SOME CYTOTAXONOMIC PROBLEMS IN THE FERN GENERA ASPLENIUM AND POLYSTICHUM.

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by

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GENERAL INTRODUCTION.

The basic aim of this research project is to attempt to obtain information concerning the phyletic affinities between species by means of a study of chromosome pairing in synthesised hybrids. Any evidence obtained is supplemented by a consideration of the morphology, ecology and geographical distribution of the species concerned.

The main problem consisted of a cytogenetic investigation into the relationships of the four European *Polystichum* species, although this soon resolved into the two immediately accessible problems of the affinities and possible origin of the two tetraploids *P. braunii* (continuing the observations of Manton & Reichstein, 1961) and *P. aculeatum*. Attempts were made to resynthesise the latter species from it s postulated parents (Manton, 1950).

Polystichum is a slow-growing fern and it was known that synthesised hybrids may need up to three years to produce sporangia. Because of the possibility that hybrids obtained might mature too slowly to yield results in the time available an additional investigation involving some *Asplenium* species was begun. In this genus many hybrids produce sporangia within a year of fertilisation, thus permitting a rapid extension of the hybridisation programme. This considerable advantage has converted a secondary investigation into the mutual affinities of

two European tetraploids (A. forisiense and A. macedonicum) into a major project, and the hybridisation programme was expanded to include a number of other European species at both diploid and tetraploid levels. Much new material was made available from the personal collections of Professor Reichstein of Basel, who was also responsible for obtaining an important collection of living Polystichum species from Japan by arrangement with Professor H. Itô of Tokyo. Both investigations have therefore extended beyond the bounds first envisaged but since the work on Asplenium is more nearly complete it will be convenient to deal with it first in the account which follows.

Each genus is treated separately as a distinct section with the exception of the account of the methods used, which follows, and the General Discussion, which will be found at the end of the thesis.

METHODS.

1. COLLECTION OF MATERIAL.

The material used in this investigation came from three main sources: a) stock plants in cultivation at Leeds, b) living plants and spores collected and sent by colleagues and well-disposed foreign correspondents, and c) my own collection. As I was particularly fortunate in having a large collection of material available to me little use has been made in this investigation of material grown from spores taken from herbarium specimens. The individual sources for plants and spores obtained from correspondents are given separately under 'Materials' at the beginning of each section.

When collecting in the field living plants were taken wherever possible. Fronds were pressed as herbarium specimens, ripe spores packeted (in the usual type of folded spore packet made from grease-proof paper), and the rhizomes wrapped in damp moss and polythene and dispatched by air mail to the experimental garden of the University of Leeds Botany Department.

Where spores of wild origin were used in the hybridisation programme the precaution was taken of selfing some of the resultant prothalli and raising stock plants. Besides serving as a future source of spores this enabled morphological comparison to be made between synthesised hybrids and their actual parents. For the same reasons selfed plants were raised from spores received from correspondents.

2. CULTURAL AND HYBRIDISATION TECHNIQUES.

A. CULTIVATION OF ADULT SPOROPHYTES.

(i) ASPLENIUM

Both species and hybrids were cultivated under green-house conditions. The plants were grown in John Innes compost and variations made according to the ecological requirements of the species concerned, e.g. extra lime was added to the compost for A. fontanum, and extra peat for species such as A. macedonicum which are normally restricted to acid soils. Fertiliser is added for the older sporophytes.

It was found that the best results were obtained with these plants if they were potted in early spring. Individual sporophytes are grown in pots known as 'Long Toms', the smallest of which are 2" wide and 3" deep. Besides giving extra depth to the roots of these rock-crevice species, they are of considerable value where space is limited. In the cultivation of these plants it has been the practice to plunge the pots to the rim in peat, sand or weathered ashes. The chief advantage of this method is that the potting medium is kept uniformly moist; sudden drying out of individual pots is eliminated and even conditions are maintained throughout. Plunging also serves to keep the roots cool and fluctuations in temperature are avoided.

When cultivating Asplenium species rock crevice conditions are simulated as far as is possible. Unimpeded surface drainage is perhaps the most important factor, and to prevent rotting of the

rhizome the crown is raised and the pot surface dressed with gravel or chipped stones.

(ii) POLYSTICHUM.

It was found most satisfactory to cultivate adult plants outside. Stock plants and wild hybrids were grown in a shady position in the Experimental Garden of Leeds University Botany Department. Synthesised hybrids were grown under green-house conditions until they had been examined cytologically, and for these plants regular fern compost was the potting medium. It consisted of 2 parts peat: 1 part loam and 1 part of coarse sand. 'Aldrin' is added to this compost to counteract soil pests. Plants were generally potted in their main growing season in the spring, although they could also be potted in early autumn when the roots are still growing actively.

Plants of both genera are grown under cool conditions. During the winter the houses are heated, but the temperature is kept as low as possible (maximum 45° - 50°F) in order to encourage a period of dormancy. If sporophytes are kept warm and forced to grow actively both summer and winter they soon loose vigour and die within a year or so.

The sporophytes of both genera are attacked by relatively few pests, the two most important being slugs and scale-insects. The former can be a particular nuisance on young fronds of Asplenium. Numerous proprietary brands of slug-bait were tried but none were really efficacious, and removal by hand seemed to be the only remedy.

Scale-insects were found on both genera (compare Walker, 1956) and this pest was combated by regular fumigation of the houses and by removal of infected fronds.

During the summer of 1964 many Asplenium hybrids died suddenly from an unknown cause, in which a healthy plant would wilt and die within a few days. The plants were examined for evidence of fungus, soil pests or bacterial infection, but nothing could be found. Many sick plants were re-potted and kept under conditions of high humidity but a large scale loss resulted. The cause of this condition is still unknown.

B. GERMINATION AND CULTURE OF GAMETOPHYTES.

Spores collected from wild plants are generally free from contamination because of periodic washing of the fronds by rain. Fertile pinnae are broken off and placed inside a spore packet (see Collection of Material). When spores are collected from stock plants it is advisable either to isolate the plant from which the spores are to be taken, or to wash the fertile frond carefully under running water before placing it between drying paper. In most cases it has been found more practical to adopt the latter procedure. Once the frond is dry spores can be sown directly from the paper and need not be transferred to a spore packet.

Spores should be sown in a draught-free atmosphere as free from contamination as possible. They are sown in $2\frac{1}{2}$" pots which have been previously sterilised by steam. The compost used is a fine mixture of 2 parts peat: 1 part loam: 1 part silver sand which is passed

through a $\frac{1}{4}$" riddle, and thoroughly steam-sterilised. Each pot is well crocked, half-filled with gravel or coarse sand and filled with the above mixture, the top-most layer being passed through a particularly fine riddle. The pots are then sprayed with orthocide Captan, prepared according to the maker's recommendations, and covered with clock-glasses. After this treatment they are left to stand for 24 hours before use. Pricking out pots are prepared in the same way.

After sowing the pots are placed in a covered frame to maintain a humid atmosphere. The frames are kept at a temperature of 70° - 75°F throughout the year. Once germination has begun the pots are removed to a frame in the cool house; these are heated during the winter months to maintain a temperature of 50°F. When individual pots became dry they were watered from below by standing them in a trough of water until the soil was moist throughout. The cultures cannot be watered from above as free surface moisture would encourage release of spermatozoids and so lead to self-fertilisation. For the same reason the pots are covered with clock-glasses in preference to flat plates of glass, as any condensation then runs to the edge of the glass and does not drip onto the culture.

During gametophyte germination and growth the accompanying weeds are algae, fungi and moss. The first of these is generally found only in cultures where the prothalli are not growing well. The alga forms a greeny-brown smooth growth over the surface of the soil from which it is removed by hand. A more serious pest is moss, which

invades the cultures quite frequently, but again is most common under conditions of poor prothallial germination. It is removed from the surface of the soil with forceps, and watering the pots from below with a dilute solution of potassium permanganate seems to be effective in reducing its growth. It has been noted that moss growth on germinating cultures can be considerably reduced by placing sowing pots under hot-house conditions until germination has begun. Much more moss appears if the spores are germinated under the cooler humid conditions favourable to Bryophyte development. However, once prothallial germination has begun the sowing pots must be removed to the cooler frames as the warmer conditions would favour the growth of fungus.

Fungal attacks present by far the greatest problem. This pathogen infects well-established growing prothalli, particularly if the spores were thickly sown. Infected patches were removed by hand, but the method was not satisfactory as within a week further contamination would develop.

Some experiments were carried out in which infected cultures were sprayed with varying dilutions of the fungicide Captan, but the results were inconclusive. It would appear that chemical treatment may be efficacious in preventing or retarding fungus growth, but once established very little can be done to eliminate it.

In an endeavour to offset any losses that might occur a greater number of pots were sown at the outset. This proved to be the best way of dealing with the problem for if subsequent fungal attack did

completely destroy some of the cultures reserve material was available at the required stage of development.

It should be noted that none of the above pests are important at the later stages of growth.

It was found that prothalli which had been sown thickly to produce a green sward over the surface of the pot were ideal for use as males in the hybridisation programme. Such prothalli, being crowded together, became narrow and attenuated in shape and were covered with antheridia. A good yield of antheridia also resulted if the 'male' cultures were put in a shaded place (e.g. under a bench) and kept rather dry. Scattered prothalli, on the other hand, attained the regular heart-shape quickly. In this state the male region is senescent while archegonia at all stages of development are borne in abundance. Such prothalli are suitable for use as female parents in a hybridisation attempt.

Sowing was begun early in January, and the cultures destined for use as females were sown some 5 - 6 weeks before those to be used as males. Female cultures were sown thinly, which facilitates later 'pricking-out', and the later cultures were sown as thickly as possible. Sowing was continued at regular intervals throughout the summer. The prothalli intended for use as females are normally big enough to be pricked out some 3 - 4 months from the date of sowing. They are separated out and placed singly in rows approximately 1 cm. apart on the surface of a fresh $5\frac{1}{2}$" pot, care being taken to isolate each prothallus from others clinging to it. These prothalli are

normally left from 4 - 8 weeks after separation before being used in a hybridisation attempt.

When it is required to self prothalli, a sexually mature culture is stood rim-deep in a pan of warm water and left in the light for several hours. Resulting sporophytes are pricked out into 2" pots when size permits.

C. HYBRIDISATION TECHNIQUES.

Prothalli to be used in the hybridisation programme are examined periodically for signs of antheridia production. Generally Asplenium prothalli are ready for use as males in 3 - 4 months, and Polystichum 4 - 5 months, from the date of sowing. A small quantity of culture in the antheridial state is teased out in a drop of warm water on a slide which is placed under a lamp for 10 - 20 minutes. The prothalli are then examined under the low power of the microscope in order to see if liberation of spermatozoids has taken place. If many spermatozoids are seen swimming actively in the field of view then the culture is deemed to be ready for use. If no spermatozoa are liberated after 20 - 30 minutes, or if the spermatozoa liberated are few in number and swimming sluggishly, then the culture is unfit for use.

About 2 sq.cm. of the culture are taken, rinsed briefly to remove adhering soil and teased out in a drop of warm water in a clock-glass. The previously-examined prothalli from this culture (if still swimming), are washed into the clock-glass by means of a pipette, and prothalli of the required female parent are added.

Ten to twenty prothalli bearing mature archegonia are selected, examined carefully and any showing signs of developing sporophytes are discarded. Any soil or juvenile prothalli adhering to the rhizoids are removed, and the cleaned prothalli are placed ventral surface downwards in the mass of male prothalli and swimming cells. The clock-glass is then covered to prevent drying out, placed in a bright light and left for from half to several hours to allow fertilisation to be effected. In a number of cases it has been found that further liberation of spermatozoids occurs if warm water is added by means of a pipette. After the period of hybridisation the female prothalli are removed from the clock-glass, rinsed briefly in water, care being taken to remove any small 'male' prothalli which may be adhering to the rhizoids. The 'female' prothalli are transferred to the surface of a fresh $3\frac{1}{2}$" pot (containing the same mixture as is used for sowing pots) at approximately 1 cm. intervals, and the details of the hybridisation are noted.

Young sporophytes of the two genera investigated begin to appear in from 5 - 8 weeks from the date of hybridisation. If no sporelings have appeared within 12 weeks the attempt is discarded as unsuccessful. In practice it was found that sporelings appearing within 2 - 4 weeks of the hybridisation attempt were the result of an early self-fertilisation, and those appearing after 9 weeks had elapsed were most likely to be late selfs. The occasional alien sporeling is recognised by its morphology and weeded out.

The time of appearance of young sporophytes does not appear to

be connected with seasonal variation as other workers have suggested. It is possible to have hybrid sporophytes appearing as late as 10 weeks after hybridisation, even in mid-summer, but this is a rare occurrence.

Hybridisations can be performed throughout the year, although it is usual to make crosses in the summer months. Particularly successful examples of winter hybridisations are A. viride x A. majoricum (February), A. obovatum x A. forisiense and P. lonchitis x P. acrostichoides (both end of November).

No method has been found of completely eliminating the self-fertilisation of female prothalli and it must be accepted as inevitable that a certain proportion of selfed plants will be obtained. In this connection it should be noted that some species produce selfed progeny more easily than others: notable in this respect is Asplenium petrarchae.

D. CULTIVATION OF YOUNG SPOROPHYTES.

After hybridisation the young sporelings are pricked out into individual 2" pots at the three-leaf stage. If a number of sporophytes have resulted from a single hybridisation attempt it is advisable to separate and pot the sporelings as soon as possible. If left the roots tend to become entangled and separating the young plants after this has happened results in damage to the roots and very often leads to the death of the plant.

The potting mixture used for young sporophytes is the regular fern compost used for older plants, which is passed through a $\frac{1}{4}$"

riddle before use to produce a fine texture. No additional fertiliser is used when the sporelings are first potted, but for larger plants bone meal is added to the mixture in the quantity of one $3\frac{1}{2}$" pot per bushel of soil.

Potting of young sporophytes is carried out throughout the spring and summer. If older sporophytes have not been potted on before the end of September it is advisable to delay re-potting until the following spring. The pots are plunged in sand under covered frames at a maximum winter temperature of 50°F. When about a year old these sporophytes can be transferred to a cooler greenhouse where they are treated in the manner described in section A.

Asplenium sporophytes generally become fertile in from 8 - 10 months from the date of hybridisation, and an extremely rapid test of positive hybridity is the examination of the spores. Hybrids may be recognised by their shrivelled mis-shapen spores, and selfed plants producing rounded well-filled spores are discarded.

3. CYTOLOGICAL TECHNIQUES.

A. MEIOTIC PREPARATIONS.

The aceto-carmine squash method has been used throughout. Young sporangia in a state of division were taken from the fronds and fixed in a solution of 1 part glacial acetic acid to 3 parts of absolute alcohol. The fixed material was generally left from 1 - 3 days in a domestic refrigerator (+4°C) to harden, but it was found that the material kept satisfactorily for an indefinite period if placed in the deep freeze (-15°C).

A small quantity of the fixed material was placed on a clean slide, covered by a drop of aceto-carmine (a saturated solution of carmine in 45% acetic acid), and mashed gently to separate the cells. A clean cover slip was applied and the slide heated over a spirit lamp until the carmine began to bubble gently. Manual pressure was quickly applied, care being taken to prevent any lateral movement of the cover slip which would shear the cells. After examination the preparations were made permanent by McClintock's method as described in Manton (1950).

B. MITOTIC PREPARATIONS.

During the course of this investigation little use has been made of mitotic preparations. Asplenium yields results so quickly that it is better to await the results of meiosis than to do a root tip count to determine the ploidy of putative hybrid plants. In Polystichum many of the hybrids made were at the diploid and tetraploid levels, where a root tip count would have given no information,

and the triploid hybrids were generally so characteristic in appearance as to make a mitotic count unnecessary.

(i) Root tip sections were used in some cases to check unsatisfactory meiotic counts from the collection of Japanese plants which were sent to Kew. Healthily growing root tips were fixed in a freshly-prepared half-strength solution of chromacetic-formalin, made up to the formula given by Manton (1950), and were subsequently embedded in paraffin wax (MP 55°C) and sectioned at 10 or 12μ. Most of the sections were stained in Heidenhain's haematoxylin, although crystal violet was also used.

(ii) Root tip squashes were made in a minority of cases, a particular instance being the expected demonstration of triploidy in putative plants of the important hybrid P. braunii x lonchitis.

