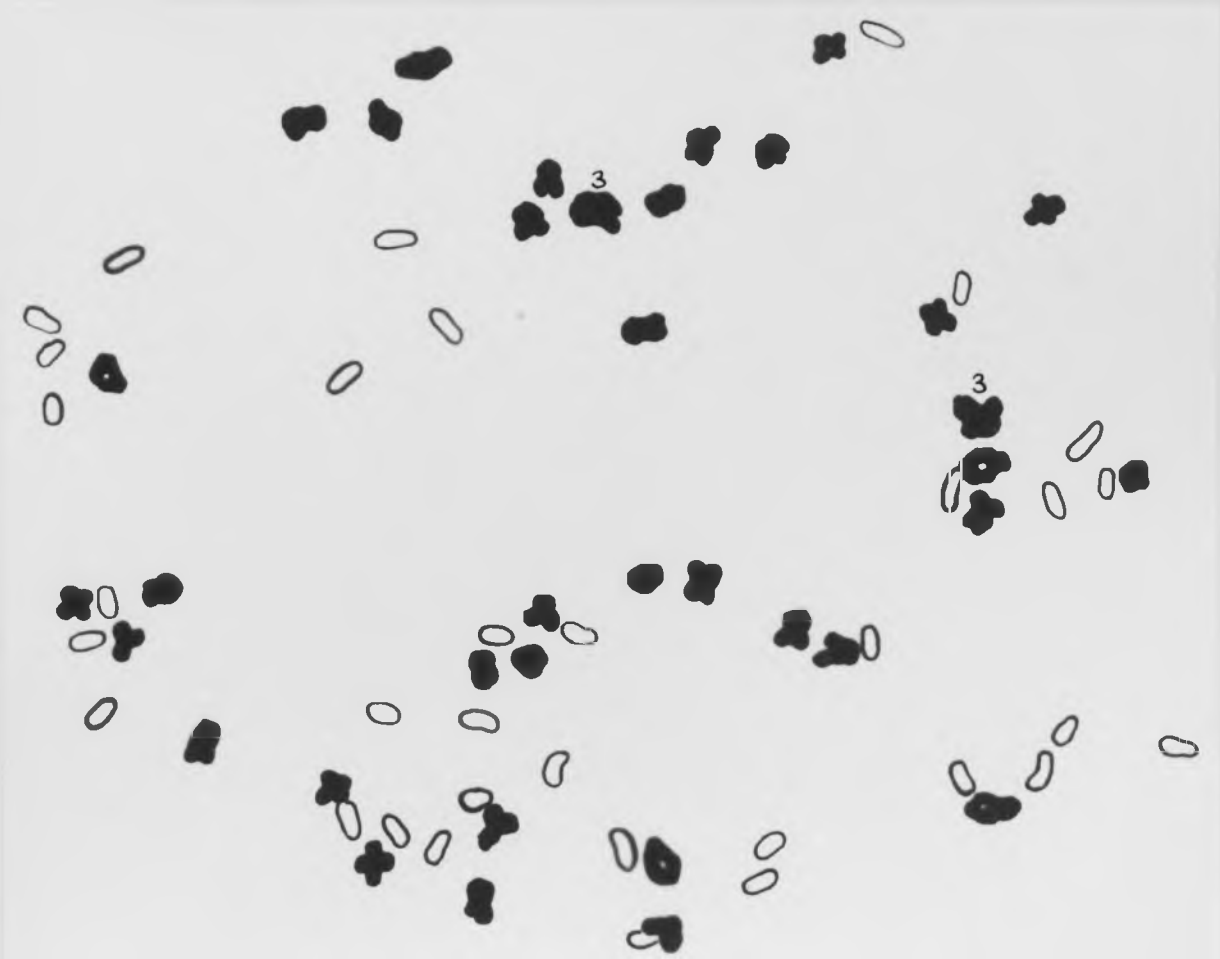
Analysis of meiotic pairing : 2 triv., 34 biv., & 34 univ.

Meiosis

X 1000



X 2000



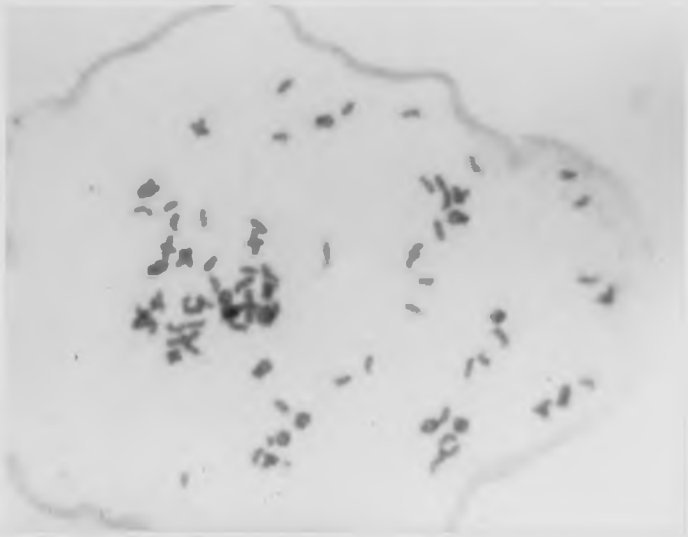
Cytology : artificial triploid hybrid. Fig. 119.

Asplenium trichomanes 2x Australia Victoria x A.trichomanes 4x Europe Devon

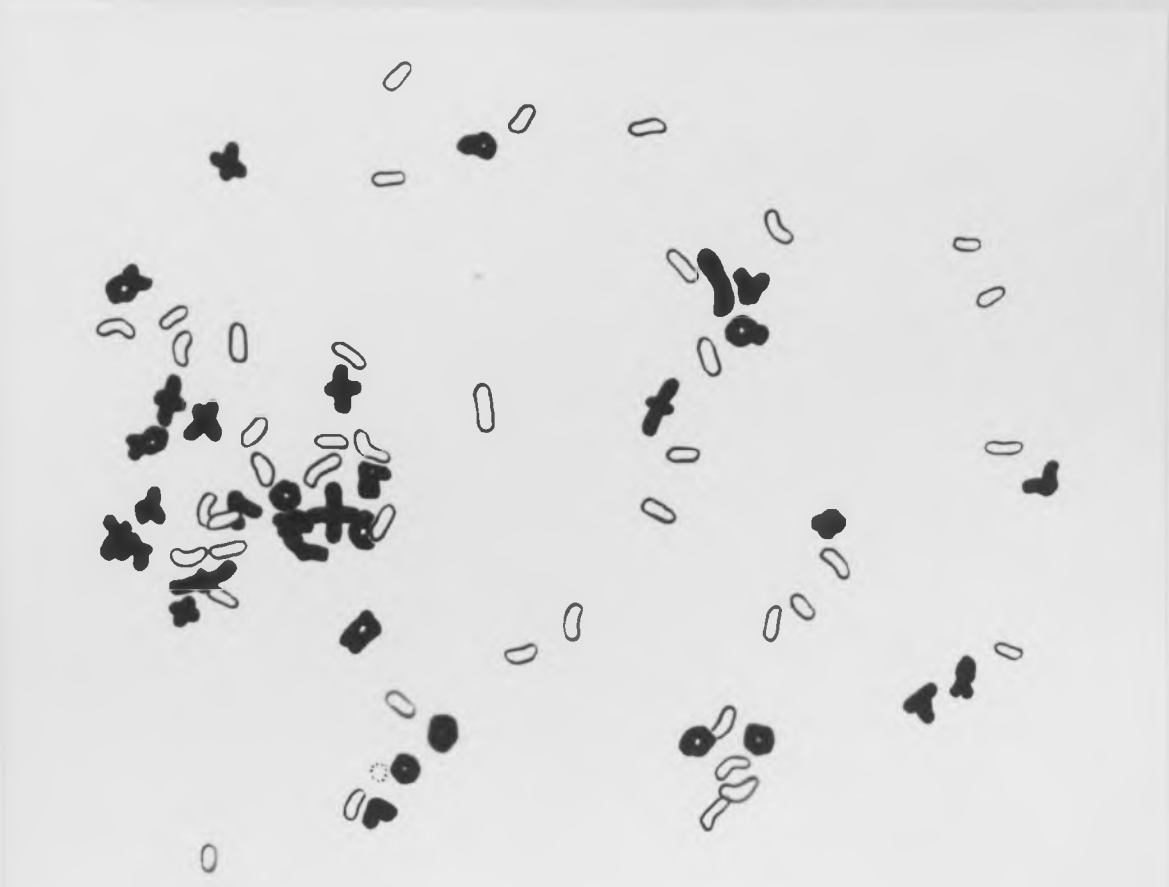
Analysis of meiotic pairing : 33 bivalents & 42 univalents.