The basic method used has been a combination of the techniques devised by Tjio & Levan (1950) and by Meyer (1952), with modifications suggested by Chambers (1955). Root tips were pre-treated for a period of $1\frac{1}{2}$ - 4 hours in a saturated aqueous solution of either 8-hydroxy-quinoline or para-dichlorbenzene in order to shrink the chromosomes, and were then fixed in a 1:3 solution of acetic alcohol. Chambers' modifications involved the use of the enzyme cytase which digests cellulose walls and so allows easy separation of the cells.

As no major results were obtained by this method it will not be described in detail.

4. SPORE AND SCALE PREPARATIONS.

A. SPORES.

Spore preparations for microscopic examination were made by mounting the spores directly in a drop of gum chloral, which consists of a gum arabic mounting medium containing chloral hydrate as a clearing agent. Sporangia and spores were removed from dried herbarium material on the point of a needle, after first moistening the sori with Alcopol, a liquid of low surface tension. This greatly facilitates the removal of the dry spores.

The spore dimensions were measured using a calibrated micrometer eye-piece and a x 40 objective, care being taken to reject all mis-shapen spores. In each case the length of the exospore was recorded, the irregular outline of the perispore being ignored. In chloral mounts the regular outline of the exospore is clearly visible within the perispore, and in this connection it may be noted that the clearing action of the chloral can be accelerated by placing the slides on a hot-plate at a maximum temperature of 40°C for a few hours.

B. SCALES.

In Asplenium the short conical rhizome is densely covered with small linear scales. These are carefully removed with needle or forceps and are first de-hydrated and de-aerated in solutions of 95% and absolute alcohol, before being briefly transferred to xylol and then mounted in canada balsam. The scales can be mounted in gum chloral, but it was found that direct mounting in this medium

caused air bubbles to be retained within the cells, and so the first procedure was adopted as the most satisfactory.

The same technique was used in the preparation of Polystichum scales for microscopic examination although in this case it was found more convenient to mount scales taken from the base of the stipe.

5. PHOTOGRAPHIC TECHNIQUES.

A. CHROMOSOME PHOTOGRAPHS.

The chromosome preparations were photographed at a magnification of approximately 800 diameters using a Reichert Biozet microscope and detachable plate camera. Particular care was taken to thoroughly clean the camera and microscope lenses before use. The microscope was set up for critical illumination, the oil immersion fluid 'Objecktol' being used between objective and slide and between the slide and the sub-stage condenser. A x 10 eyepiece was used in conjunction with a x 100 oil immersion objective, and maximum contrast was obtained by the use of a green filter.

Preparations of good contrast were photographed on Ilford Special Rapid Panchromatic plates (R.20), at exposures of approximately 10 seconds, and were developed for 2 minutes in a 1:2 solution of ID 36 at 20°C. Poorly stained preparations were photographed on Ilford Thin Film Half Tone plates (N.50), at exposures varying from 20 - 30 seconds, and were developed for the above time in a 1:2 solution of ID 2.

Preparations of Asplenium are illustrated throughout at a

magnification of x 1500, and Polystichum at a magnification of x 1200.

B. CHROMOSOME DIAGRAMS.

For the production of an analytical diagram an enlarged print at a uniform magnification of x 2000 was made onto Kodak Bromide matt paper, no.3. The image was then inked over using waterproof India ink, and after thorough drying the photographic image was bleached leaving the drawing only. The method of bleaching is described by Manton (1950).

The diagrams are duplicated by means of a paper negative, which is prepared by placing the diagram in contact with the sensitive surface of Ilford Reflex Document paper, 50 M. in a Kodak contact printing machine. Illumination is through the back of the diagram, and exposure results in a negative print from which positives can be obtained by a repetition of the process.

Paper negatives may also be obtained by the reflex copying process, in which illumination is through the back of the Reflex Document paper. The direct method was however preferred. The developer used was a 1:1 solution of ID 36.

C. SILHOUETTES.

(i) ASPLENIUM.

Mature fronds are reproduced at natural size throughout. A paper negative was produced by placing the chosen fronds (spore-bearing surface uppermost) on the sensitive side of a sheet of Ilford Reflex Document paper 50 M. Good contact was ensured by

placing the photographic paper on a foam rubber pad and by covering with a heavy glass sheet held down by weights or by manual pressure. Illumination was by two 60W. bulbs placed approximately 2 ft. directly above the specimens, and the exposure varied from 3 - 5 seconds. Development was effected in a 1:1 solution of ID 36 for 45 seconds at 20°C. A number of positives were obtained in the manner described above for the duplication of chromosome diagrams.

(ii) POLYSTICHUM.

In this case the fronds were often so large that paper negatives were produced in two or even three overlapping sections by the process described above. The prints of each frond were subsequently joined up, mounted on card, and photographed at a reduced magnification onto Ilford Panchromatic plates ($4\frac{1}{4}$" x $3\frac{1}{4}$"). Enlarged prints were then made at a suitable magnification and were re-mounted in the required arrangement. The resulting plates were then photographed at a suitable scale of reduction and were finally enlarged back to the chosen magnification onto bromide matt paper. Polystichum fronds are reproduced in this thesis at approximately half natural size, apart from the plate showing entire fronds of the wild hybrids (see page 166), which is reproduced at approximately one-sixth natural size. The individual pinnae figured are all natural size. A scale is given beside all reduced fronds.

6. THE USE OF COLCHICINE.

Colchicine is a chemical substance which inhibits spindle formation in dividing cells, resulting in the direct formation of restitution nuclei. As it's use can bring about a doubling of chromosome number within the cells affected it is a valuable tool in the artificial synthesis of polyploids.

In the attempted re-synthesis of P. aculeatum, diploid hybrids between P. setiferum and P. lonchitis were treated with colchicine in an attempt to obtain tetraploid plants. Young fertile fronds were subjected to treatment with varying concentrations of colchicine solution with the object of inducing chromosome doubling during the meiotic divisions of the spore mother cells.

A colchicine solution of the required strength was placed in a boiling tube and the chosen frond was immersed for a period of time varying from 24 - 48 hours. The exact treatments used are given in Table I, below. During and after immersion the plants were shaded to prevent scorching, and after treatment the frond under experiment was carefully washed.

Ripe spores were collected about six weeks after treatment, sown in $2\frac{1}{2}$" pots on the usual mixture and signs of germination were awaited. Germination was sparse in all cases, but the resulting prothalli were nurtured carefully and when sexually mature the cultures were selfed by immersion in water. Several sporophytes were obtained but unfortunately most of them proved to be alien. So far no Polystichum sporelings have been successfully raised,

but further experiments are planned.

TABLE I.

Hybrid.	Strength of colchicine solution.	Length of treatment.	Date of treatment.	No. of pots sown.
AS/485(i)	0.01%	24 hrs.	12.6.64.	3
AS/486(i)	0.05%	24 hrs.	28.7.64.	2
AS/485(i)	0.05%	24 hrs.	28.7.64.	1
AS/486(ii)	0.05%	48 hrs.	28.7.64.	2
AS/485(i)	0.1%	24 hrs.	7.9.64.	3
AS/485(i)	0.1%	36 hrs.	1.9.64.	1
AS/485(i)	0.1%	48 hrs.	30.7.64.	3

7. THE ATTEMPTED INDUCTION OF APOGAMY.

A diploid sporophyte is produced by the induction of apogamy in the tetraploid under investigation. Meiosis in such a sporophyte would be examined for any signs of bivalent formation.

The two following techniques can be applied in the attempted induction of apogamy:

A. HORTICULTURAL TECHNIQUES.

Production of apogamous sporophytes with the reduced chromosome number has been successfully accomplished in Scolopendrium vulgare Sm. (Manton, 1950) and in two species of Dryopteris (Manton & Walker, 1954). The method involves the withholding of water from the prothalli and consequent failure of self-fertilisation. This was attempted with prothalli of Polystichum braunii and P. aculeatum, but in neither case did the experiments meet with any success.

B. STERILE CULTURE METHODS.

The method used was that of Whittier & Steeves (1960), who successfully produced apogamous outgrowths in Pteridium by treatment with glucose of varying concentrations.

Spores were germinated on a sterile nutrient medium containing a suitable concentration of glucose. Spores of P. braunii and P. aculeatum were first sterilised by application of a dilute solution of one of the following substances: Mercuric chloride, copper sulphate, formaldehyde and household Domestos. The latter poison was completely unsatisfactory as it killed the fern spores at all dilutions tried. After this treatment the spores were spun down in a centrifuge and the excess liquid decanted off before they were introduced onto the culture medium under sterile conditions.

The medium used was Knudson's solution of mineral salts supplemented by the addition of 1 cc. of a minor elements solution per litre. Glucose was added at concentrations varying from 0.5% to 2.5%, and the medium was solidified with 0.5% agar. The cultures were grown on 25 cc. of the above medium in steam-sterilised glass jars sealed with cotton-wool plugs. The experiment was maintained at a temperature of 24°C with 12 hours daily illumination from fluorescent tubes.

These preliminary experiments yielded poor results. In spite of the precautions taken most of the cultures became contaminated, and as no apogamous outgrowths were obtained the method was pursued no further. It may be noted that although apogamous outgrowths are initiated by this method, actual apogamous sporophytes have never been raised to maturity (Steeves, personal communication, 1962).

Frontispiece: Plants of A. forisiense Le Grand growing in a shaded cleft in granitic rocks opposite the ruins of Retourtour, near Lamastre, Ardèche, France.

PART I. ASPLENIUM.

CHAPTER I. INTRODUCTION.

At the outset of the present investigation in 1962 four morphologically similar European *Asplenium* species were in cultivation at Leeds, namely *A. forisiense* (4x) from Switzerland, *A. macedonicum* (4x) from Jugoslavia, *A. fontanum* (2x) from France and *A. obovatum* from Sardinia (see Manton & Reichstein, 1962). From their morphology it was thought possible that the two tetraploids were closely related, and that the two diploids, *A. fontanum* and *A. obovatum*, could possibly be involved in the ancestry of one or both of these plants. In order to test this hypothesis experimental hybridisations were set up in all possible combinations between these four taxa.

The results obtained were sufficiently promising to encourage an expansion of the hybridisation programme, and other species at both diploid and tetraploid levels were included as material became available. Although crosses were attempted between all the species listed on page 74 , the chief emphasis has been on the production of triploid hybrids between species suspected of being related. Supplementary evidence to confirm the exact nature of any chromosome pairing observed in such hybrids is obtained by crossing the tetraploid under investigation with other species unrelated to it, either diploids of distinct morphology or other tetraploids of known affinity. Successfully produced hybrids of this type involve the diploid species *A. onopteris* and *A. viride*.

As will be seen, the final results obtained confirm the above hypothesis regarding the tetraploids A. forisiense and A. macedonicum, which have been shown to share a common ancestry involving alloploidy from the two diploids A. fontanum and A. obovatum. These four taxa will therefore be discussed throughout the thesis as the A. forisiense complex. Two further European tetraploids successfully incorporated into the hybridisation programme and for which cytological results have been obtained are A. majoricum and A. petrarchae. Each species will be discussed independently in the following chapters.

All species used in the hybridisation programme are listed in Table II on pages 26 to 28. As can be seen, most of the material used came originally from the personal collections of Professor T. Reichstein, although some was already in cultivation at Leeds (e.g. A. majoricum). Additional material of A. macedonicum was collected personally in 1962 during a visit to the Balkan Peninsula which was financially supported by the D.S.I.R.

Chromosome counts have been made on the species listed in Table III, page 29. A. macedonicum has been counted for the first time, and additional records are given for other previously-examined species. Wild and cultivated fronds of the six taxa investigated are illustrated on pages 36 and 37 and throughout Chapter III at the beginning of each corresponding sub-section.

CHAPTER II. SOURCES OF MATERIAL.

Listed below are all the species of Asplenium which were used in the hybridisation programme, together with their localities of origin and other relevant details. The letter H indicates that a collection has been successfully incorporated in a hybrid combination and further particulars of these are listed separately as an appendix.

TABLE II.

	SPECIES.	LOCALITY OF ORIGIN.	SOURCE OF SPORES.	COLLECTOR.	SUCCESS.
1	A. fontanum	Roche, Vaud, Switzerland.	Stock plants at Leeds AS/24; AS/25.	A. Sleep	H
2	A. fontanum	Grotte de Niaux, Ariège, France.	Stock plant at Leeds. Raised from spores from Herb.B.M.	Exsc. Walter-Callé No. 180.	H
3	A. fontanum	Villard de Lans, Isère, France.	Stock plant at Leeds. Spores from Herb.B.M.	A.H.G.Alston.	H
4	A. fontanum	Mt. d'Ottan, Valais, Switzerland.	Spores from Herb.B.M.	A.H.G.Alston. No. 11806	
5	A. fontanum	Val di Llo, Pyrénées-Orientales, France.	Plant cultivated in Basel. TR 16.	Professor Reichstein.	H
6	A. fontanum	Fort d'Ecluse, Haute-Savoie, France.	Plant cultivated in Basel. TR 69.	Professor Reichstein.	H
7	A. fontanum	Schloss Waldenburg, Basel, Switzerland.	Plants cultivated in Basel. TR 340.	Professor Reichstein.	
8	A. obovatum	Mt. Caruso, Ischia, Italy.	Plants cultivated in Basel. TR 520.	Professor Reichstein.	
9	A. obovatum	Trinité nr. Bonfacio, S.W. Corsica.	Plant cultivated in Basel. TR 621.	Professor Reichstein.	H

	SPECIES.	LOCALITY OF ORIGIN.	SOURCE OF SPORES.	COLLECTOR.	SUCCESS.
10	A. obovatum *	Capo di Testa, Sardinia.	Plant cultivated in Basel. TR 123.	Professor Reichstein.	H
11	A. forisiense *	Brissago, Ticino, Switzerland.	Plant cultivated in Basel. TR 34.	Professor Reichstein.	H
12	A. forisiense	Val Vizézy, nr. Montbrison, Auvergne. France.	Plant cultivated in Basel.	Professor Reichstein.	H
13	A. forisiense	Col de Maz de l'Air, Cevennes, France.	Plant cultivated in Basel.	Professor Reichstein.	H
14	A. forisiense	Lot, Penchot, France.	Spores from Herb.B.M.	Exsc. Walter-Callé No. 365.	
15	A. macedonicum	Markovgrad, N. of Prilep, Macedonia, S. Jugoslavia.	Stock plants at Leeds. Alston 17776.	A.H.G.Alston.	H
16	A. macedonicum	Treskavec planina, Macedonia, S. Jugoslavia.	Stock plants at Leeds. AS/62/32.	A. Sleep.	H
17	A. petrarchae *	Salon, Bouches-du- Rhône, France.	Plant cultivated in Basel. TR 358.	Professor Reichstein.	H
18	A. petrarchae	Karlobak, Croatia, Jugoslavia.	Plant cultivated in Basel. TR 838.	Professor Reichstein.	
19	A. majoricum	C'as Patro Lau, Barranc, Sóller, Mallorca.	Stock plants at Leeds.	J. Orell.	H
20	A. billotii	Bosa, Sardinia, Italy.	Plant cultivated in Basel. TR 109.	Professor Reichstein.	
21	A. billotii	Ronco, Ticino, Switzerland.	Plant cultivated in Basel. TR 276.	Professor Reichstein.	H
22	A. onopteris	Aritzo, Sardinia, Italy.	Plant cultivated in Basel. TR 114.	Professor Reichstein.	H

Footnote: Progeny of collections marked by an asterisk were in cultivation at Leeds at the outset of the investigation.

	SPECIES.	LOCALITY OF ORIGIN.	SOURCE OF SPORES.	COLLECTOR.	SUCCESS.
23	A. cuneifolium	Kirchdorf, Styria, Austria.	Plant cultivated in Basel.	Professor Reichstein.	
24	A. cuneifolium	Kraubath, Styria, Austria.	Plant cultivated in Basel.	Professor Reichstein.	
25	A. cuneifolium	Lac Lanet, Davos, Grisons, Switzerland.	Wild collection.	A. Sleep.	
26	A. jahandiezii	Gorge du Verdon, Basses-Alpes, France.	Plants cultivated in Basel.	Professor Reichstein.	
27	A. trichomanes	Borrowdale, Cumberland, Great Britain.	Stock plants at Leeds.	J.D.Lovis	
28	A. trichomanes	Aber Falls, Caernarvonshire, Great Britain.	Stock plants at Leeds.	J.D.Lovis	
29	A. viride	Hutton Roof, Westmorland, Great Britain.	Wild collection.	A. Sleep.	H
30	A. viride	Saalbach, Salzburg, Austria.	Stock plants at Leeds AS/2; AS/6.	A. Sleep.	
31	A. viride	Gorge Chauderon, Montreux, Vaud, Switzerland.	Stock plant at Leeds. AS/19.	A. Sleep.	
32	A. viride	Bains de Tredos, Pyrenees, Spain.	Stock plant at Leeds.	D. Bartley.	
33	A. viride	Scar Close, Ribblehead, Yorks., Great Britain.	Stock plant at Leeds.	J.D.Lovis.	
34	A. pseudo-fontanum	Agok Pass, north-east Afghanistan.	Spores from stock plant at Leeds (now dead). Originally raised from Herb.B.M. collection.	H.F. Neubauer. No. 893.	

CHAPTER III. THE SPECIES.

SECTION A. CYTOLOGY.

A cytological examination was made of the plants listed. All are, at the time of writing, in cultivation at Leeds.

Chromosome counts were obtained from aceto-carmine squash preparations of spore mother cells undergoing meiotic division. Counts are most easily and accurately made when the dividing cells have reached the stage of diakinesis or first metaphase, when the chromosomes are contracted to a maximum.

The results obtained are given in the following Table. Asterisks denote new records.

TABLE III.

A. fontanum	Roche, Switzerland	n = 36
"	Isère, France *	n = 36
"	Ariège, France *	n = 36
A. obovatum	Corsica	n = 36
"	Sardinia	n = 36
A. forisiense	Brissago, Switzerland	n = 72
"	Cevennes, France *	n = 72
"	Auvergne, France *	n = 72
A. macedonicum	Markovgrad, Macedonia *	n = 72
"	Treskavec, Macedonia *	n = 72
A. petrarchae	Salon, France	n = 72
A. majoricum	Sóller, Mallorca	n = 72
A. onopteris	Sardinia	n = 36

SECTION B. TAXONOMY, DISTRIBUTION AND ECOLOGY.

All the species of Asplenium involved in this investigation exist as named taxa in the literature. They are small plants inhabiting rock crevices and many are not only variable but rather alike. There is consequently a great deal of confusion in floras and in some herbaria, more especially concerning the species numbered 1 - 4. It will therefore be convenient to enumerate the main morphological characters of these species (the A. forisiense complex) in tabular form (Table IV), from which it can be seen that while A. forisiense and A. macedonicum are very much alike, both are intermediate between A. fontanum and A. obovatum, with the latter closer to A. forisiense than to A. macedonicum. Species 5 and 6 are adequately described in the literature and present no difficulty. Silhouettes of adult fronds are illustrated in Figs. 1-5+13-14 and in most cases come from both wild and cultivated specimens.

Further information is given individually under each of the species enumerated. Additional taxa such as A. onopteris L. and A. viride Huds., which were used in the hybridisation programme in attempts to elucidate the relationships of these species, are omitted. In the preparation of detailed distributions citations in floras have been found to be of little use because of the confusion that has existed between the members of the A. forisiense complex. The data presented have therefore been compiled from a study of dried material in National (British Museum (N.H.), Kew and Vienna) and private herbaria (notably that of Professor T. Reichstein) and from cytologically-examined plants in cultivation. Distribution maps will be found on pages 44, 61 and 73.

TABLE IV. COMPARATIVE MORPHOLOGY OF MEMBERS OF THE A. FORISIENSE COMPLEX. [1]

	A. FONTANUM	A. OBOVATUM	A. BILLOTII	A. FORISIENSE	A. MACEDONICUM
Appearance.	Fine & delicate, deeply cut.	Robust.	Robust, lax in general appearance.	Robust.	Robust; generally of more delicate appearance than A. forisiense.
Rhizome.	Erect, conical c.1 cm. Densely covered with shining dark brown scales.	Erect, conical 0.8-1.0 cm. Densely covered with chestnut-coloured filiform scales.	Erect, conical c.1 cm. Densely covered with tapering dark-brown filiform scales.	Erect, conical 0.6-1.0 cm. Densely covered with narrow scales of two types, the outer lanceolate & dark brown, those nearer the apex becoming filiform and chestnut-coloured.	Erect, conical 0.8-1.0 cm. Densely clothed with a mixture of dark lanceolate scales and chest-nut-coloured filiform scales.
Rhizome scales.	Robust, multi-cellular, lanceo-late in shape, consisting of regular rectang-ular shaped cells with thick walls, often heavily thickened at base of scale, but cell cavity never occluded. Central dark stripe completely lacking.	Narrow and tapering, multi-cellular, consisting of regular rectang-ular shaped cells. Walls thinner than in A. fontanum, the cells having a larger central lumen. No dark stripe.	Long & tapering, dark brown, multicellular, the rectangular shaped cells with thick walls and a large central lumen. No dark central stripe.	Long & tapering, multicellular, cells elongate, rectangular, with a wide lumen and brownish walls of medium thickness (intermediate between A. obovatum and A. fontanum). No central stripe of occluded cells.	Long & tapering, multicellular. Cells regular, rectangular in shape with thick yellow-brown walls. Central stripe of occluded cells completely lacking.

	A. FONTANUM	A. OBOVATUM	A. BILLOTII	A. FORISIENSE	A. MACEDONICUM
Frond colour.	Pale or dark green.	Pale green.	Bright green.	Bright green.	Bright to dark green.
Frond length.	(6)9-15cm.	(8)12-25cm.	(10)12-20cm.(30)	(8)10-20cm.(25)	(6)9-12cm.(14)
Frond shape.	Narrowly lanceolate, broadest in the middle.	Deltoid to ovate and ovate-lanceolate.	Lanceolate to ovate-lanceolate.	Lanceolate to ovate.	Lanceolate.
Widest part of frond.	Middle to upper third.	Lower third.	Lower third.	Middle to lower third.	Middle to lower third.
Basal pinnae.	Broadest in the middle, with the pinnae decreasing markedly in size towards the base of the frond.	Basal pinnae equalling or slightly exceeding the middle pinnae in length.	Basal pinnae sometimes just equalling the middle ones in length, generally slightly smaller.	Widest in middle. Basal pinnae smaller than middle ones, but not markedly so.	Widest in middle. Pinnae decreasing in size towards the base, but not as markedly as in A. fontanum.
Insertion of basal pinnae.	Not reflexed. Markedly smaller than middle pinnae.	Basal and lowermost pinnae often markedly reflexed.	Often slightly reflexed.	Lowermost pinnae frequently reflexed.	Often slightly reflexed.
Frond dissection.	Bipinnate.	1 - 2-pinnate.	Bipinnate.	1 - 2-pinnate.	Sub-bipinnate.
Frond apex.	Long, tapering pinnatisect terminal segment. Apex much shorter in cultivated plants.	Long terminal segment (0.8-1.2cm.), irregularly lobed but not deeply divided; lobes bearing obtuse mucronate teeth.	Tip acute, sharply toothed.	Often with long tapering terminal segment which is pinnatifid to pinnatisect, each division bearing a sharp mucro.	Frond tapering shortly to the subacute dentate tip.

	A. FONTANUM	A. OBOVATUM	A. BILLOTII	A. FORISIENSE	A. MACEDONICUM
Lamina.	Thin.	Thick.	Thick.	Thick.	Fairly thick.
Length of petiole.	$\frac{1}{4}$-$\frac{1}{3}$ of total length of frond.	$\frac{1}{4}$-$\frac{1}{3}$ of total length of frond.	$\frac{1}{4}$ of total length of frond, often much less.	$\frac{1}{3}$ of total length of frond in wild material, much less in cultivated plants.	$\frac{1}{4}$-$\frac{1}{3}$ of total length of frond.
Colour of petiole.	Petiole bearing occasional dark filiform scales, green above, brown on underside at base. Dark coloration generally poorly developed, but in some cases may extend as far as the second pair of pinnae.	Deep reddish-brown both above and below, and bearing fine reddish scales, particularly near the base.	Deep brown both above and below and bearing occasional dark filiform scales.	Pale green above, red-brown below and bearing occasional reddish-brown filiform scales.	Pale green above, red-brown below; bearing occasional reddish-brown filiform scales.
Rhachis.	Robust. Uniformly green throughout.	Robust, green above. Red-brown below for $\frac{1}{3}$-$\frac{1}{2}$ it's length and bearing reddish filiform scales which are particularly frequent at the pinnae insertions.	Robust, green above and red-brown below for $\frac{1}{2}$ it's length. Bearing occasional filiform scales.	Robust. Green above. Red-brown below for $\frac{1}{3}$ of it's length. Bearing occasional filiform scales.	Robust, green above and red-brown for $\frac{1}{4}$-$\frac{1}{3}$ of it's length. Bearing occasional filiform scales.
Number of pinnae.	15 - 20	10 - 15	15 - 20	12 - 20+	11 - 20

	A. FONTANUM	A. OBOVATUM	A. BILLOTII	A. FORISIENSE	A. MACEDONICUM
Pinna length.[2]	0.8 - 2.0 cm.	0.6 - 3.0 cm.	1.5 - 3.0 cm.	0.8 - 1.5 cm.	0.9 - 1.5 cm.
Pinna shape.	Triangular.	Oblong.	Oblong or deltoid.	Oblong - triangular	Ovate to triangular.
Dissection of pinnae.	Pinnate for most of their length.	Variable. Well grown wild specimens may have 5-7 pairs of pinnules; plants growing under poor conditions or in cultivation have the pinnae divided only in the proximal half, being pinnatifid distally.	Pinnate for most of their length.	Variable; when deeply subdivided bearing 3-7 pairs of pinnules with pinnatifid tip; otherwise bearing 1-3 pairs of pinnules & having a terminal segment half as long as pinna and not deeply cut.	Pinnate to pinnatifid; where few in number the rest of the pinna is pinnatifid, bearing deeply-cut, toothed segments.
Apex of pinna.	Acute, pinnatifid.	Tapering to rounded, obtuse tip.	Acute to subacute and markedly dentate.	Obtuse, dentate.	Toothed apex which may be obtuse or subacute.
Number of distinct pinnules.	3-8 pairs/pinna.	1-7 pairs/pinna.	4-6 pairs/pinna.	Up to 3-7 pairs/ pinna.	Up to 3-6 pairs/ pinna.
Dissection of pinnules.	Pinnatifid with sharply mucronate lobes.	Lobed, with tiny obtuse mucronate teeth.	Proximal ones lobed, all bearing acute mucronate teeth.	Lobed and bearing obtuse mucronate teeth.	Lobed, with obtuse teeth bearing a tiny mucro.

	A. FONTANUM	A. OBOVATUM	A. BILLOTII	A. FORISIENSE	A. MACEDONICUM
Pinnule shape.	Proximal pinnules stalked, oval to ovate-truncate, generally with the proximal acroscopic pinnule somewhat distant and larger than the rest.	Shortly stalked, sub-orbicular.	Stalked, oval to oblong, narrower than in A. obovatum.	Shortly stalked and sub-orbicular. Proximal pinnules more oval in shape, the acroscopic one somewhat distant and larger than the rest.	Shortly stalked, oval to sub-orbicular.
Shape of sorus.	Oval.	Oval.	Oval.	Oval.	Oval.
Position of sorus.	Nearer vein than margin.	Nearer margin than midrib.	Close to margin.	Mid-way between margin and midrib.	Mid-way between margin and midrib.
Indusium.	Persistent, membraneous, white.	Persistent, membraneous, white.	Persistent, thin, white, membraneous and recurved.	Persistent, white, membraneous.	Persistent, white, membraneous.
2n	72	72	144	144	144

Footnotes:

1. Table compiled from a study of both wild and cultivated specimens of all taxa.
2. Measurements taken from the median pair of pinnae (mid-way between frond apex and insertion of basal pinnae).


A
B
C

Fig. 1. Frond silhouettes from adult plants of A. fontanum. Natural size.

Wild (B) and cultivated (A) fronds from the same plant originally collected from Roche, Switzerland.

Wild collection from Ramsfluh, Switzerland. (C).

Cultivated frond from Val di Llo, Pyrénées-Orientales, France (D).

Cultivated fronds from Isère, France (E).

Cultivated fronds from Ariège, France (F).

Further details of these collections will be found in Appendix I.

1. A. FONTANUM (L.) Bernh. (Fig. 1).

This fern was first described by Linnaeus (1753) as Polypodium fontanum and was transferred to the genus Asplenium by Bernhardi (in Schrader, 1799), although subsequently placed in other genera by different authors (e.g. Athyrium (Roth, 1799), Aspidium (Willd., 1810). The name Asplenium Halleri DC in Lam. & DC., (1815) which appears widely in the literature, is regarded (Christensen, 1906) as a synonym. Several named varieties and forms are cited in floras, for example A. fontanum var. Halleri Mett., which is generally interpreted as having a markedly dissected frond. However, the species as a whole is very variable and these varieties may be regarded as only extreme types of a continuous range. This is to some extent illustrated by the fronds in Fig. 1 which include both wild and cultivated specimens, some of which were taken from the same individual plant.

Distribution: A.fontanum is found on limestone rocks, from Spain to the north of Switzerland. It occurs in the mountains of eastern Spain, in the central and eastern Pyrenees, Alpes Maritimes, and along the whole length of the French Alps and the Jura, where it is particularly frequent. It's distribution becomes sporadic from Basel eastwards, and only isolated records are to be found. This species is rare in Germany, where there are scattered records from Baden, Freiburg and Württemberg, this area being the limestone continuation of the Jura to the east of the Black Forest. It is also

of infrequent occurrence in Italy where there are sporadic records from the mountainous areas of Piedmont. The fact that some authors e.g. Fiori (1943) used the taxon A. fontanum in a very wide sense to include A. obovatum, A. billotii and A. forisiense adds to the difficulty of tracing records of this species. A. fontanum was once recorded from Austria but has not been seen there since, and a similar situation pertains in Mallorca, where until fairly recently it used to be found on the limestone walls of olive terraces in the vicinity of Sóller. The only Balkan locality for this fern is a record of Heldreich's (quoted n.v. by Halácsy (1901) and Rechinger (1943) from the island of Crete (2300m.)). In contrast to these diminishing records in eastern Europe Vida recently discovered this species in Hungary, from Fanietal, Vértas-Gebirge, [redacted] (Vida, 1963, personal communication). A. fontanum itself does not apparently occur outside Europe but A. pseudofontanum (Kossinksky, 1922), a species which may well be of close affinity to the European taxon, occurs in certain mountainous regions of Central Asia. It is recorded from Turkestan (Province of Samarkand, at 1600m.), Afghanistan and northern India. It's cytology is however as yet unknown.

Herbarium specimens of A. fontanum have been seen from the following regions:

Spain: Valencia, Cuenca, Teruel, Huesca and the Pyrénées espagnols (Aragona) and catalanes.

France: Pyrénées-Orientales, Ariège, Hautes-Pyrénées, Alpes-Maritimes, Basses-Alpes, Savoie, Haute-Savoie, Isère, Ain, Rhône, Jura, Doubs, Terr.-de-Belfort and Haut-Rhin.
Switzerland: Valais, Vaud, Neuchâtel, Basel, Aargau and St. Gallen.
Italy: Piedmont (Tanaro valley).

Ecology: A. fontanum is restricted to shaded crevices of limestone rocks which generally have a north-exposed aspect. In France and Switzerland it is found at relatively low altitudes (300 - 700m.) but it extends to 1300 - 1800m. in Spain.

This species has been collected personally from two localities in Switzerland: Roche (Vaud) and Ramsfluh (Aargau). In the former it was accompanied by much Polypodium australe Fée among calcareous block scree in steep woodland. In the latter many plants were found in shaded crevices on a relatively inaccessible rock face in beech woodland. Other trees present were Sycamore and Abies, with a ground flora consisting mainly of Mercurialis perennis L. and Asarum europaeum L. Other ferns accompanying A. fontanum in the same rocks were A. trichomanes L., A. viride Huds., A. ruta-muraria L. and Polystichum aculeatum (L.) Roth, together with a number of bryophytes.

Fig. 2. Silhouettes of A. obovatum s.s. Natural size.

A. Cultivated fronds from Capo di Testa, Sardinia, Italy.

B. Wild frond from Mt. Caruso, Ischia, Italy.

Fig. 2a. Silhouettes of two cultivated fronds of A. billotii from Ronco, Ticino, Switzerland. Natural size.

2. THE A. OBOVATUM GROUP.

a) A. OBOVATUM Viv. (Fig. 2).