Meiosis

X 1000



X 2000



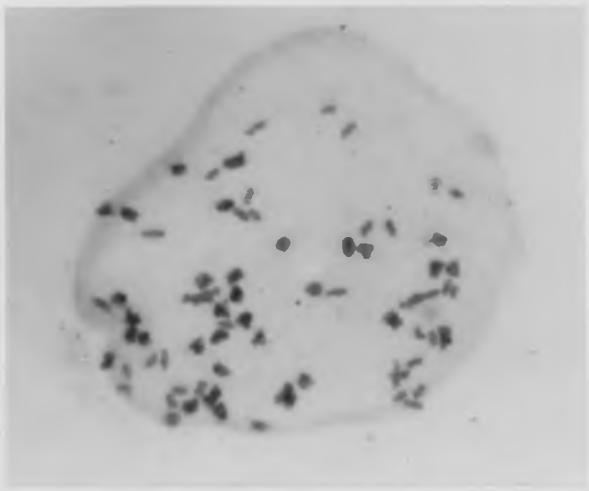
Cytology : artificial triploid hybrid. Fig. 120.

Asplenium trichomanes 4x N.America Ontario x A.trichomanes 2x Europe Caernarvon

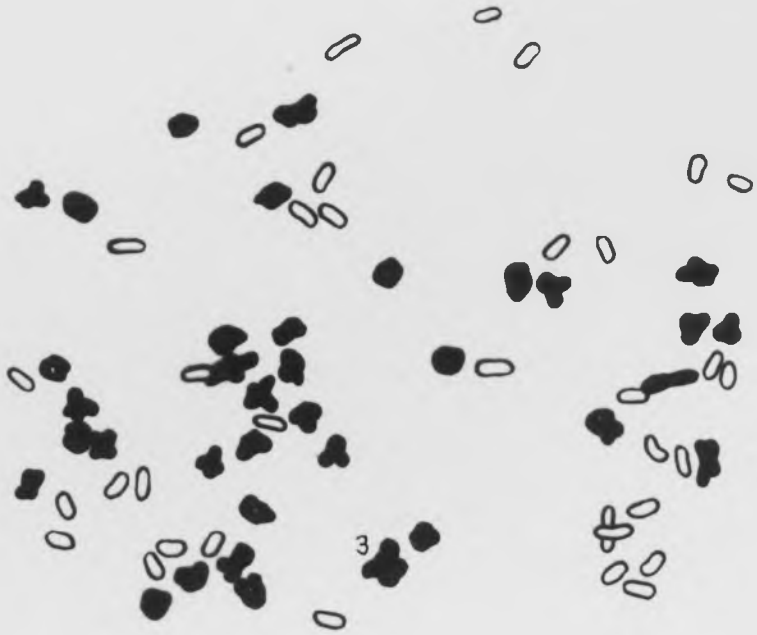
Analysis of meiotic pairing : 1 triv., 35 biv., & 35 univ.

Meiosis

X 1000



X 2000



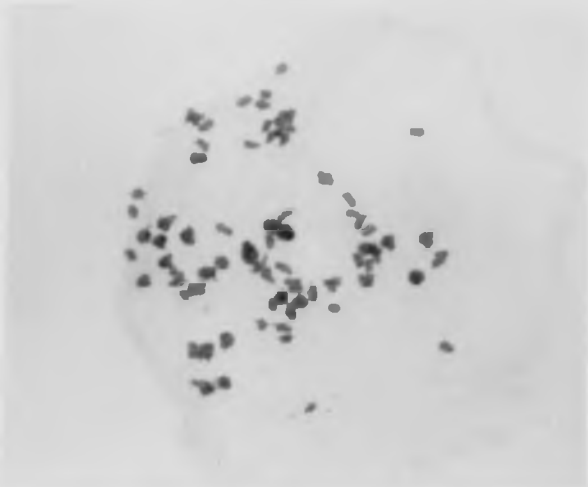
Cytology : artificial triploid hybrid. Fig. 121.

Asplenium trichomanes 4x S.Africa Basutoland x A.trichomanes 2x Europe Caernarvon

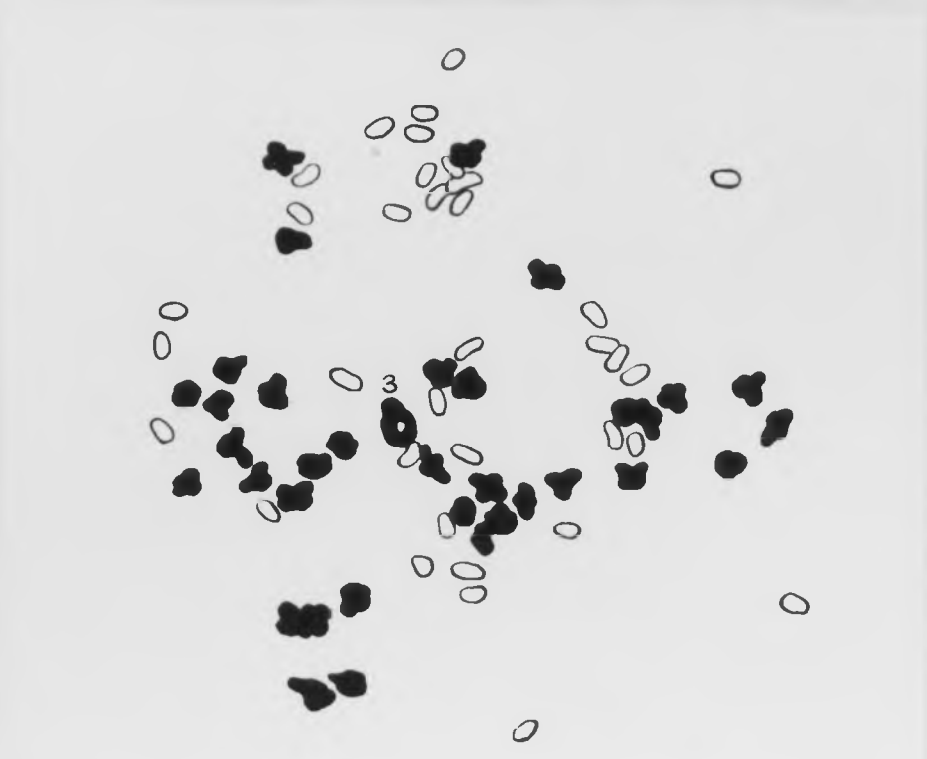
Analysis of meiotic pairing : 1 triv., 35 biv., & 35 univ.

Meiosis

X 1000



X 2000



Cytology : artificial triploid hybrid. Fig. 122.

Asplenium trichomanes 4x Hawaii x A.trichomanes 2x Europe
Hawaii Caernarvon

Analysis of meiotic pairing : 2 triv., 35 biv., & 32 univ.

Meiosis

X 1000

X 2000

Cytology : artificial triploid hybrid. Fig. 123.

Asplenium trichomanes 2x Europe x A.trichomanes 4x Australia
Caernarvon New South Wales

Analysis of meiotic pairing : 1 triv., 35 biv., & 35 univ.

Meiosis

X 1000

X 2000

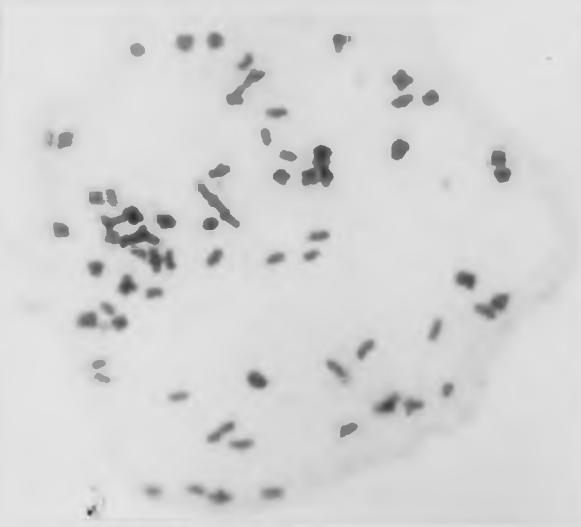
Cytology : artificial triploid hybrid. Fig. 124.

Asplenium trichomanes 2x Europe Caernarvon x A.trichomanes 4x Japan Kyushu

Analysis of meiotic pairing : 1 triv., 35 biv., & 35 univ.