This species and the next (A. billotii) collectively make up the former complex known in the literature either as A. lanceolatum auct. non Forskål, or more recently as A. obovatum Viv. sensu lato (which includes A. lanceolatum Huds.). The name A. obovatum was first introduced by Viviani (1824) to describe Corsican material, although subsequently many authors reduced this taxon to a variety or sub-species of A. lanceolatum. A full account of the synonomy will be found in Becherer (1929). It is sufficient to say here that when the existence of two chromosome numbers within this complex was first detected by Manton & Reichstein (1962) comparison with the type specimen of A. obovatum showed that this name was in fact referable to the diploid cytotype and not to the tetraploid.

A. obovatum s.s. is morphologically close to A. billotii, as shown by the characters listed in Table IV. It may be distinguished from A. billotii by the following criteria: the chestnut-coloured, filiform rhizome scales, 1-2 pinnate frond, often less dissected than in A. billotii, and the leaf shape almost deltoid, the basal pinnae equal to or longer than the middle pinnae and often markedly reflexed. The pinnae have obtusely rounded apices, and generally bear fewer and less distinct pinnules. These are lobed, suborbicular in shape and not sharply toothed.

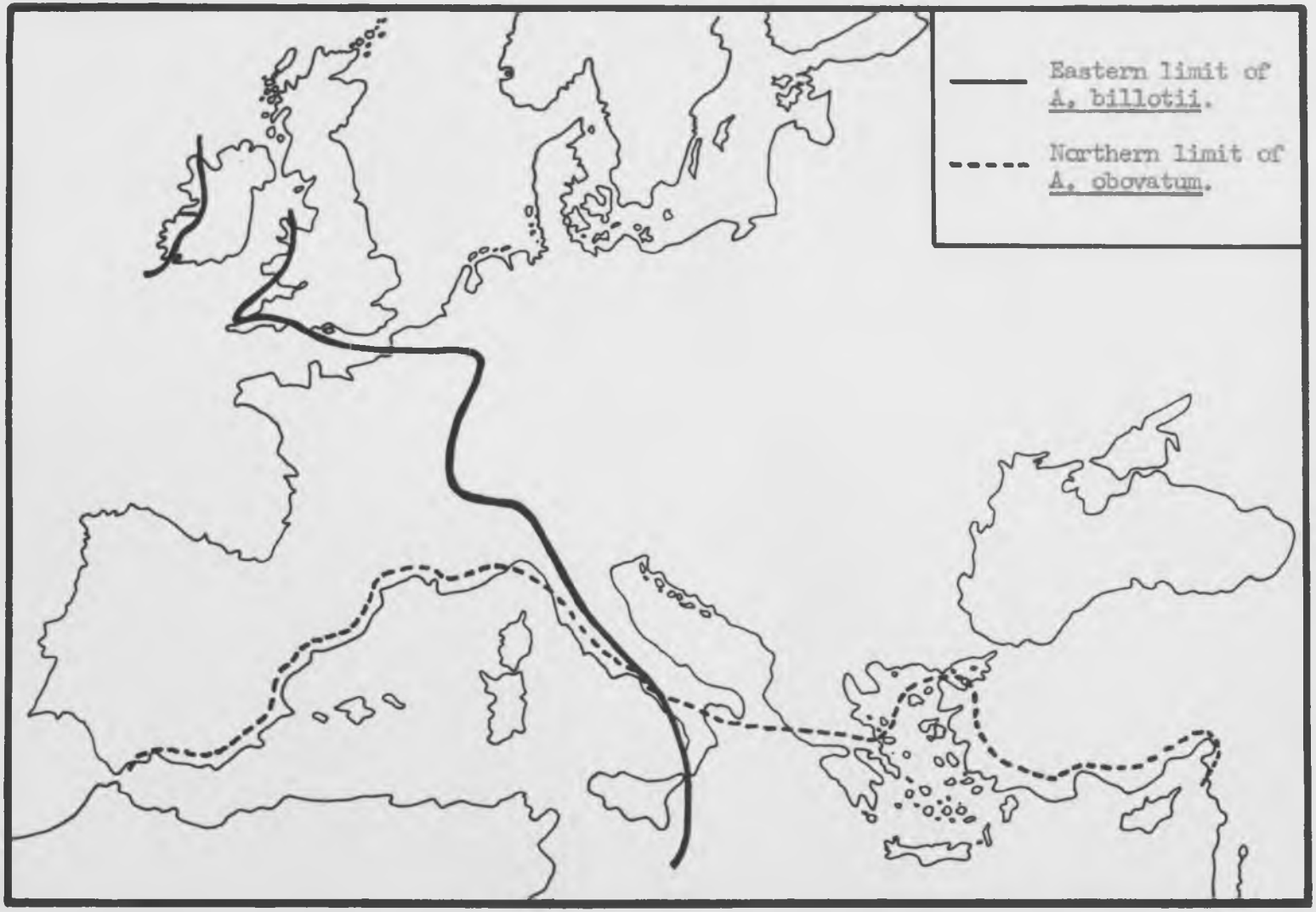


Fig. 3. Map to show the distribution of A. obovatum (2x) and A. billotii (4x).

Distribution: Owing to the frequent confusion of this species with A. billotii it has been necessary to rely on living material and dried specimens in National and private herbaria for tracing the geographical distribution. Of critical importance in this respect have been the rich collections in the Naturhistorisches Hofmuseum at Vienna which greatly exceed those in British herbaria. Though probably still incomplete, the available information nevertheless indicates fairly clearly that this species possesses a disrupted (presumably relict) distribution confined to the Mediterranean region, where it occurs frequently on islands.

Herbarium specimens have been seen from southern France, Corsica, Sardinia and Italy. There are numerous collections of this fern from the eastern Mediterranean where it is locally common on the islands of the Cyclades group. There are specimens from the Greek mainland (Arcadia and Argolis (Peloponnese); Euboea) and from the following islands: Samothrake, Nikaria, Delos, Naxos, Andros, Melos, Tenos and Mykonos. It is also recorded from Turkey (Davis, 1965). On the western side of the Balkan peninsula it is rare, and is in fact probably absent from the Jugoslavian coast as it is not recorded by Rossi (1911). Mayer (1963) gives only a doubtful record for Croatia. It is probable that A. obovatum occurs in Spain: records exist in the literature but no specimens have been seen. On the other hand, Fernandes (1960) and Pinto da Silva (1951, 1959) both suggest that the records for Portugal are the result of confusion between A. obovatum and A. billotii; this seems highly probable in view of the generally

'Atlantic' distribution of the latter.

The plants in cultivation came from the islands of Hyères (south France), Ischia (Italy) and from several localities on Corsica and Sardinia. This species is particularly abundant around the Straits of Bonifacio between these two islands.

Ecology: A. obovatum is generally found on ancient siliceous rocks (granite, gneiss and schist), never on limestone. It invariably grows close to the sea, and although most often found in rock crevices it may also grow on old, dry stone walls. It occurs at altitudes from sea level to 150+m. in it's western localities, and up to 900m. on some of the Aegean islands. On the latter it occurs without A. billotii although these two ferns are often found growing together in the western Mediterranean region.

b) A. BILLOTII F.Schulze. (Fig. 2a).

As already explained, there has been much taxonomic confusion between this species and the preceding one (A. obovatum Viv.). British material was described as A. lanceolatum by Hudson (1778), and although invalidly published, the name was subsequently used by a number of authors (e.g. Luerssen (in Rabenhorst, 1889), Briquet, 1910, Rouy, 1913). It is now known (Manton & Reichstein, 1962) that two chromosome numbers exist within this complex, and that British material is referable to the tetraploid cytotype. The name of A. lanceolatum Huds. is however not available for the tetraploid species since it is a pre-occupied homonym of A. lanceolatum Forskål

(1775), and the first validly published alternative name is A. billotii F.Schulze (1845). For a detailed account of the synonomy the reader is referred to Becherer (1929).

The most useful criteria for distinguishing this species from A. obovatum (cf. Table IV) are: the lanceolate rhizome scales which are dark brown in colour, the fully bipinnate frond, ovate-lanceolate in shape and generally larger than in A. obovatum. The pinnae have acute, dentate apices and are generally sub-divided into distinct pinnules for most of their length; the pinnules are narrower than in A. obovatum and bear characteristic sharp teeth.

Distribution: It is only possible to give the distribution of this species in outline because the published records suffer from the same confusion already noted with regard to A. obovatum. A. billotii has also frequently been confused with A. forisiense. There can be no doubt however that A. billotii possesses a markedly Atlantic distribution.

Although often encountered together with A. obovatum in parts of the Mediterranean region, notably Corsica and Sardinia, it penetrates further inland and further west, reaching the coastal counties of the south and west of England (Manton, 1950) and the west coast of Ireland. It is also recorded from the Channel Islands (McClintock, 1961), Madeira, the Canary Islands and the Azores. It is of frequent occurrence throughout France, where it is particularly common in the south, and in Spain and Portugal. It is absent from Germany but small

plants occur infrequently on the sandstones of the Vosges between Bitsch and Weissenburg (Alsace), the locus classicus. Also on the fringe of it s range, this fern is found in southern Switzerland, at Ronco, near Ascona (compare A. forisiense, page 52). Rechinger (1943) quotes records from a number of Aegean localities, but a study of herbarium material confirms that these refer to A. obovatum and that in all probability A. billotii is absent from the eastern Mediterranean.

Living material has been collected personally from Merioneth and South Devon, and plants are in cultivation from a number of European localities, in particular from Ronco (Switzerland), Sardinia and Corsica. Dried specimens have been seen from several localities on Corsica and Sardinia, Ischia (Italy), Ronco (Switzerland), Wasigenstein (Alsace), Oporto (Portugal) and from the following departments of France: Pyrénées-Orientales (Banyuls), Manche, Deux-Sèvres, Indre and Aveyron.

Ecology: Like A. obovatum, this species occurs on siliceous Palaeozoic rocks (granite, schists and sandstones), never on limestone. It too is often found on islands, but it seems to be a more adaptable and vigorous plant than A. obovatum. It generally occurs in sheltered rock crevices but can be found growing on dry stone walls, as at Ronco. It is recorded at altitudes of 100 - 500m.

Fig. 4. A. forisiense.

Silhouettes of wild fertile fronds from Retourtour, Lamastre, France. Natural size.

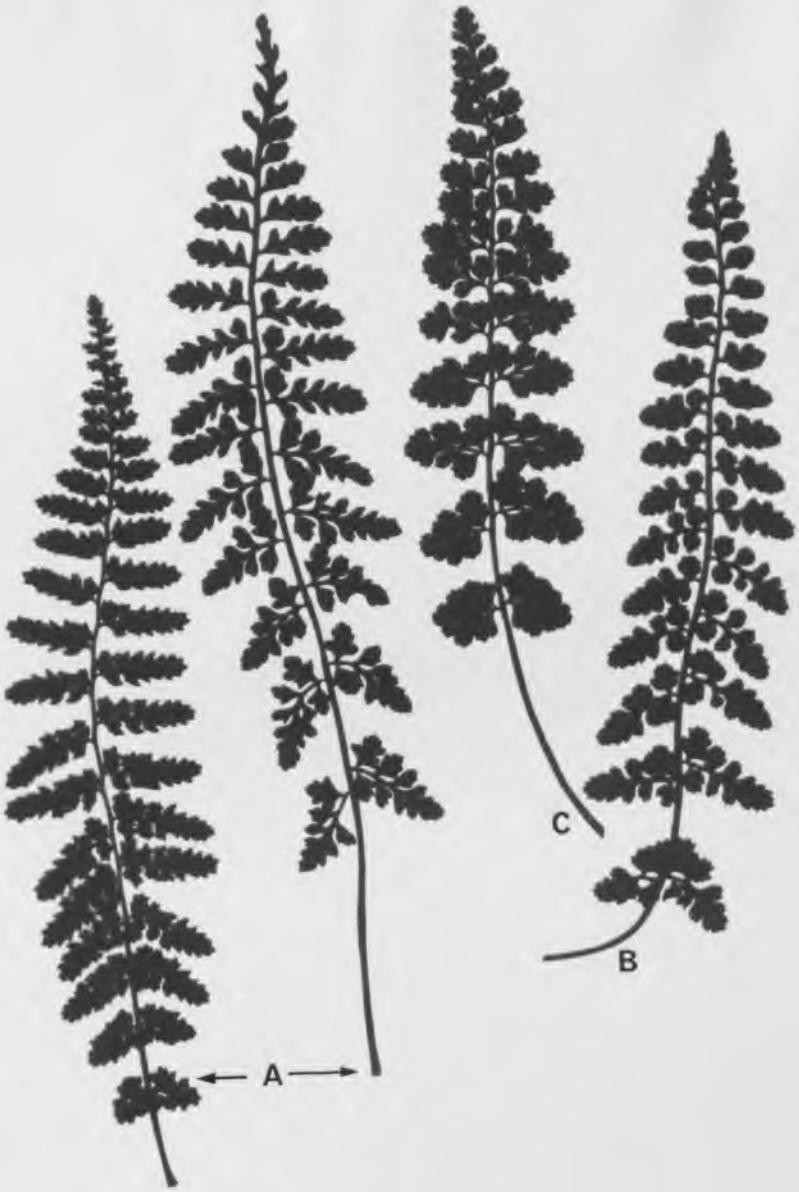


Fig. 4a. Silhouettes of cultivated fronds of A. forisiense. Natural size.

A. From Auvergne, France.

B. From Brissago, Ticino, Switzerland.

C. From Cevennes, France.

3. A. FORISIENSE Le Grand. (Fig. 4).

This fern was first described as A. Halleri var. foresiacum by Le Grand in 1869, who stated that the plants growing at Montbrison in the Central Massif of France differed from A. Halleri (= A. fontanum (L.) Bernh.) in "the large proportions, the segments two or three times larger, the divisions less pronounced and often only toothed". Subsequent synonomy is confused. According to Flora Europaea (1964) the date of first publication of the name A. forisiense at the species level is Le Grand (1873). Further discussion will however be found in Christ (1900) and Becherer (1935), from which it is clear that the first really detailed description and illustration for A. forisiense Le Grand is that given by Sudre (1894). From time to time this fern has also been regarded as a variety or sub-species of A. fontanum (e.g. A. fontanum var. foresiacum (Fiori, 1943), A. fontanum ssp. foresiacum (Aschers. & Graebn., 1912)) or of A. lanceolatum auct. (e.g. A. lanceolatum ssp. foresiacum (Rouy, 1913)). These alternative treatments do in fact reflect genuine affinity between the species named.

A. forisiense is a variable plant, particularly with regard to the degree of dissection of the lamina. This is to some extent illustrated by the silhouettes of wild and cultivated fronds figured on pages 49 and 50. It is also morphologically close to A. macedonicum, a species described from the Balkan peninsula by Kümmerle (1916). A brief comparison of the morphology of these two taxa will be found on page 57.

Distribution: Some difficulty is encountered in plotting the detailed distribution of this species since in both literature and herbaria it

has been confused with A. fontanum, A. obovatum, and most of all with A. billotii. The following account has been compiled from a study of plants in cultivation and reasonably confident identifications of herbarium material.

A. forisiense is found throughout the Massif Central of France, which constitutes it's centre of distribution. It is recorded from: Lot, Aveyron, Lozère, Ardèche, Haute-Loire, Loire, Rhône and Isère, and probably occurs also in other departments within this region. It's presence in parts of Spain, and more particularly, in the Pyrenees, is highly probable, but so far specimens referable with certainty to A. forisiense have been recorded only from Banyuls, in the Pyrénées-Orientales. An outlier of the main distribution is found in southern Switzerland, on the west side of Lake Maggiore just south of Locarno. Here it occurs quite frequently in the vicinity of Brissago, Piodina and Ronco in the crevices of dry stone walls bordering vineyards. It is also found on rock. This species is recorded from northern Italy by Dr. H. Christ, who designates plants collected from Liguria as a distinct variety, A. foresiacum var. italicum (Christ, 1902). Dried material of this variety has been seen, but as the material was somewhat depauperate it could not with confidence be referred to A. forisiense. These plants would well repay further investigation, and if they are indeed A. forisiense and not small forms of A. billotii it will be an interesting extension to the distribution of A. forisiense as known at present.

Ecology: This fern is frequently found on ancient igneous rocks, generally granite (and other related siliceous rocks such as diorite

and aplite) although it also occurs on metamorphic schists and gneisses. It's overall distribution is undoubtedly largely influenced by the geological incidence of these rocks. The altitudinal range in France is from 300 - 800m., and at Brissago (Switzerland) it occurs at approximately 300m. It is a shade-loving plant, invariably found deep in crevices, beneath overhangs or behind a growth of Rubus. In sunny situations it becomes small, stunted, yellowish-green in colour and coriaceous in texture.