Meiosis

X 1000



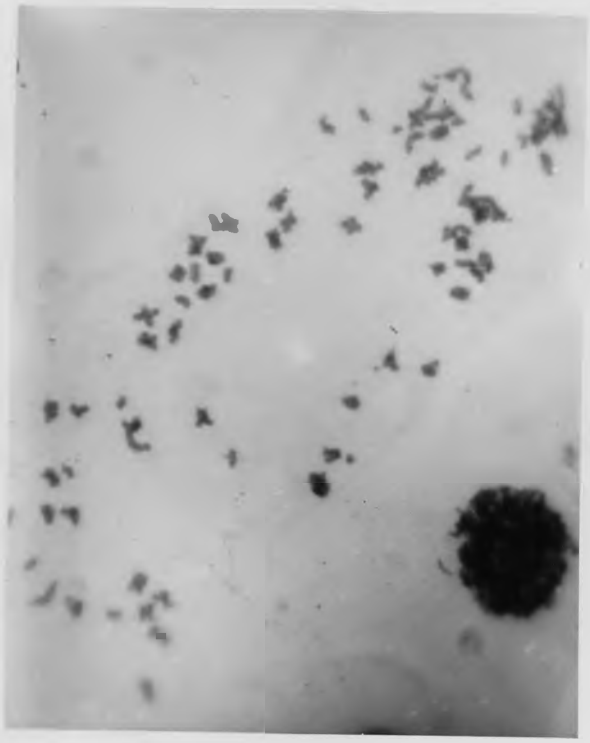
X 2000

Cytology : artificial tetraploid hybrid. Fig. 125 a.

Asplenium trichomanes 6x N.Z. Canterbury x A.trichomanes 2x Europe Caernarvon

Analysis of meiotic pairing : 4 triv., 48 biv., & 36 univ.

Meiosis X 1000. See also fig. 125 b.



Cytology : artificial tetraploid hybrid. Fig. 125 b.

Asplenium trichomanes 6x N.Z. Canterbury x A.trichomanes 2x Europe Caernarvon

Analysis of meiotic pairing : 4 triv., 48 biv., & 36 univ.

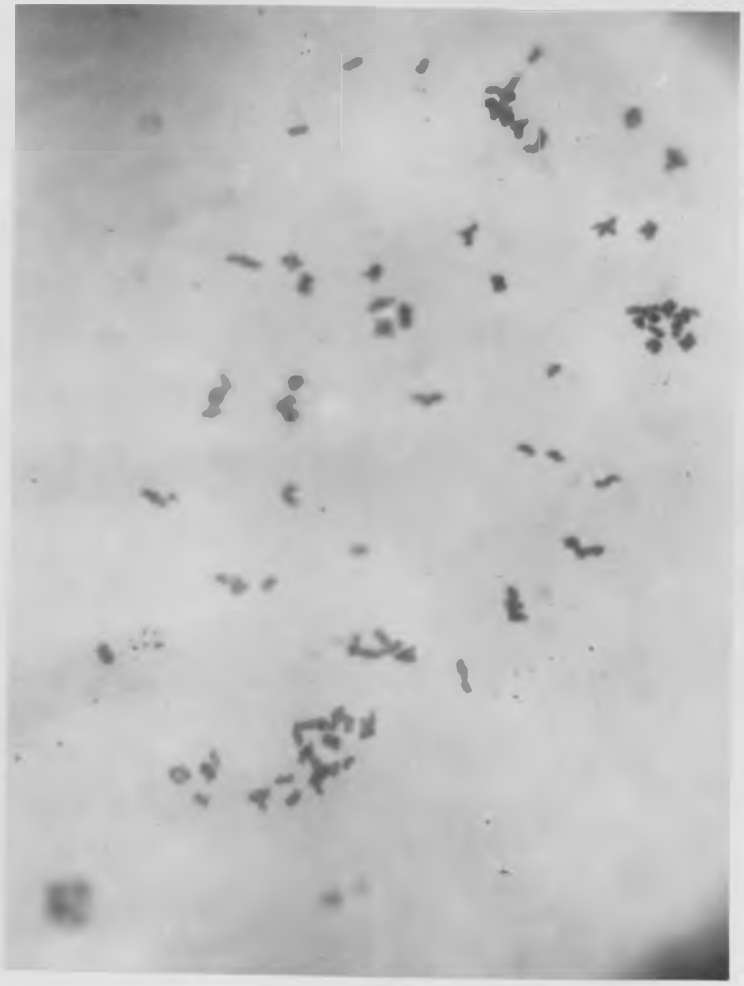
Meiosis X 2000. See also fig. 125 a.

Cytology : artificial tetraploid hybrid. Fig. 126 a.

Asplenium trichomanes 6x N.Z. Canterbury x A.trichomanes 2x Europe Caernarvon

Analysis of meiotic pairing : 3 triv., 45 biv., & 45 univ.

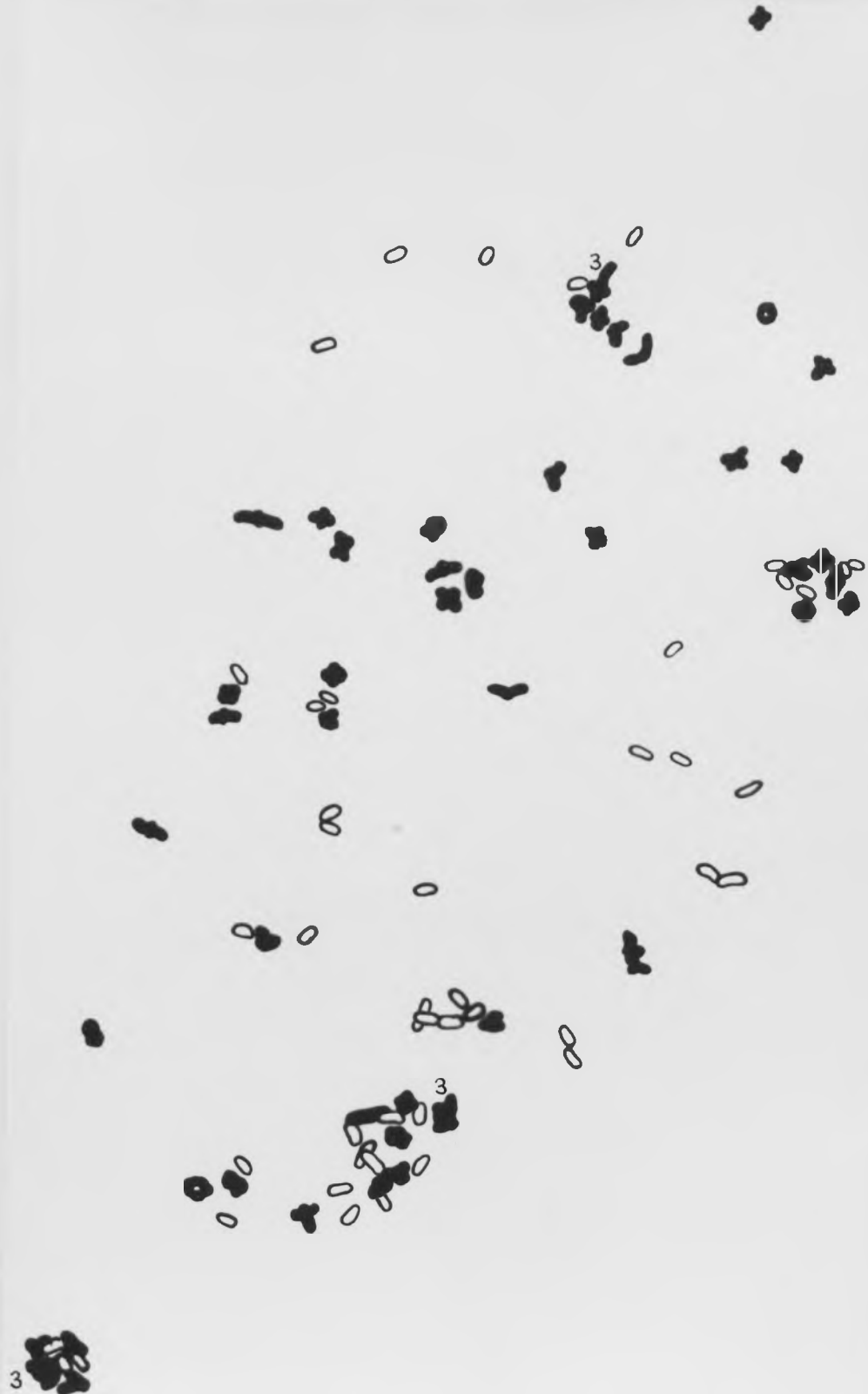
Meiosis X 1000. See also fig. 126 b.



Cytology : artificial tetraploid hybrid. Fig. 126 b.

Asplenium trichomanes 6x N.Z. Canterbury x A.trichomanes 2x Europe Caernarvon

Meiosis X 1500. See also fig. 126 a.



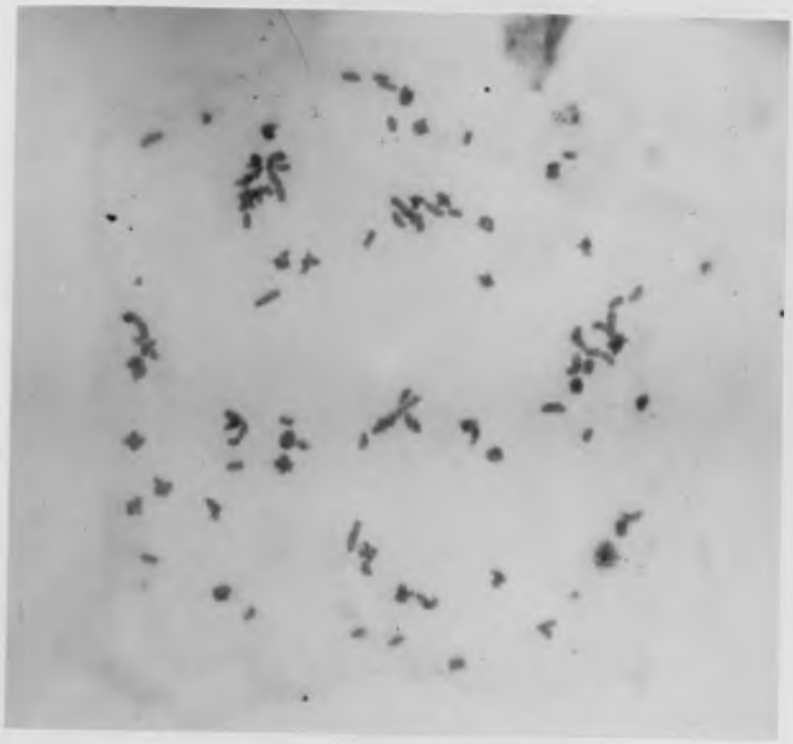
Cytology : artificial tetraploid hybrid. Fig. 127 a.

Asplenium trichomanes 6x N.Z. Canterbury x A.trichomanes 2x Europe Caernarvon

Analysis of meiotic pairing : 1 triv., 46 biv., & 49 univ.

Meiosis X 1000.

See also fig. 127 b.



Cytology : artificial tetraploid hybrid. Fig. 127 b.

Asplenium trichomanes 6x N.Z. Canterbury x A.trichomanes 2x Europe Caernarvon

Analysis of meiotic pairing : 1 triv., 46 biv., & 49 univ.

Meiosis X 1500. See also fig. 127 a.

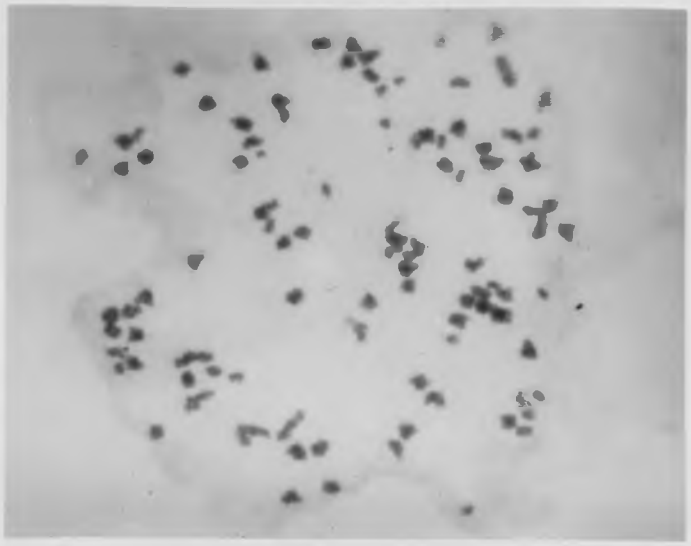
Cytology : artificial pentaploid hybrid. Fig. 128.

Asplenium trichomanes 6x N.Z. x A.trichomanes 4x Europe
Marlborough Devon

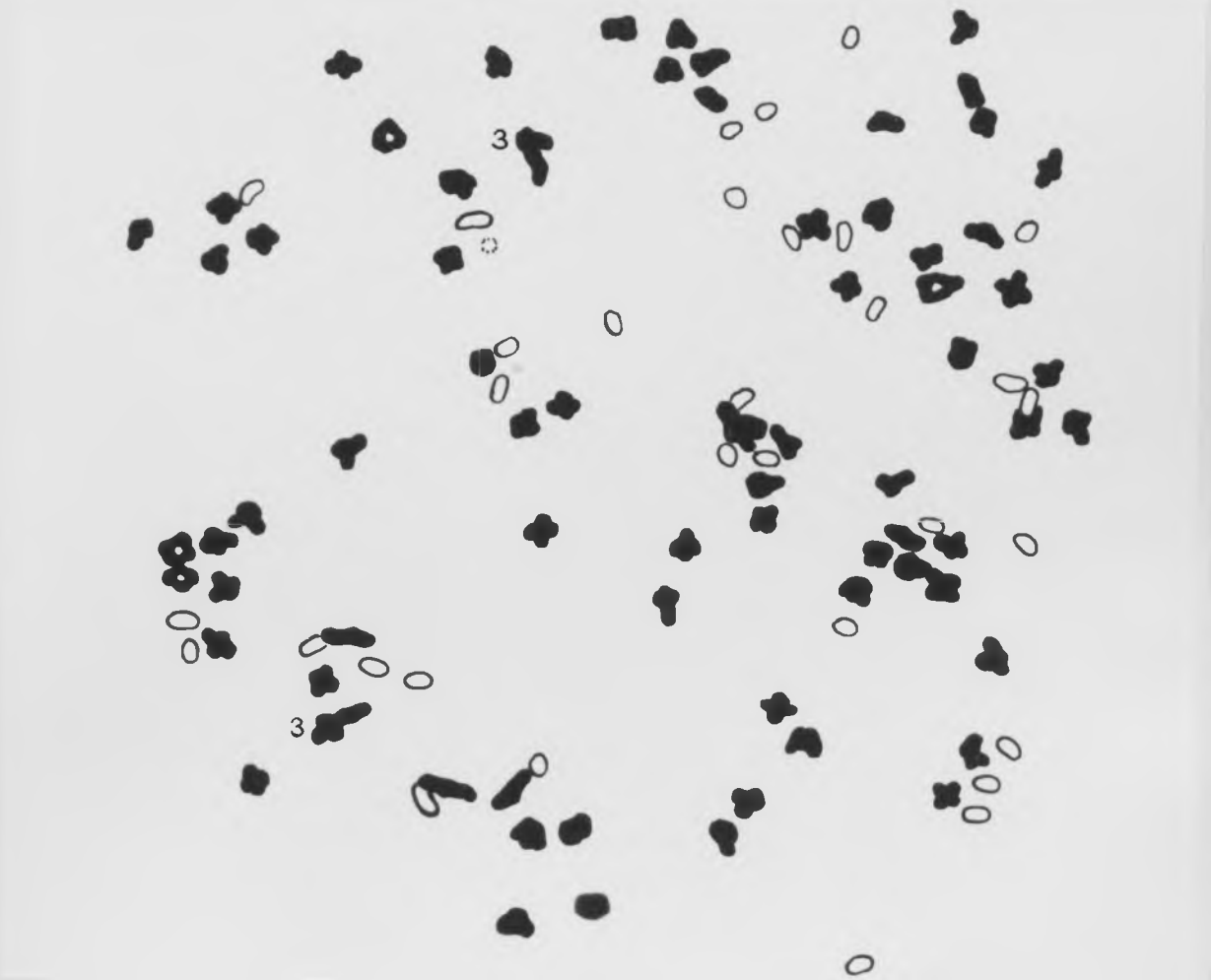
Analysis of meiotic pairing : 2 triv., 71 biv., & 32 univ.

Meiosis

X 1000



X 2000



Cytology : artificial pentaploid hybrid. Fig. 129 a.

Asplenium trichomanes 6x N.Z. Marlborough x A.trichomanes 4x Europe Devon

Analysis of meiotic pairing : 1 quadriv., 7 triv., 62 biv., & 31 univ.