This species has been collected personally from three localities in central France: Val Vizézy, west of Montbrison (Loire), and at Lamastre and Retourtour, south-west of Tournon (Ardèche). Habitat photographs are shown on pages 64 to 65. In the Val Vizézy, A. forisiense grows at an altitude of 550m. in crevices of south-exposed rocks which geologically are part of the vast outcrop of eruptive granite that forms the chain of mountains known as the Mtns. du Forez. Accompanying it in this locality were: A. septentrionale (L.) Hoffm., A. onopteris L. and the hybrid A. x costei (= A. foresiacum x A. septentrionale Litard.). In a number of places between Annonay and Lamastre A. forisiense was found growing in crevices of red-brown metamorphic rocks by the road-side, at an altitude of c.300m., and was accompanied by A. trichomanes L., A. septentrionale (L.) Hoffm., A. x alternifolium Wulfen, A. onopteris L., A. adiantum-nigrum L. and Ceterach officinarum DC. In exposed south-facing rocks opposite the ruins of Retourtour, near the town of Lamastre, numerous plants of A. forisiense were growing in deep crevices and narrow clefts, always in deep shade, at an altitude of approximately 400m.

Fig. 5. Silhouettes of cultivated fronds of A. macedonicum (AS/62/32) from the Treskavec planina, near Prilep, Macedonia. Natural size.

Fig. 5a. Silhouettes of cultivated fronds of A. macedonicum (progeny of Alston 17776) from Markovgrad, Prilep, Macedonia. Natural size.

4. A. MACEDONICUM Kümmerle. (Fig. 5).

In 1916 and 1921 respectively Kümmerle described and named two supposedly endemic species of Asplenium from south Macedonia as A. macedonicum (Kümmerle, 1916) and A. bornmülleri (Kümmerle, 1921). The close resemblance of the former taxon to A. fontanum, A. forisiense and 'A. lanceolatum' was noted. Subsequently Hayek (1924) cited A. bornmülleri as a sub-species of A. fontanum (L.) Bernh. (but accepted A. macedonicum as a species). The original collector J. Bormüller, (1928) considers, however, that A. macedonicum, A. bornmülleri, A. obovatum and A. foresiacum should be united as sub-species within the taxon A. lanceolatum. Mayer, in his check-list of Jugoslavian Pteridophytes (1963) recognises only the taxon A. macedonicum, quoting A. bornmülleri as a later synonym of this species. This interpretation is almost certainly correct (see below).

As may be seen by reference to Table IV, A. macedonicum is morphologically close to A. forisiense. These two taxa together form one broad morphological unit which is intermediate between A. fontanum and A. obovatum in the characters listed. A. forisiense is in general appearance much closer to A. obovatum, and A. macedonicum, although more robust in appearance than the delicate A. fontanum, more nearly approaches this species in overall dissection of the frond.

Unfortunately very little wild material of A. macedonicum has been available for study and it is thus impossible to know the complete range of variation of this plant. The closely related species A. forisiense is in it's natural habitat a very variable fern, and such

characters as size and frond dissection seem to be correlated with position and exposure, plants growing in deep shade producing large, fully bipinnate fronds with up to 7 pairs of distinct pinnules per pinna (1-3 in cultivated plants). None of the Asplenium species investigated grows well in cultivation (with the exception, perhaps, of A. billotii); all are much smaller and less dissected than in the wild state. It was however found that small but constant differences were maintained in cultivation between A. macedonicum and A. forisiense when grown under the same conditions. Fronds of these taxa are generally lanceolate in shape and sub-bipinnate, although A. macedonicum is often of smaller size. The most constant differences are:

1) Basal pinnae generally markedly smaller than the middle ones in A. macedonicum, and only slightly reflexed.

2) Apex of frond tapering shortly to the dentate tip; A. forisiense often having a long, tapering pinnatisect apex.

3) Pinnae generally more divided than in A. forisiense. This species has 1-2 pairs of toothed rounded pinnules with a terminal segment about half as long as pinna, not deeply cut. A. macedonicum, on the other hand, has pinnae ovate to triangular, with 1-3 rounded toothed pinnules and an obtusely rounded dentate apex.

Silhouettes of cultivated fronds of A. macedonicum are illustrated in Fig. 5, pages 54 and 55.

Distribution: The type locality for A. macedonicum is described (Kümmerle, 1916) as "Morihovo, above a village" (un-named). The Morihovo is an extensive mountain range, about 20 miles long and 400 - 800m. high,

extending from the east of the valley of the Crna Reka (Black River) almost to the Greek frontier. Information about the further distribution of this fern cannot be obtained from the literature, but a local botanist, Dr. Bačar (1965, personal communication) has reported that ferns referable to A. macedonicum occur quite frequently in a number of the mountainous parts of south Macedonia, notably near Prilep, in the mountains of Bigla, Morihovo and Kožuf, and in the Vardar valley. In all these regions A. macedonicum is said to be somewhat variable, but no specimens referable to A. bornmülleri have been found in recent years; the existence of more than one taxon is therefore doubtful.

Ecology: There is no ecological information included in Kümmerle's description of A. macedonicum, but Dr. Bačar states that he has found this fern in the association of Andropogon ischaemi in the Morihovo mountains, and in the Quercus cocciferae association near Prilep and in the Vardar valley. Geologically south Macedonia shows a number of parallels with the Massif Central of France; the mountains of this area once formed part of the ancient Rodopi Massif, (Turrill, 1929, Darby (Ed.), 1944) and now consist for the most part of crystalline schists and gneisses with outcrops of granite. Further information with regard to the ecology of the second Balkan taxon (A. bornmülleri) is given by J. Bornmüller (1928) as follows: "On granite, in shaded rock fissures and narrow crevices near Markovgrad, both above Varoš and on the whole length of the mountains of the Treskavec planina at 800 - 900m., and on the way up to the monastery at Treskavec at an altitude of 1000m." Dr. Bačar (personal communication) states that

the altitudinal range of A. macedonicum is 200 - 1200m. (average 400 - 800m.), which therefore overlaps that attributed to A. bornmülleri.

There are only four collections of Balkan Aspleniums referable to either of these taxa in the herbaria examined (London and Vienna); two came from crevices of granitic rocks at Markovgrad, north-west of Prilep (coll: Alston no. 17776 (1957) and Soska s.n. (1933)) and two from the area of the Treskavec planina, also on granitic rocks (coll: Bornmüller no. 3263 (1913) and Kiril Micev s.n. (1957)).

The plants of A. macedonicum in cultivation at Leeds at the outset of the present investigation were originally raised from spores derived from a collection of Alston's (1957), although at that time the locality of origin was unknown (see below). Subsequently, in 1962, an attempt was made to collect the second endemic Balkan Asplenium, namely A. bornmülleri. A visit was made to the type locality at Prilep but without success. However, six plants were collected from the vicinity of the Treskavec planina, the locality mentioned by Bornmüller (1928). These plants in cultivation became indistinguishable from those of A. macedonicum already under investigation at Leeds. The new material was incorporated into the hybridisation programme and crosses previously made with the Alston material were repeated. A number of hybrids were obtained and these gave results which completely confirmed the previous findings. It was not until 1965, when Alston's notebooks and two dried specimens of A. macedonicum relating to his 1957 journey first became available, that the origin of the Leeds material of this species could be traced. It was found that these plants were raised originally from

spores derived from either Kiril Micev s.n. or Alston 17776, almost certainly the latter, although for reasons connected with the tragic and premature death of A. H. G. Alston in 1957 there is still some slight uncertainty which cannot now be rectified.

A more surprising discovery was that the locality corresponding to Alston's specimen no. 17776 was in fact the locus classicus of A. bornmülleri. There is however no reason to doubt the correctness of Alston's own determination of his specimen as A. macedonicum. Close comparison of the living material with Kümmerle's descriptions of A. macedonicum and A. bornmülleri has shown that the Leeds plants could in fact be referred to either taxon. There seems little doubt, therefore, that the opinion of Mayer mentioned above, to the effect that A. bornmülleri should be treated as a later synonym of A. macedonicum, is correct.

On this assumption it is possible to add some further ecological information regarding A. macedonicum. The six plants collected in 1962 were all found in sheltered crevices of granitic rocks on the east face of the Treskavec planina at an altitude of approximately 1000m. The terrain consisted for the most part of stony ground with an open herbaceous vegetation (including Euphorbia mercanites and Dianthus sp.) and extensive outcrops of bare siliceous rock. At that time of year (August) most of the vegetation had died down, giving a characteristic bare and scorched landscape and almost the only green plants to be found were the ferns, hidden deep in rock clefts which afforded shelter and slight humidity.

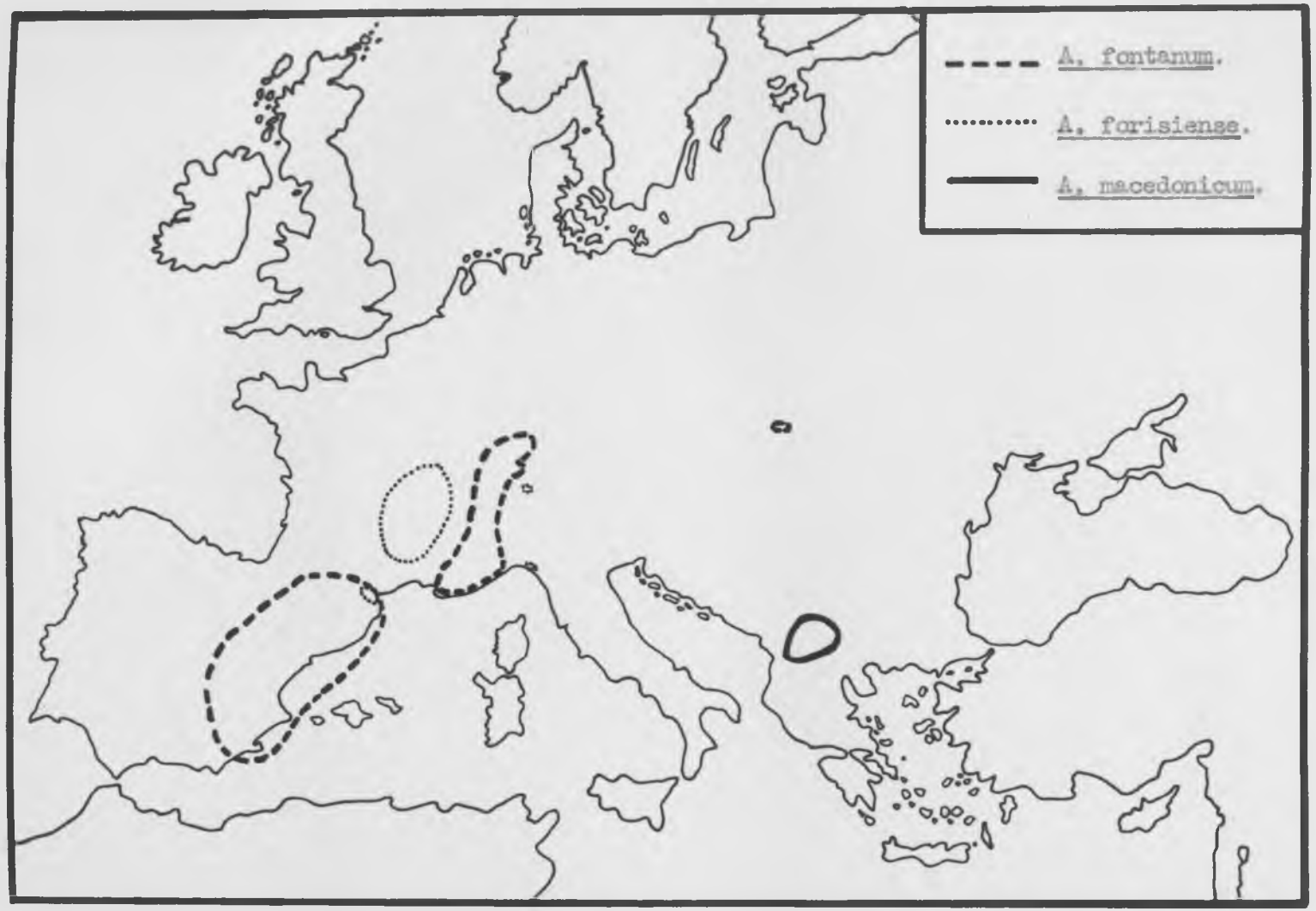


Fig. 6. Map to show the distribution of A. fontanum (2x) and the two tetraploids A. forisiense and A. macedonicum.

Fig. 7. Markovgrad, near Prilep, Macedonia. General view of the locus classicus of A. bornmülleri.

Fig. 8. A. fontanum (L.) Bernh. growing in deep shade on moss-covered limestone rocks in beech wood at Ramsfluh, Aargau, Switzerland.

Fig. 9. A. obovatum Viv. growing in shaded crevice of granitic rocks on Mt. Caruso, Ischia, Italy.
Photo: T. Reichstein.

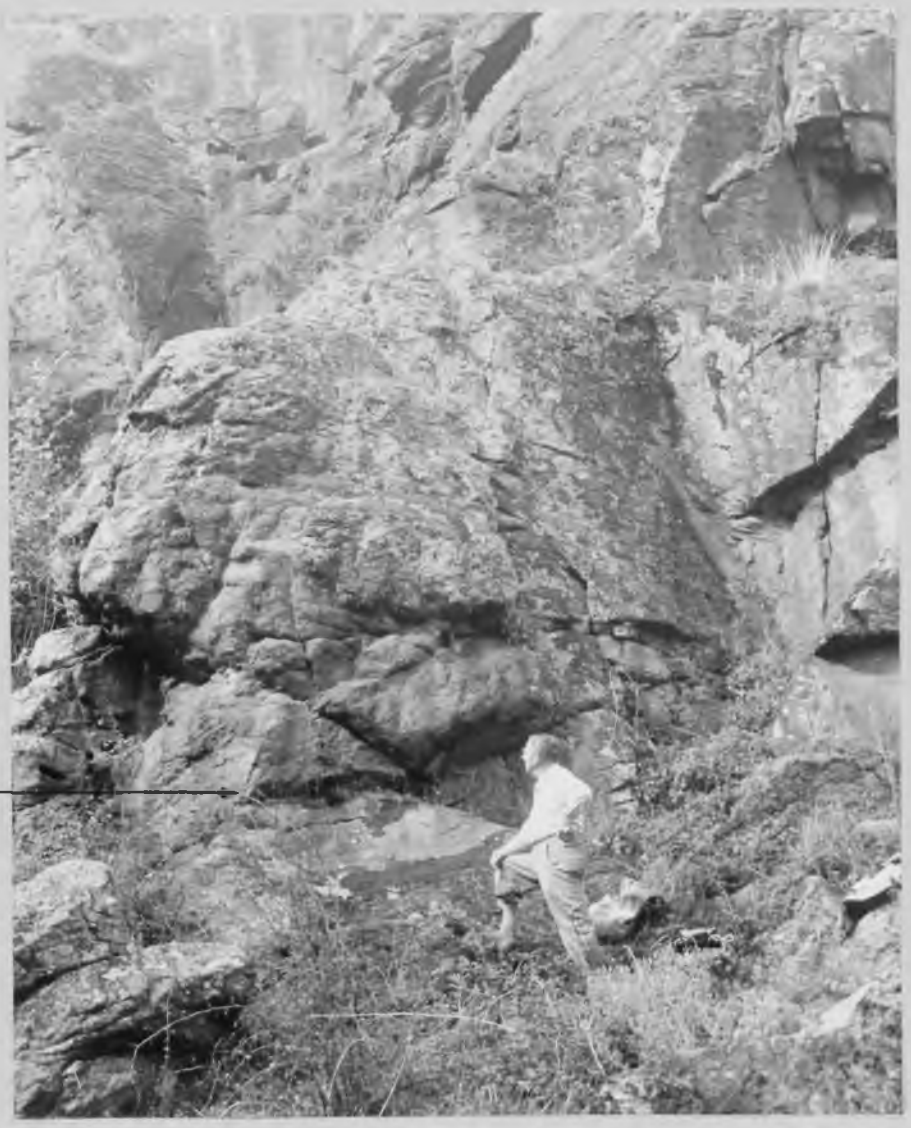


Fig. 10. Exposed south-facing outcrop of granitic rocks in the Val Vizézy, Loire, France. The plants of A. forisiense and A. septentrionale shown in Fig. 11 are growing in the crevice marked.

Fig. 11. A. forisiense growing together with A. septentrionale in shaded crevice of granitic rock. (See also Fig. 10).

Fig. 12. Val Vizézy, west of Montbrison, Loire, France. Habitat of A. forisiense in crevices of granitic rocks.