Meiosis X 1000 See also fig. 129 b.

Cytology : artificial pentaploid hybrid. Fig. 129 b.

Asplenium trichomanes 6x N.Z. Marlborough x A.trichomanes 4x Europe Devon

Analysis of meiotic pairing : 1 quadriv., 7 triv., 62 biv., & 4 univ.

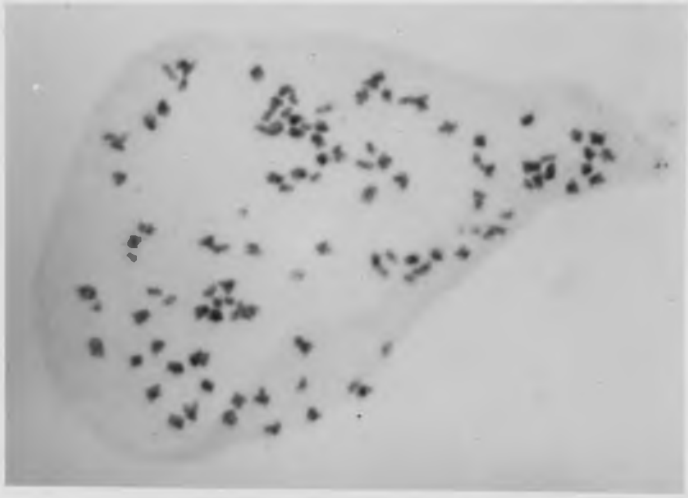
Meiosis X 1500. See also fig. 129 a.

Asplenium trichomanes 6x N.Z. Marlborough x A.trichomanes 4x Europe Devon

Analysis of meiotic pairing : 3 triv., 64 biv., & 43 univ.

Meiosis

X 1000



X 2000

HYBRIDISATION EXPERIMENTS

TABLES OF RESULTS

Figs. 131 - 132.

Table : Results of hybridisation experiments.

HYBRIDISATION EXPERIMENTS : TETRAPICID X DIPLOID PROTHALLI USED : HYBRIDS OBTAINED						
4x \ 2x	Australia	Asia	N.America	Europe	Total	Total - Australia
Europe	160:2	91:20	59:22	183:63	493:107	333:105
Hawaii	83:1	44:2	23:3	93:29	243:35	160:34
N.America	48:0	30:4	12:3	53:4	143:11	95:11
S.Africa	34:3	26:1	-	29:10	89:14	55:11
Australia	58:0	-	26:7	6:1	90:8	32:8
Total	383:6	191:27	120:35	364:107	1058:175	675:169
Japan	94:0	69:11	39:15	89:20	291:46	197:46
Total&Japan	477:6	260:38	159:50	453:127	1349:221	872:215
A.adulterinum	146:1	72:11	23:7	417:7	658:26	512:25

Table showing numbers of female prothalli used, and numbers of hybrids obtained, in attempts to produce various triploid hybrid combinations. Compare fig. 132.

Table : Results of hybridisation experiments.

HYBRIDISATION EXPERIMENTS : TETRAPLOID X DIPLOID : PERCENTAGE SUCCESS								
4x \ 2x	Australia	Asia	N.America	Europe	Total	Total- (A) Australia	Australia (B)	Ratio A:B
Europe	1.25	22	36	34	22	32	1.25	26:1
Hawaii	1.5	4.5	13	31	14	21	1.5	14:1
N.America	-	13	25	7.5	7.5	12	-	
S.Africa	8.75	3.75	-	34	16	20	8.75	2.5:1
Australia	-	-	27	13	9	25	-	
Total	1.5	14	29	29	17	25	1.5	17:1
Japan	-	16	38	22	16	23	-	
Total&Japan	1.25	15	31	28	16	25	1.25	20:1
A.adulterinum	0.75	15	30	1.75	4	5	0.75	6.5:1

Table showing percentage success obtained in attempts to produce various triploid hybrid combinations. Compare fig. 131.

SUPPLEMENT

ASPLENIUM ADULTERINUM

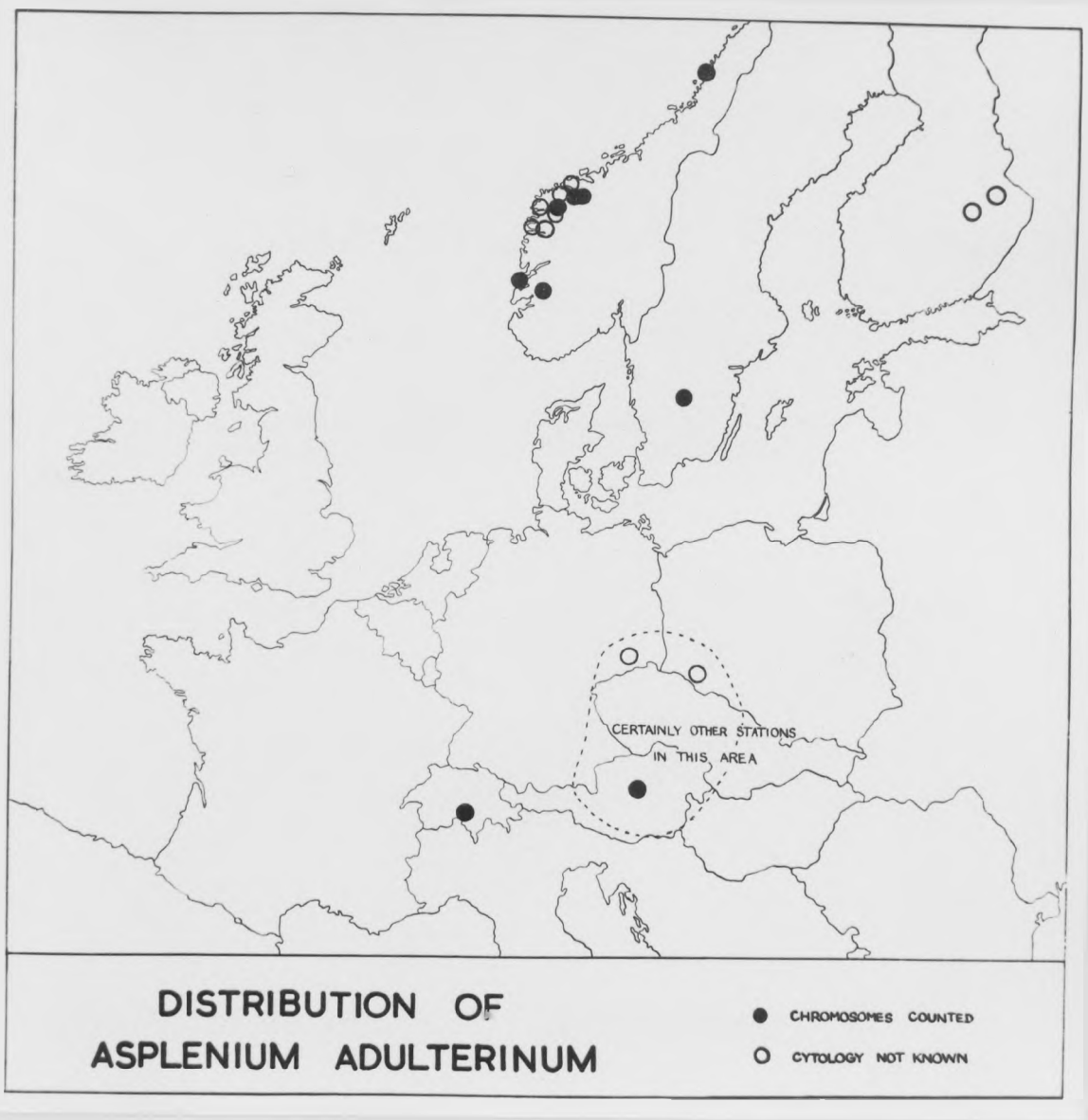
ASPLENIUM ADULTERINUM

DISTRIBUTION MAP

Fig. 133.

Fig. 133.

Distribution map.



Distribution of Asplenium adulterinum.

ASPLENIUM ADULTERINUM

AUTECOLOGY

HABITAT PHOTOGRAPHS

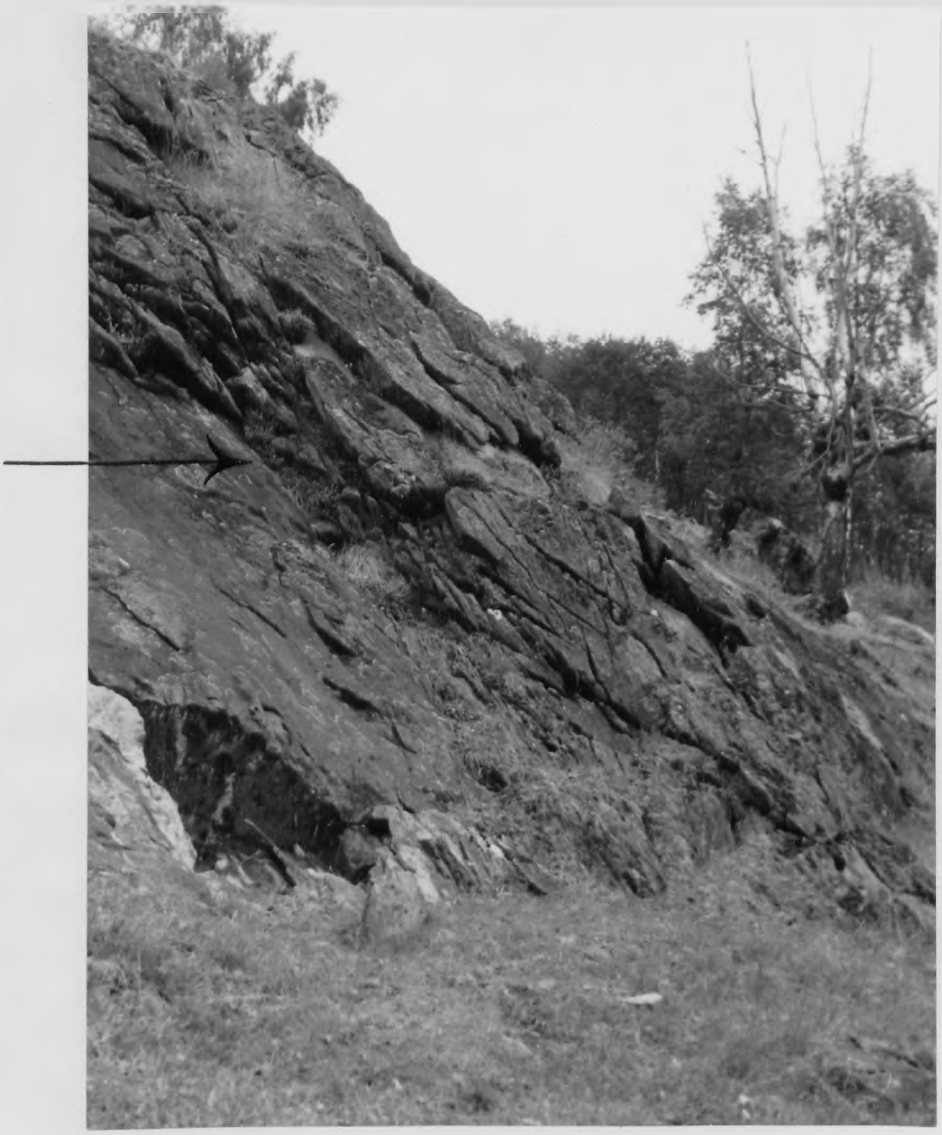
Figs. 134 - 139.

Asplenium adulterinum.

Norddal, Sunnmore, Norway.

Habitat of Asplenium adulterinum, in crevices of bare serpentine rock, viewed from south. Norddalsfjord in background. Compare fig. 135.

Asplenium adulterinum.



Norddal, Sunnmore, Norway.

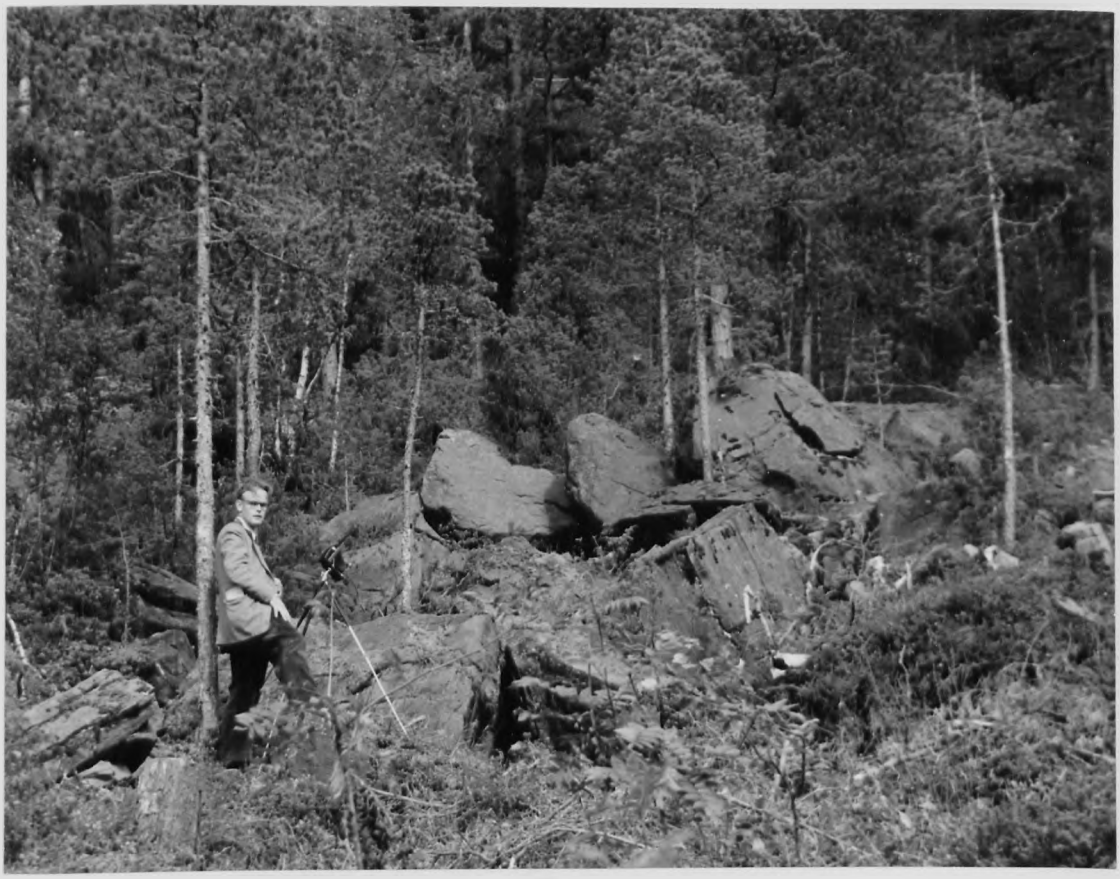
Habitat of Asplenium adulterinum, in crevices of bare serpentine or dunite rock, viewed from north. Compare fig. 134.

Asplenium adulterinum.

Rodbergvik, Sunnmore, Norway.

Habitat of Asplenium adulterinum and the diploid cytotype of A.trichomanes, in crevices of dunite rock exposed on a grassy slope below Picea forest. Sunnylven fjord in foreground.

Asplenium adulterinum.



Bjorkedalen, Sunnmore, Norway.

Habitat of Asplenium adulterinum, A.viride, and the diploid cytotype of Asplenium trichomanes, in crevices of tumbled dunite blocks on the edge of Picea forest.

Fig. 138.

Asplenium adulterinum.

Norddal, Sunnmore, Norway.

Two plants of Asplenium adulterinum growing on dunite rock. Note position on frond where rachis changes in colour from brown to green.

Asplenium adulterinum x viride.

Bjorkedalen, Sunnmore, Norway.

A plant of Asplenium x poscharskyanum (A. adulterinum x A.viride), growing in a crevice of a serpentine boulder. Note the position on the frond where the rachis changes in colour from brown to green.

ASPLENIUM ADULTERINUM

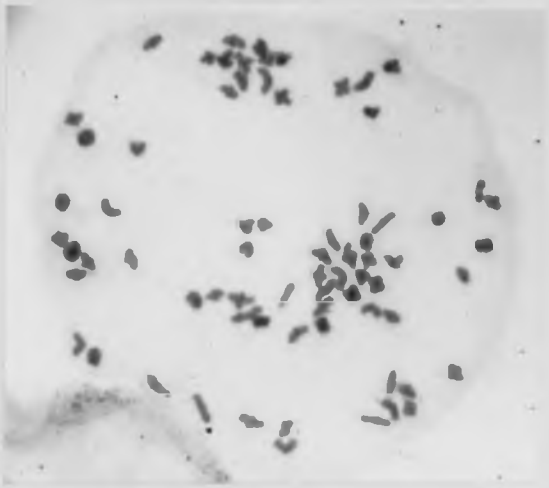
CYTOLOGY

Figs. 140 - 146.