Fig. 13. A. petrarchae. Silhouettes of cultivated fronds from tetraploid specimens originating from two localities in the south of France (Bouches-du-Rhône: A and B) and from Croatia (Karlobak: C). Natural size.

Fig. 14. Natural-size silhouettes of A. majoricum.

Unmarked fronds from plants in cultivation at Leeds, originally collected from Barranc, near Sóller, Mallorca.

Wild fronds (w) collected from Chemin Coma de S'Arron, near Sóller, Mallorca. September, 1965.

5. A. MAJORICUM Litard. (Fig. 14).

This fern was first described by de Litardière (1911) from walls near the town of Sóller, Mallorca. It's general resemblance to A. fontanum was pointed out, the main distinguishing feature being the dark coloration of the petiole and underside of the rhachis in A. majoricum. This fern was mentioned in Flora Europaea (1964) but was omitted from the key. Subsequently a full description was published by Jermy & Lovis (1964) together with a key for distinguishing it from other European Aspleniums.

Distribution: A. majoricum is endemic to the island of Mallorca where it is found only in certain localities in the mountains bordering the west coast. It grows in several places close to the town of Sóller at altitudes of 100 - 300m. and at Teix, south-west of Sóller, at 530m. Further north, at Puig Roig (close to the tourist resort of Torrent de Pareis) A. majoricum reaches altitudinal levels of 500 - 700m.

Ecology: In the original description (de Litardière, 1911) the only ecological information is "Mallorca, walls near the town of Sóller, with A. trichomanes L. and Ceterach officinarum DC.". This can be amplified following a personal visit to several localities in the vicinity of Sóller under the guidance of a local botanist, Sr. J. Orell. A. majoricum occurs on the north-exposed side of dry limestone walls bounding the terraces of olive groves, rarely on rock (see habitat photographs on pages 71 to 72). Always in shade, it is often found in

deep pockets of red base-rich soil between the stones. It is accompanied by much A. trichomanes L., and by Ceterach officinarum DC., Selaginella denticulata (L.) Link and Sedum dasyphyllum L. Polypodium australe Fée is also present on the same walls. In one locality south-east of Sóller A. majoricum was associated with A. petrarchae (Guérin) DC. It is interesting to note that in all these localities the rock is Jurassic limestone. Other types of limestone present in Mallorca (Triassic and Miocene) do not appear to harbour this species (Orell, 1965, personal communication). Like many other Mediterranean ferns A. majoricum dies down completely in the summer months. New fronds make their appearance at the end of September and active growth continues during the winter and early spring. A succession of fertile fronds is produced throughout the growing season.

6. A. PETRARCHAE (Guérin) DC. (= A. glandulosum Lois.) (Fig. 13).

This species appears first under the name of Polypodium petrarchae in a rare work (Guérin, 1804) on the vegetation of the Fontaine de Vaucluse near Avignon, a very brief description of the plant appearing as a footnote "Polypodium frondibus pinnatis, pinnis sub-pinnatifidis, foliis petiolisque ciliatis, serratis. Habitat in speluncis vallis-clausae". The second edition of this work (1813) is more readily available, and in this publication the name was included but the description omitted. A more detailed description of the same species under the name of A. glandulosum (Lois., 1810) was therefore

for long thought to be the earliest valid publication in spite of the fact that de Candolle (1815) had transferred Guérin's species into the genus Asplenium as A. petrarchae. The name petrarchae was however validly published by Guérin in 1804 (Lanjouw, 1961) and so antedates glandulosum as a specific epithet by six years.

This species has been adequately described in a number of floras, and for a concise description the reader is referred to Flora Europaea (1964).

Distribution: This species has a disjunct Mediterranean distribution which is shown on the map on page 73 (redrawn partly from Giacomini, in Fiori, 1943). It is recorded from the following countries: France (Alpes-Maritimes, Var, Basses-Alpes, Bouches-du-Rhône, Vaucluse, Hérault and Aude), Spain (from the Pyrenees to the south coast), Italy, Sicily, Algeria, Greece, Albania and Jugoslavia (Croatia, where it is tolerably rare and difficult to find). Plants are in cultivation from several localities from the south of France, southern Spain, Mallorca and from Croatia (Karlobak).

Ecology: A. petrarchae is found on limestone rocks and is never far from the sea. It is generally found at altitudes up to 700 or 800m., but in the Pyrenees it can reach an altitudinal level of 1200m.

Fig. 15.

General view of Biniaralx, near Sóller, Mallorca. Note the terraced olive groves in the foreground.

Fig. 16.

Terraced olive groves in limestone terrain south-east of Sóller.

Fig. 17. Habitat of A. majoricum Litard. on limestone walls bounding olive terraces. Near Sóller, Mallorca.

Fig. 18. A. majoricum growing in crevice on limestone wall accompanied by A. trichomanes and Polypodium australe. Photo: T. Reichstein.

Fig. 19. Map to show the distribution of A. petrarchae (4x) and A. majoricum (4x).

CHAPTER IV. THE HYBRIDS.

SECTION A. THE HYBRIDISATION PROGRAMME.

The species used in the hybridisation programme are enumerated in Table II (see page 26). Crosses were attempted between these taxa in the combinations shown in Table V, below. Unsuccessful hybridisation attempts are represented by X and successfully produced hybrids by H. The numerical data relating to each hybridisation attempt are given as an appendix (see page 231). Synthesised hybrids are listed in Table VI.

TABLE V.

♀		♂ Ft. 2x	Ob. 2x	On. 2x	C 2x	J 2x	T 2x	Mc. 4x	Fr. 4x	Mj. 4x	P 4x	B 4x
A. fontanum	2x		H	X	X		X	H	H		X	X
A. obovatum	2x	X						H	H		X	H
A. onopteris	2x	X	X					H	H	H	X	H
A. cuneifolium	2x	X						X	X			
A. jahandiezii	2x	X						X	X			X
A. viride	2x	X						X	X	H	X	
A. trichomanes*	2x	X						X	X	X	X	
A. macedonicum	4x	H	H		X		X		H		X	H
A. forisiense	4x	H	H	X	X		X	H			X	X
A. majoricum	4x	H	H	X		X	X	H	H		X	H
A. petrarchae	4x	H	X		X			X	X			X
A. billotii	4x	X	H	X				H	H	X	H	
A. pseudo-fontanum	?	X						X	X			

* The sub-species used was A. trichomanes ssp. trichomanes.

TABLE VI. SYNTHESISED HYBRIDS.

	PLOIDY.	HYBRID.			NO. OF PLANTS.
	(3x)	A. fontanum	x	A. obovatum *	2
R	3x	A. obovatum	x	A. forisiense *	39
R	3x	A. obovatum	x	A. macedonicum *	38
R	3x	A. fontanum	x	A. forisiense *	24
R	3x	A. fontanum	x	A. macedonicum *	70
	3x	A. onopteris	x	A. forisiense *	22
	3x	A. onopteris	x	A. macedonicum *	2
	3x	A. onopteris	x	A. majoricum *	2
	3x	A. viride	x	A. majoricum *	1
	3x	A. majoricum	x	A. fontanum *	7
	3x	A. petrarchae	x	A. fontanum *	1
	3x	A. onopteris	x	A. billotii	1
R	3x	A. obovatum	x	A. billotii	3
R	4x	A. forisiense	x	A. macedonicum *	6
	4x	A. majoricum	x	A. forisiense *	2
	4x	A. majoricum	x	A. macedonicum *	9
	(4x)	A. majoricum	x	A. obovatum *	1
	4x	A. majoricum	x	A. billotii *	1
	4x	A. billotii	x	A. petrarchae *	2
	4x	A. billotii	x	A. forisiense	4
R	4x	A. billotii	x	A. macedonicum	14

R indicates that hybrids of the reciprocal cross were also obtained.

( ) indicates the presumed formation of a doubled gamete.

All hybrid combinations marked by an asterisk have been examined cytologically and these, together with the respective parents, are illustrated by silhouettes on pages 83 to 116. The cytology of each hybrid will be found on the corresponding facing page. Wherever possible meiotic analyses were made from at least two different plants of any one combination.

SECTION B. RESULTS OBTAINED.

In the following section details of each type of hybrid successfully raised are given together with an analysis of meiosis. A minimum of six cells were generally examined, sometimes more if the material was particularly suitable. If available more than one plant of each combination was examined and wherever possible hybrids involving different parental stocks of the species concerned were tested.

Photographs of cells at meiosis, each accompanied by an explanatory diagram, are illustrated in Figs. 20 to 41 (pages 84 to 117).

In the following tables individual localities are referred to by a single place name and for clarity these are underlined in the list of Materials on pages 26 and 28. In the presentation of the results of the hybridisation experiments, figures are given of the numbers of female prothalli used (P), sporelings obtained (Sp), self-fertilised plants produced (Se) and the number of sporophytes which died before their identity could be established (D). Successfully produced hybrids are represented by H and morphological hybrids not yet examined cytologically are listed under Unc. (unconfirmed). The figure given for the percentage success is calculated from the ratio: number of female prothalli used : number of hybrids produced. The procedure is standard throughout the thesis from this point onwards.

1. HYBRIDS WITHIN THE A. FORISIENSE COMPLEX.

Within this group the emphasis has naturally been on the production of triploid hybrids, as outlined in the introduction. However, crosses were also attempted between A. fontanum and the other diploid species available, in order to demonstrate whether or not any chromosome homology was present at the diploid level. Complete failure of pairing in such a hybrid could generally be taken as demonstrating the lack of any such homology. In spite of a large number of hybridisation attempts no such hybrids were obtained. Two plants listed in Table 1, column 5, below, were putative hybrids of A. fontanum (2x) x A. obovatum (2x). Both proved, surprisingly, to be triploid and not diploid as expected.

Details of each hybrid synthesised are given below:

I. DIPLOID : DIPLOID CROSSES.

1. A. fontanum (2x) x A. obovatum (2x).

♀				P	Sp.	Se.	D	H	Unc.
	Isère	x	Ischia	53	0	0	0	0	0
	Roche	x	Corsica	15	5	5	0	0	0
	Valais	x	Sardinia	27	2	2	0	0	0
	Isère	x	Sardinia	50	11	10	1	0	0
	Ariège	x	Sardinia	239	39	37	0	2	0
R	Sardinia	x	Roche	315	101	99	2	0	0
				699	158	153	3	2	0

Percentage success: 0.29%

Analysis of Meiosis.

Two triploid hybrids were obtained. Meiotic plates from both plants regularly showed 36 bivalent and 36 univalent chromosomes. The most likely explanation to account for the production of triploid hybrids instead of the expected diploids is to postulate the formation of an unreduced gamete by one parent. However, the possibility of a chance fertilisation by a gamete coming from a stray prothallus of either A. macedonicum or A. forisiense cannot be completely excluded. For this reason the results from these two plants will not be considered further.

II. DIPLOID : TETRAPLOID CROSSES.

2. A. fontanum (2x) x A. macedonicum (4x). Triploid hybrid.

♀			P	Sp.	Se.	D	H	Unc.
Isère	x	Markovgrad	51	18	8	0	10	0
Roche	x	Markovgrad	78	14	5	0	9	0
Val di Llo	x	Markovgrad	9	1	0	0	1	0
Ariège	x	Markovgrad	68	10	7	0	3	0
Ariège	x	Treskavec	21	6	0	1	5	0
Fort d'Ecluse	x	Treskavec	43	22	8	1	13	0
R Treskavec	x	Isère	20	8	2	2	4	0
R Markovgrad	x	Isère	76	27	13	0	14	0
R Markovgrad	x	Roche	47	15	7	0	8	0
R Markovgrad	x	Ariège	38	10	7	0	3	0
			451	131	57	4	70	0

Percentage success : 15.5%

Analysis of Meiosis. (Fig. 20, p. 84).

Meiosis was examined from a representative selection of the hybrids obtained, and in all, 36 bivalent and 36 univalent chromosomes were regularly seen.

3. A. fontanum (2x) x A. forisiense (4x). Triploid hybrid.

♀				P	Sp.	Se.	D	H	Unc.
	Ariège	x	Auvergne	20	10	7	0	3	0
	Val di Llo	x	Auvergne	11	7	4	0	3	0
	Roche	x	Auvergne	10	4	4	0	0	0
	Isère	x	Auvergne	39	5	1	0	4	0
	Isère	x	Cevennes	9	3	1	0	2	0
	Isère	x	Brissago	28	9	5	1	3	0
	Roche	x	Brissago	33	1	1	0	0	0
R	Brissago	x	Roche	34	5	5	0	0	0
R	Brissago	x	Isère	41	15	8	0	7	0
R	Brissago	x	Ariège	33	5	3	0	2	0
				258	64	39	1	24	0

Percentage success : 9.3%

Analysis of Meiosis. (Fig. 21, p. 86).

36 bivalent and 36 univalent chromosomes were regularly seen in all hybrids examined.

4. A. obovatum (2x) x A. macedonicum (4x). Triploid hybrid.

	♀			P	Sp.	Se.	D	H	Unc.
	Sardinia	x	Markovgrad	237	129	89	6	34	0
R	Markovgrad	x	Sardinia	81	8	3	3	2	0
R	Markovgrad	x	Corsica	45	8	7	1	0	0
R	Markovgrad	x	Ischia	14	2	2	0	0	0
R	Treskavec	x	Corsica	16	3	2	0	1	0
R	Treskavec	x	Sardinia	40	5	3	1	1	0
				433	155	106	11	38	0

Percentage success : 8.78%

Analysis of Meiosis. (Fig. 22, p. 88).

36 bivalent and 36 univalent chromosomes were regularly seen in all hybrids examined.

5. A. obovatum (2x) x A. forisiense (4x). Triploid hybrid.

	♀			P	Sp.	Se.	D	H	Unc.
	Sardinia	x	Auvergne	112	68	31	0	37	0
R	Auvergne	x	Sardinia	12	0	0	0	0	0
R	Brissago	x	Sardinia	52	9	7	2	0	0
R	Cevennes	x	Sardinia	19	3	3	0	0	0
R	Cevennes	x	Corsica	54	2	2	0	0	0
R	Brissago	x	Ischia	11	1	0	1	0	0
R	Brissago	x	Corsica	14	7	4	1	2	0
				274	90	47	4	39	0

Percentage success : 14.2%

Analysis of Meiosis. (Fig. 23, p. 90).

36 bivalent and 36 univalent chromosomes were regularly seen in the selection of hybrids examined.

6. A. onopteris (2x) x A. macedonicum (4x). Triploid hybrid.

♀			P	Sp.	Se.	D	H	Unc.
Sardinia	x	Markovgrad	76	15	13	0	1	1
Sardinia	x	Treskavec	31	5	4	1	0	0
			107	20	17	1	1	1

Percentage success : 1.87%

Analysis of Meiosis. (Fig. 26, p. 93).

One plant only has so far become fertile. Meiotic squashes performed at different seasons invariably showed complete failure of pairing, i.e. 108 univalent chromosomes.

7. A. onopteris (2x) x A. forisiense (4x). Triploid hybrid.

♀			P	Sp.	Se.	D	H	Unc.
Sardinia	x	Auvergne	18	0	0	0	0	0
Sardinia	x	Brissago	140	36	7	7	22	0
R Brissago	x	Sardinia	10	0	0	0	0	0
			168	36	7	7	22	0

Percentage success : 15.0%

Analysis of Meiosis. (Fig. 27, p. 94).

108 univalent chromosomes were regularly seen in all hybrids examined.

III. TETRAPLOID : TETRAPLOID CROSSES.

8. A. forisiense (4x) x A. macedonicum (4x). Tetraploid hybrid.

	♀			P	Sp.	Se.	D	H	Unc.
	Brissago	x	Markovgrad	18	7	0	3	4	0
R	Markovgrad	x	Penchot	11	0	0	0	0	0
R	Markovgrad	x	Auvergne	9	7	2	3	2	0
				38	14	2	6	6	0

Percentage success : 15.8%

Analysis of Meiosis. (Fig. 31, p. 96).

The two parental species are so alike in their morphology that it is only with the greatest difficulty that hybrids may be distinguished from self-fertilised plants. Of the eight plants that were raised to maturity all produced normal-looking well-filled spores. Meiotic pairing in these plants was completely regular, 72 bivalents being found throughout. No evidence of univalent formation was seen. The cytology was thus of no help in the confirmation of possible hybridity but from a close study of the morphology of these plants it is suggested that six of them had resulted from cross-fertilisations.

An attempt to demonstrate segregation by raising an F_2 generation failed as a result of accidental circumstances (fungus attack), and time did not permit repetition of the experiment.