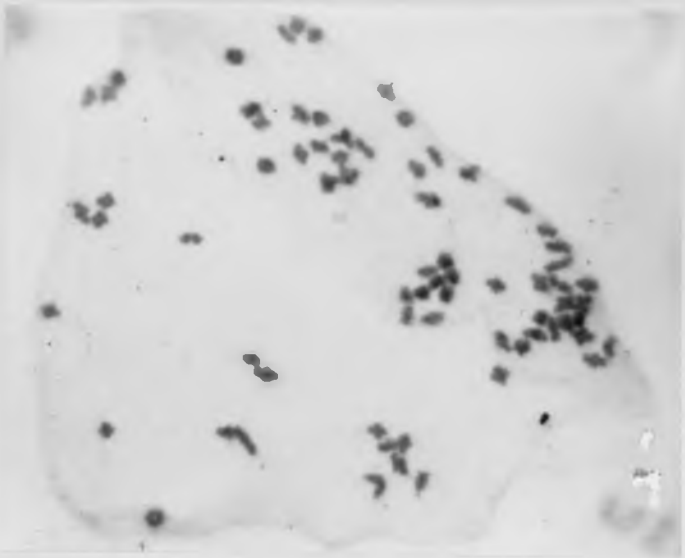
Objective : Watson 2mm NA 1.28 X 1000 Parachromatic Oil Immersion. Filter : Monochromatic Wratten Green. Magnification : X 1000. Negative : Ilford Special Rapid Panchromatic & Thin Film Half Tone Plates. Print : Contact. Diagrams : Derived from X 2 Matt Bromide enlargements.

Cytology : meiosis, X 1000. Fig. 140.

Asplenium adulterinum : tetraploid, n = 72.



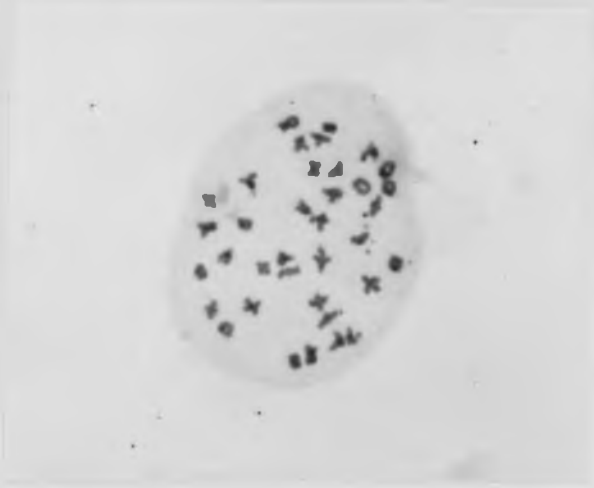
A. Kraubath, Austria.



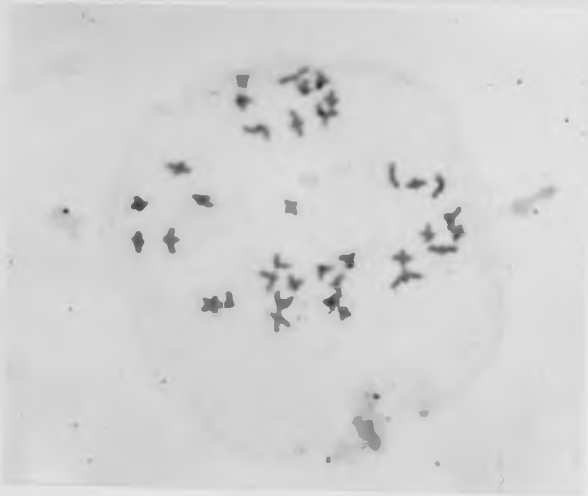
B. Norddal, Sunnmore, Norway.

Cytology : meiosis, X 1000. Fig. 141.

Asplenium viride : diploid, n = 36.



A. Kraubath, Austria.



B. Bjorkedalen, Sunnmore, Norway.

Cytology : natural triploid hybrid. Fig. 142.

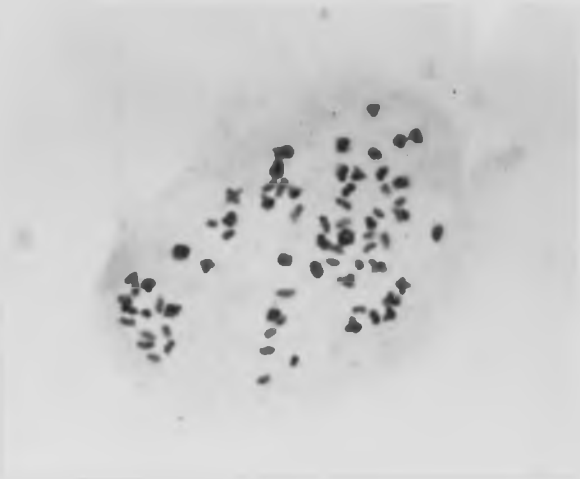
Asplenium poscharskyanum A.adulterinum x viride.

Wild triploid hybrid from Kraubath, Austria.

Analysis of meiotic pairing : 36 bivalents & 36 univalents.

Meiosis

X 1000.



X 2000.

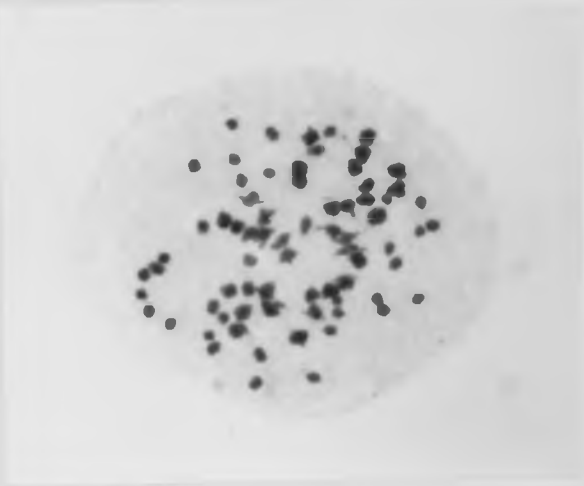
Asplenium x poscharskyanum A.adulterinum x viride.

Wild triploid hybrid from Bjorkedalen, Norway.

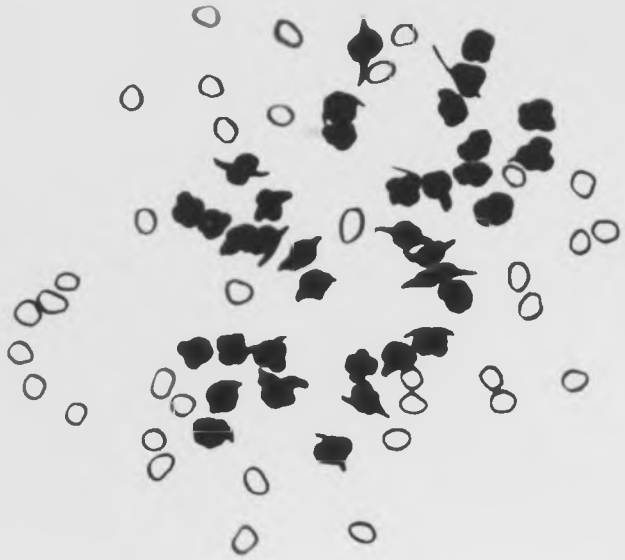
Analysis of meiotic pairing : 36 bivalents & 36 univalents.

Meiosis

X 1000.



X 2000.



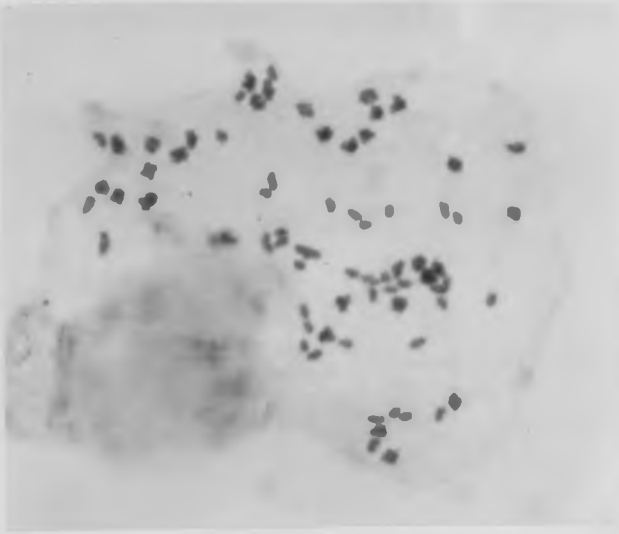
Cytology : artificial triploid hybrid. Fig. 144.