Fig. 20. Frond silhouettes of the synthesised hybrid A. fontanum x A. macedonicum and it s parents. Natural size.

A. A. macedonicum (4x). Markovgrad, Macedonia. ♂ parent.

B. AS/322. Synthesised hybrid A. fontanum x A. macedonicum in it s second year of growth.

C. A. fontanum (2x). Roche, Switzerland. ♀ parent.

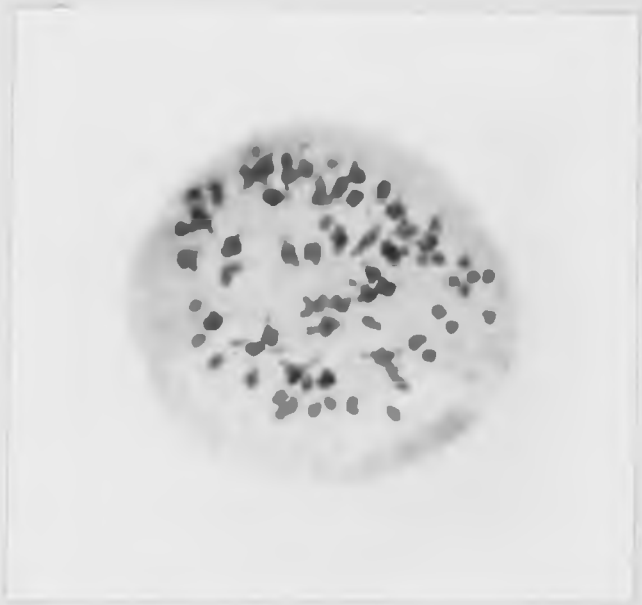


Fig. 20a. Meiosis in triploid hybrid A. fontanum 2x (Roche) x A. macedonicum 4x (Markovgrad). Magnification x 1500.

Fig. 20b. Explanatory diagram to Fig. 20a showing 36 bivalents (black) and 36 univalents (in outline). Magnification x 2000.

Fig. 21. Frond silhouettes of the synthesised hybrid A. fontanum x A. forisiense and it s parents. Natural size.

A. A. forisiense (4x). Auvergne, France. ♂ parent.

B. AS/281. Synthesised hybrid A. fontanum x A. forisiense in it s second year of growth.

C. A. fontanum (2x). Isère, France. ♀ parent.

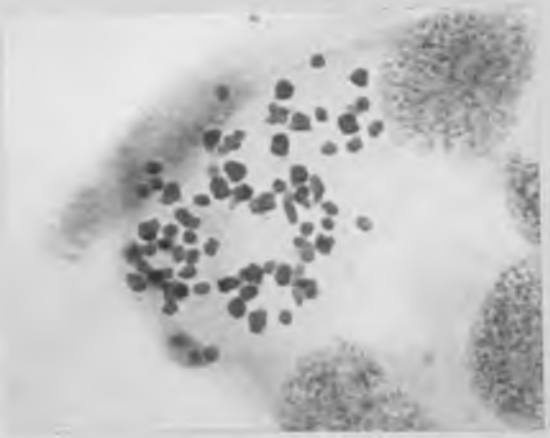


Fig. 21a. Meiosis in triploid hybrid A. fontanum 2x (Isère) x A. forisiense 4x (Auvergne). Magnification x 1500.

Fig. 21b. Explanatory diagram to Fig. 21a showing 36 bivalents (black) and 36 univalents (in outline). Magnification x 1500.

Fig. 22. Frond silhouettes of the synthesised hybrid A. obovatum x A. macedonicum and it s parents. Natural size.

A. A. obovatum (2x). Capo di Testa, Sardinia. ♀ parent.

B. AS/899. Synthesised hybrid A. obovatum x A. macedonicum in it s second year of growth.

C. A. macedonicum (4x). Markovgrad, Macedonia. ♂ parent.

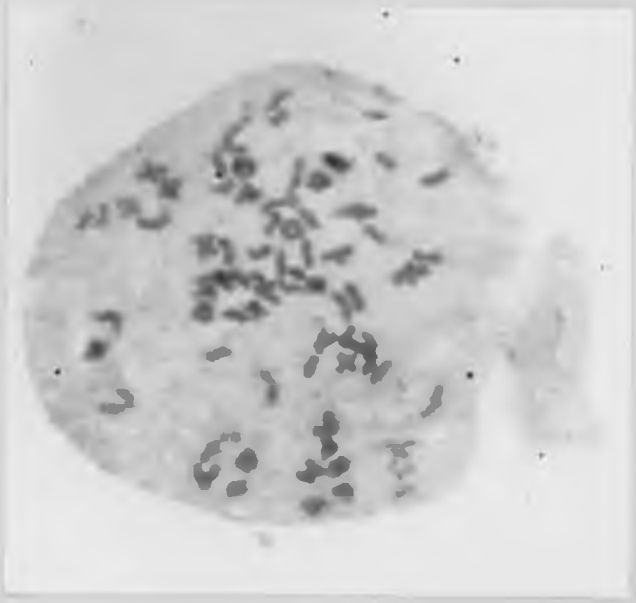


Fig. 22a. Meiosis in triploid hybrid A. obovatum 2x (Sardinia) x A. macedonicum 4x (Markovgrad). Magnification x 1500.

Fig. 22b. Explanatory diagram to Fig. 22a showing 36 bivalents (black) and 36 univalents (in outline). Magnification x 2000.

Fig. 23. Frond silhouettes of the synthesised hybrid A. obovatum x A. forisiense and it s parents. Natural size.

A. A. obovatum (2x). Capo di Testa, Sardinia. ♀ parent.

B. AS/890. Synthesised hybrid A. obovatum x A. forisiense in it s second year of growth.

C. A. forisiense (4x). Auvergne, France. ♂ parent.

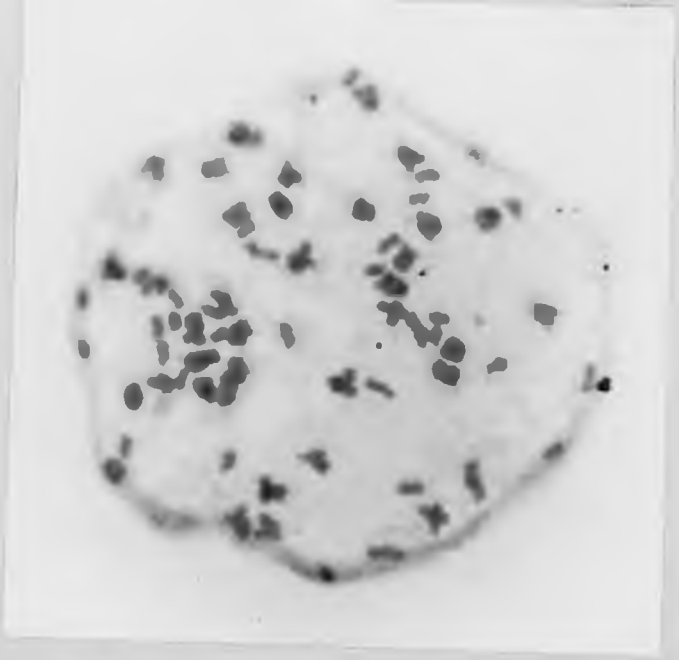


Fig. 23a. Meiosis in triploid hybrid A. obovatum 2x (Sardinia) x A. forisiense 4x (Auvergne). Magnification x 1500.

Fig. 23b. Explanatory diagram to Fig. 23a showing 36 bivalents (black) and 36 univalents (in outline). Magnification x 2000.

Fig. 24. Silhouette of cultivated frond of A. onopteris, a diploid species from Aritzo, Sardinia, Italy. Natural size.

Fig. 25. Frond silhouettes of the two synthesised triploid hybrids involving A. onopteris with the two tetraploids A. macedonicum and A. forisiense respectively. (See also Figs. 5a and 4a). Natural size.

A. AS/319. ♀ A. onopteris (2x, Sardinia) x A. macedonicum (4x, Markovgrad) in it s third year of growth.

B. AS/847. ♀ A. onopteris (2x, Sardinia) x A. forisiense (4x, Brissago, Switzerland) in it s second year of growth.

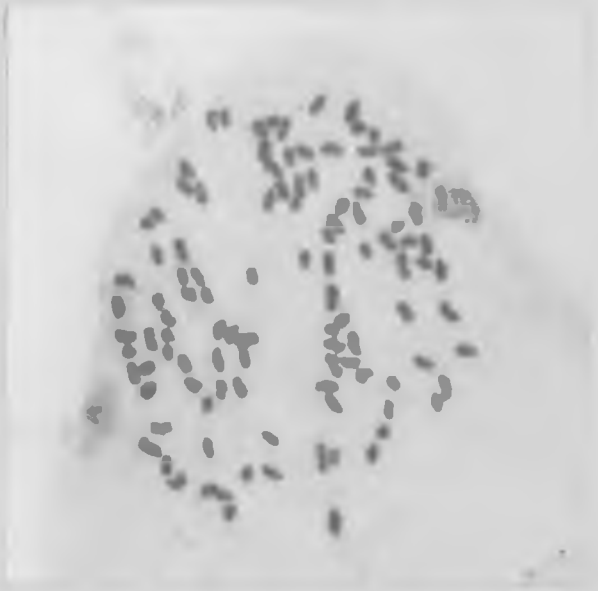


Fig. 26. Meiosis in triploid hybrid A. onopteris 2x (Sardinia) x A. macedonicum 4x (Markovgrad). Magnification x 1500.

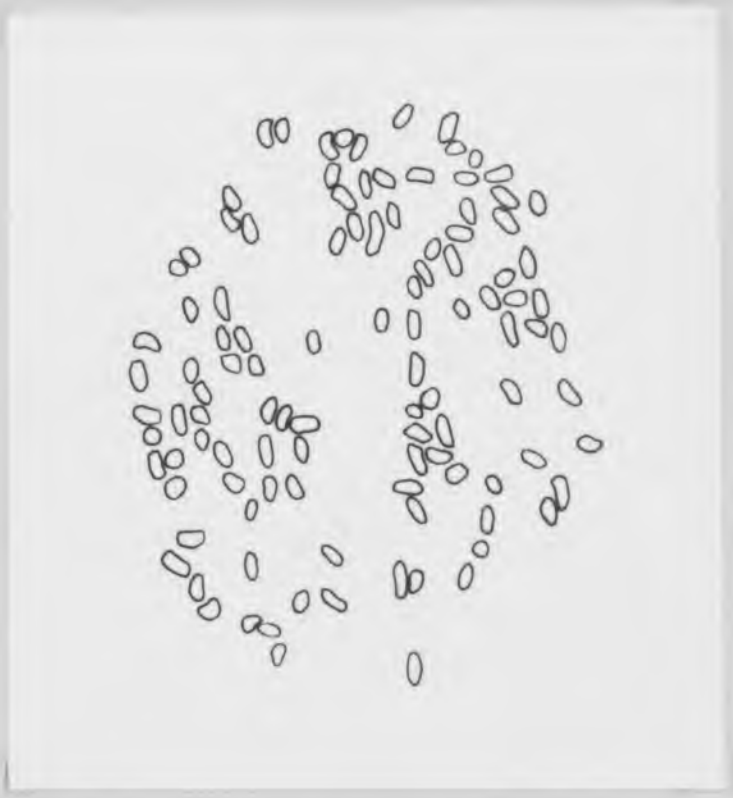


Fig. 26a. Explanatory diagram to Fig. 26 showing 108 univalent chromosomes. Magnification x 2000.

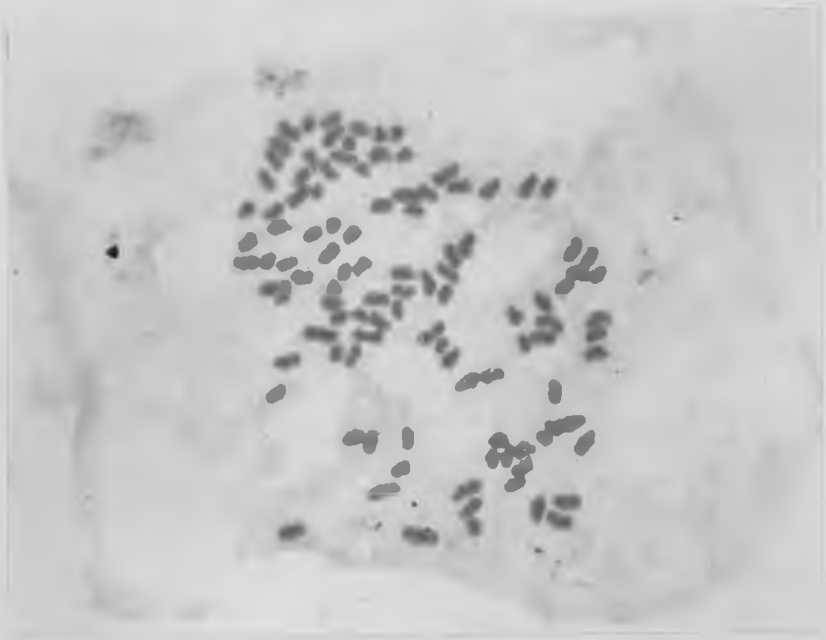


Fig. 27. Meiosis in triploid hybrid A. onopteris 2x (Sardinia) x A. forisiense 4x (Brissago). Magnification x 1500.

Fig. 27a. Explanatory diagram to Fig. 27 showing 108 univalent chromosomes. Magnification x 2000.

Fig. 28. Frond silhouettes of the synthesised hybrid A. macedonicum x A. forisiense and it s parents. Natural size.

A. A. forisiense (4x). Auvergne, France. ♂ parent.

B. AS/230. Two fronds of the synthesised hybrid A. macedonicum x A. forisiense in it s third year of growth.

C. A. macedonicum (4x). Markovgrad, Macedonia. ♀ parent.

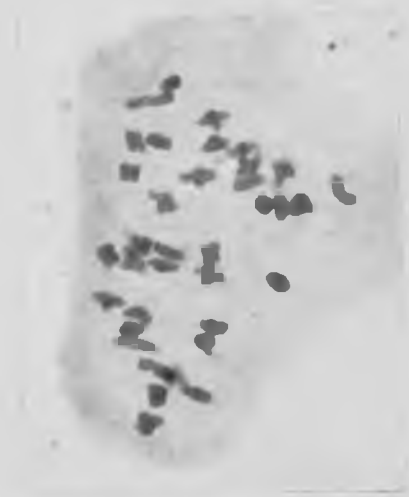


Fig. 29.

Meiosis in A. fontanum (L.) Bernh. from Roche, Switzerland, showing 36 bivalent chromosomes. Magnification x 1200.

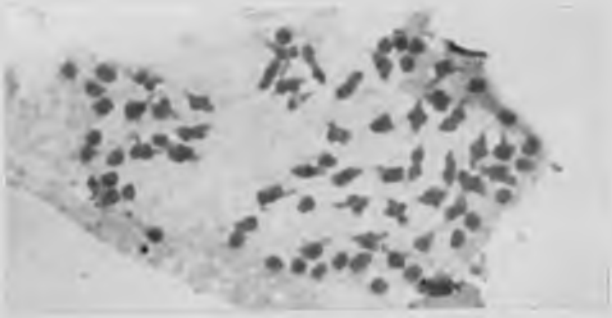


Fig. 30.

Meiosis in A. macedonicum Kümmerle from Prilep, Macedonia, showing 72 bivalent chromosomes. Magnification x 1200.

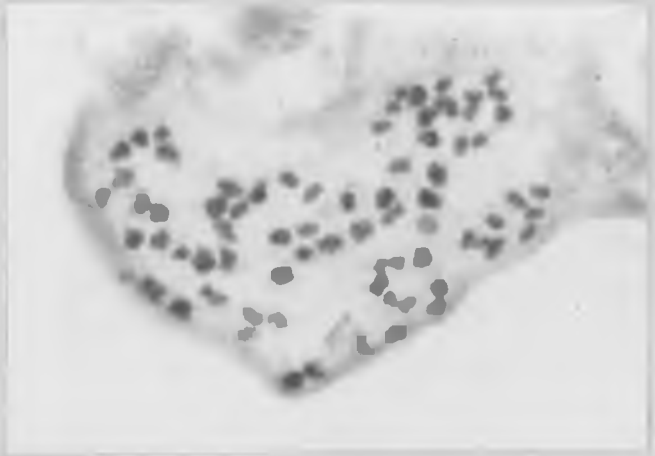


Fig. 31.