Asplenium viride x *A.adulterinum*.
Yorkshire Austria.

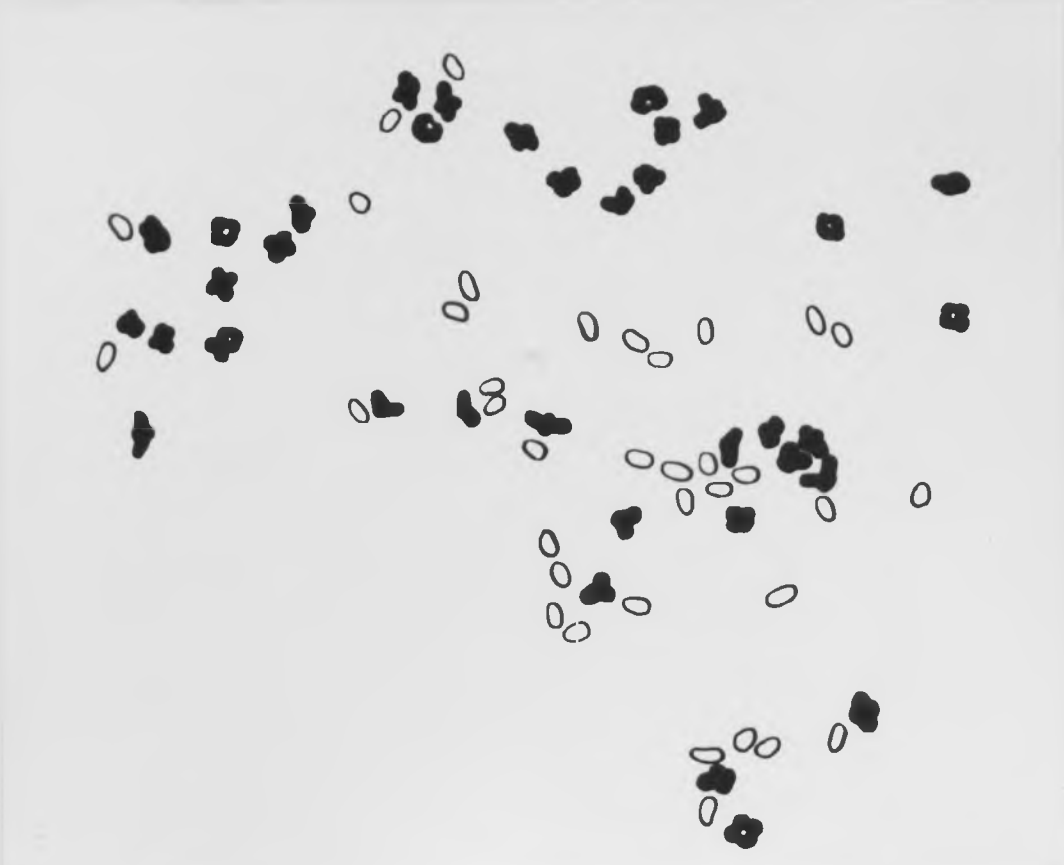
Analysis of meiotic pairing : 36 bivalents & 36 univalents.

Meiosis

X 1000.



X 2000.



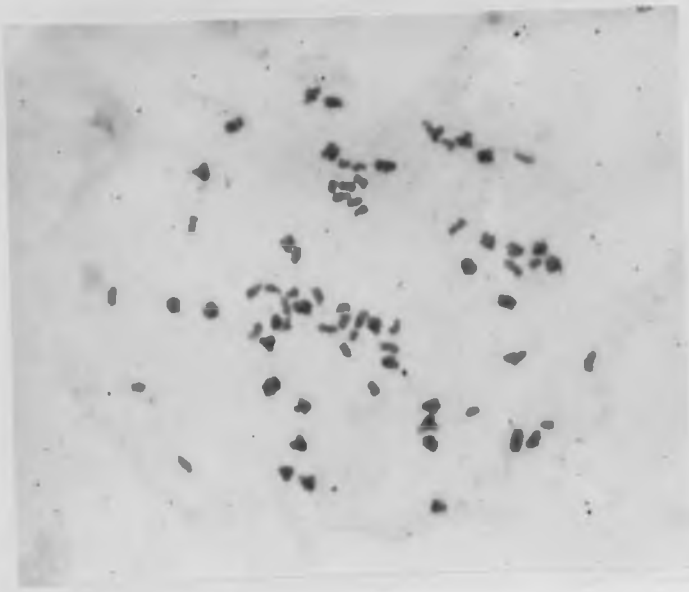
Cytology : artificial triploid hybrid. Fig. 145.

Asplenium adulterinum x A.trichomanes 2x.
Switzerland Caernarvon

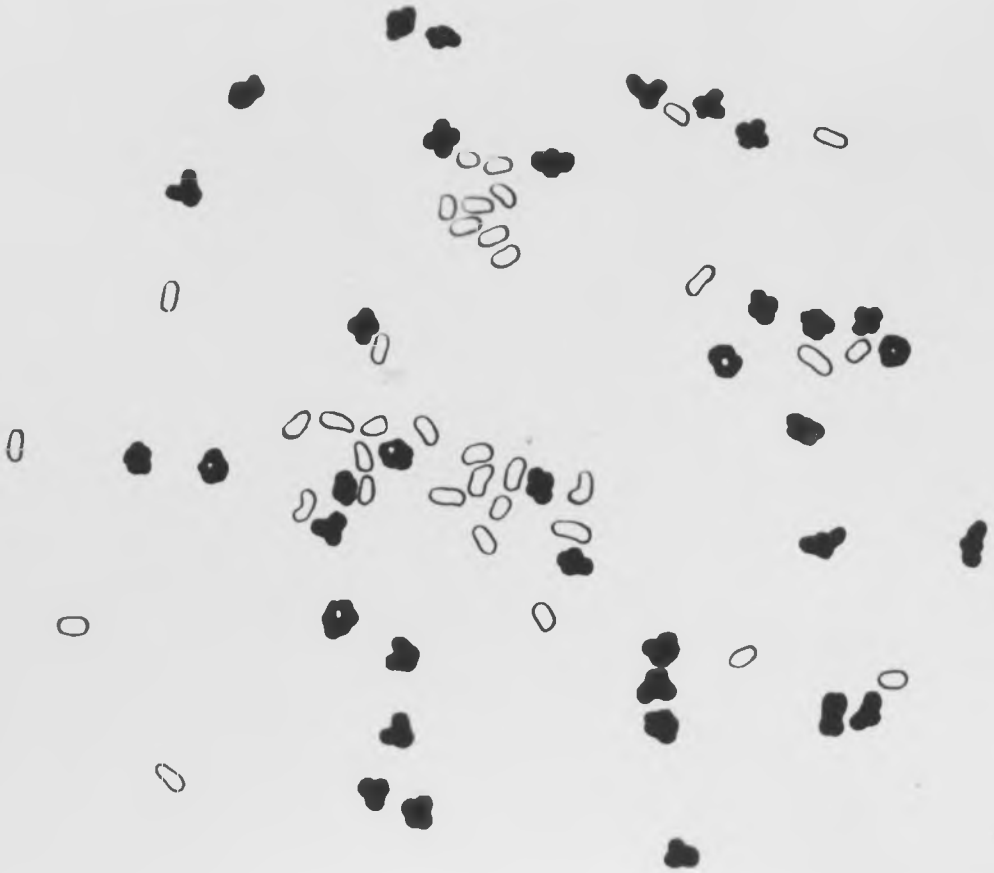
Analysis of meiotic pairing : 36 bivalents & 36 univalents.

Meiosis

X 1000.



X 2000.



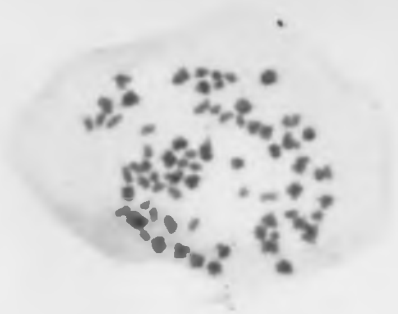
Cytology : artificial triploid hybrid. Fig. 146.

Asplenium trichomanes 2x (France) x *A. adulterinum* (Austria).

Analysis of meiotic pairing : 36 bivalents & 36 univalents.

Meiosis

X 1000



X 2000