Meiosis in synthesised tetraploid hybrid A. macedonicum 4x (Markovgrad) x A. forisiense 4x (Auvergne) showing 72 bivalent chromosomes.
Magnification x 1200.

2. HYBRIDS INVOLVING A. MAJORICUM (4x).

Crosses were made between this species and a number of diploid species of Asplenium in the hope that an analysis of chromosome pairing in any resultant hybrids would shed light on the genetical affinities of this interesting plant. Crosses were also made between A. majoricum and tetraploid species of known (or suspected) affinity.

I. DIPLOID : TETRAPLOID CROSSES.

9. A. majoricum (4x) x A. fontanum (2x). Triploid hybrid.

♀			P	Sp.	Se.	D	H	Unc.
Sóller	x	Isère	24	13	1	6	6	0
Sóller	x	Roche	70	11	8	2	1	0
Sóller	x	Fort d'Ecluse	15	5	1	4	0	0
			109	29	10	12	7	0

Percentage success : 6.42%

Analysis of Meiosis. (Fig. 32, p.102).

36 bivalents and 36 univalents were regularly seen. The same results were obtained from hybrids having the male parent from a different locality.

10. A. onopteris (2x) x A. majoricum (4x). Triploid hybrid.

♀			P	Sp.	Se.	D	H	Unc.
Sardinia	x	Sóller	26	9	7	0	1	1
R Sóller	x	Sardinia	44	4	4	0	0	0
			70	13	11	0	1	1

Percentage success : 2.86%

Analysis of Meiosis. (Fig. 33, p.104).

One hybrid has so far become fertile. Fixations taken on different occasions invariably gave the same result : namely 108 univalent chromosomes.

11. A. viride (2x) x A. majoricum (4x). Triploid hybrid.

♀			P	Sp.	Se.	D	H	Unc.
Saalbach	x	Söller	12	0	0	0	0	0
Montreux	x	Söller	40	3	2	1	0	0
Hutton Roof	x	Söller	213	6	3	2	1	0
			265	9	5	3	1	0

Percentage success : 0.38%

Analysis of Meiosis. (Fig. 34, p.106).

This hybrid showed complete failure of pairing, 108 univalents being regularly seen.

12. A. majoricum (4x) x A. obovatum (2x).

♀			P	Sp.	Se.	D	H	Unc.
Söller	x	Sardinia	390	8	7	0	1	0
Söller	x	Corsica	24	5	3	2	0	0
			414	13	10	2	1	0

Percentage success : 0.24%

Analysis of Meiosis. (Fig. 35, p.108).

One hybrid was obtained after repeated hybridisation attempts,

and this proved, surprisingly, to be tetraploid instead of triploid. At meiosis 36 bivalents and 72 univalents were regularly seen, and this suggested the possibility that the male parent, A. obovatum, had produced a gamete with an unreduced chromosome number. This result can usefully be compared with the situation discussed on page 78, above, the morphology in this instance being somewhat more informative. (see page 107).

II. TETRAPLOID : TETRAPLOID CROSSES.

13. A. majoricum (4x) x A. macedonicum (4x). Tetraploid hybrid.

♀			P	Sp.	Se.	D	H	Unc.
Söller	x	Markovgrad	123	16	7	0	9	0
			—	—	—	—	—	—

Percentage success : 7.32%

Analysis of Meiosis. (Fig. 37, p. 110).

36 bivalent, and 72 univalent chromosomes were regularly seen.

14. A. majoricum (4x) x A. forisiense (4x). Tetraploid hybrid.

♀			P	Sp.	Se.	D	H	Unc.
Söller	x	Brissago	9	2	0	0	2	0
			—	—	—	—	—	—

Percentage success : 22.2%

Analysis of Meiosis. (Fig. 38, p. 110).

36 bivalents and 72 univalents were regularly seen.

15. A. majoricum (4x) x A. billotii (4x). Tetraploid hybrid.

♀			P	Sp.	Se.	D	H	Unc.
	Sóller	x Bosa	14	0	0	0	0	0
	Sóller	x Ronco	349	10	7	2	1	0
R	Ronco	x Sóller	37	24	24	0	0	0
			400	34	31	2	1	0

Percentage success : 0.25%

Analysis of Meiosis. (Fig. 39, p. 112).

Contrary to expectation this hybrid did not show complete failure of pairing. 34 - 36 bivalents were regularly seen in the cells examined, and the particularly fine cell figured on page 112 shows unequivocally 36 bivalents and 72 univalents. The implication of this result will be discussed on page 131.

Other crosses made in an attempt to elucidate the further relationships of this species were:
A. trichomanes (2x) x A. majoricum (4x), A. trichomanes (2x) x A. fontanum (2x) and A. viride (2x) x A. fontanum (2x). (See pages 126 and 231). These attempts were, however, completely unsuccessful.

Fig. 32. Frond silhouettes of the synthesised hybrid A. majoricum x A. fontanum and it's parents. Natural size.

A. A. majoricum (4x). Sóller, Mallorca. ♀ parent.

B. AS/256. Synthesised hybrid A. majoricum x A. fontanum in it's second year of growth.

C. A. fontanum (2x). Isère, France. ♂ parent.

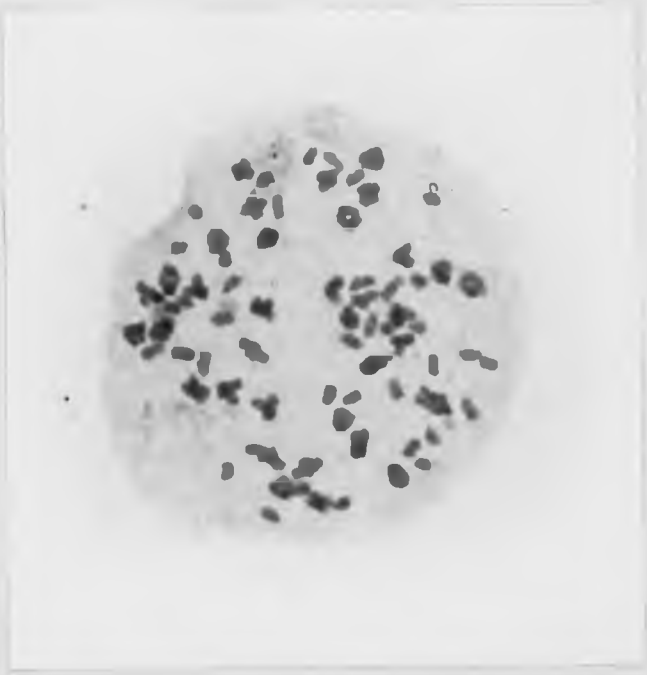


Fig. 32a. Meiosis in triploid hybrid A. majoricum 4x (Sóller) x A. fontanum 2x (Isère). Magnification x 1500.

Fig. 32b. Explanatory diagram to Fig. 32a showing 36 bivalents (black) and 36 univalents (in outline). Magnification x 2000.

Fig. 33. Frond silhouettes of the synthesised hybrid A. onopteris x A. majoricum and it s parents. Natural size.

A. A. majoricum (4x). Sóller, Mallorca. ♂ parent.

B. AS/830. Synthesised hybrid A. onopteris x A. majoricum in it s second year of growth.

C. A. onopteris (2x). Aritzo, Sardinia. ♀ parent.

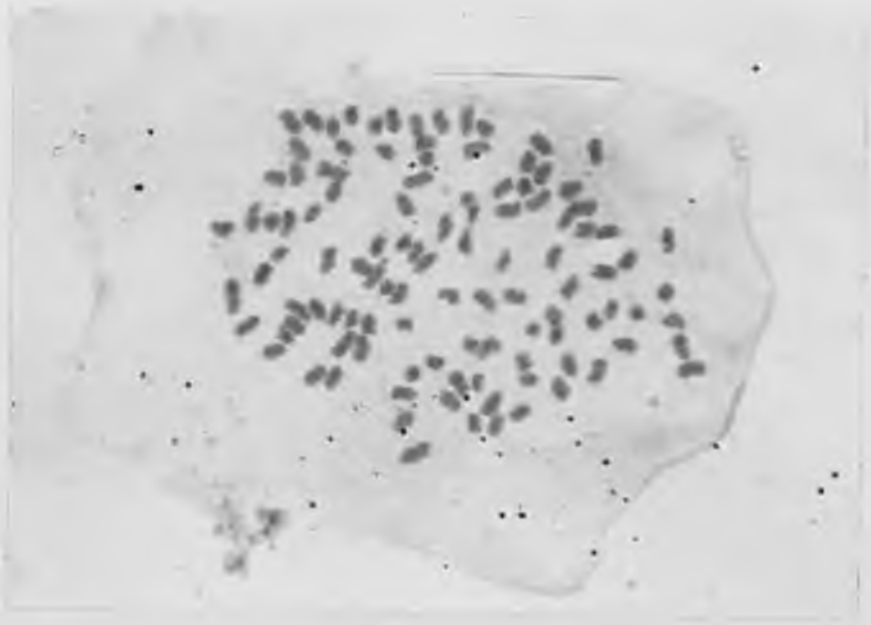


Fig. 33a. Meiosis in triploid hybrid A. onopteris 2x (Sardinia) x A. majoricum 4x (Söller). Magnification x 1500.

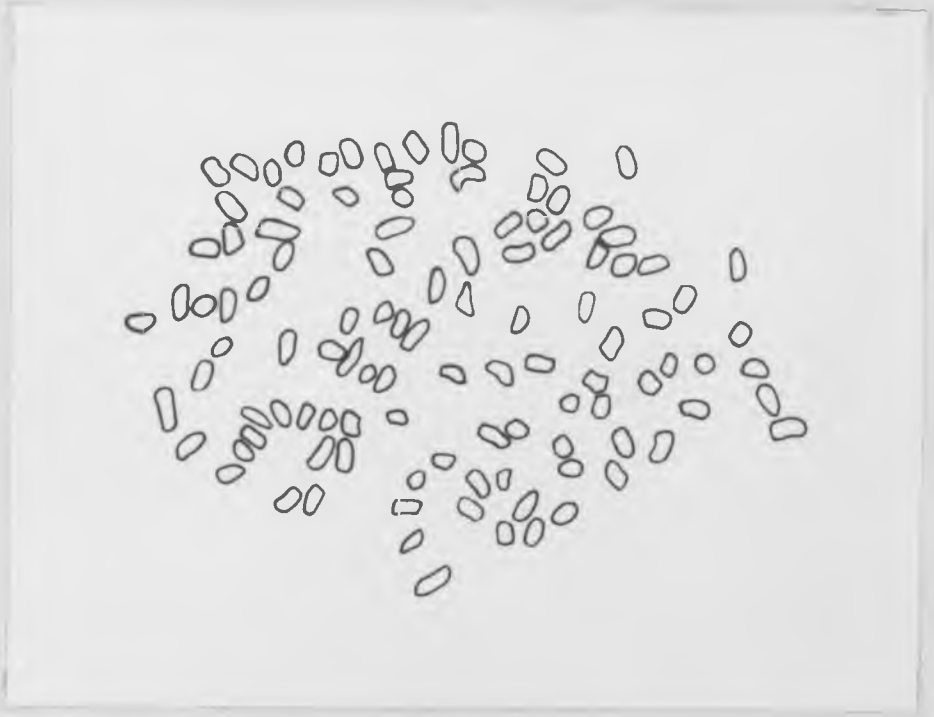


Fig. 33b. Explanatory diagram to Fig. 33a showing 108 univalent chromosomes. Magnification x 2000.

Fig. 34. Frond silhouettes of the synthesised hybrid A. viride x A. majoricum and it s parents. Natural size.

A. A. majoricum (4x). Sóller, Mallorca. ♀ parent.

B. AS/961. Synthesised hybrid A. viride x A. majoricum in it s second year of growth.

C. A. viride (2x). Hutton Roof, Westmorland. ♂ parent.

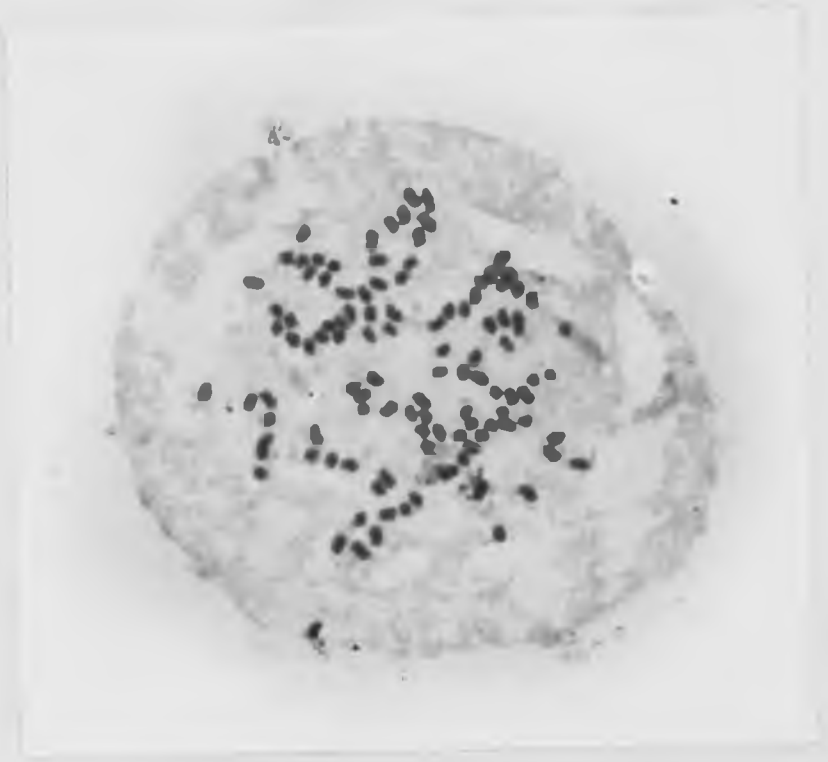


Fig. 34a. Meiosis in triploid hybrid A. viride 2x (Hutton Roof) x A. majoricum 4x (Söller). Magnification x 1500.

Fig. 34b. Explanatory diagram to Fig. 34a showing 108 univalent chromosomes. Magnification x 2000.

Fig. 35. Frond silhouettes of the synthesised hybrid A. majoricum x A. obovatum and it s parents. Natural size.

A. A. majoricum (4x). Sóller, Mallorca. ♀ parent.

B. AS/856. Synthesised hybrid A. majoricum x A. obovatum in it's second year of growth.

C. A. obovatum (2x). Capo di Testa, Sardinia. ♂ parent.

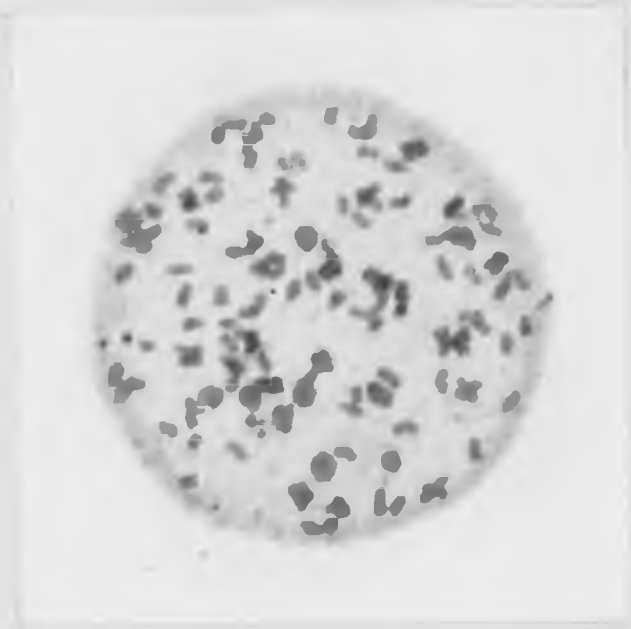


Fig. 35a. Meiosis in tetraploid hybrid A. majoricum 4x (Söller) x A. obovatum 2x (x2) (Sardinia). Magnification x 1500.

Fig. 35b. Explanatory diagram to Fig. 35a showing 36 bivalents (black) and 72 univalents (in outline). Magnification x 2000.

Fig. 36. Frond silhouettes of A. majoricum and the two synthesised tetraploid hybrids involving this species with A. macedonicum (Fig. 5a) and A. forisiense (Fig. 4a) respectively. Natural size.

A. A. majoricum (4x). Sóller, Mallorca. ♀ parent.

B. AS/681. A. majoricum x A. macedonicum (4x, Markovgrad) in it's second year of growth.

C. AS/846. A. majoricum x A. forisiense (4x, Brissago) in it's second year of growth.

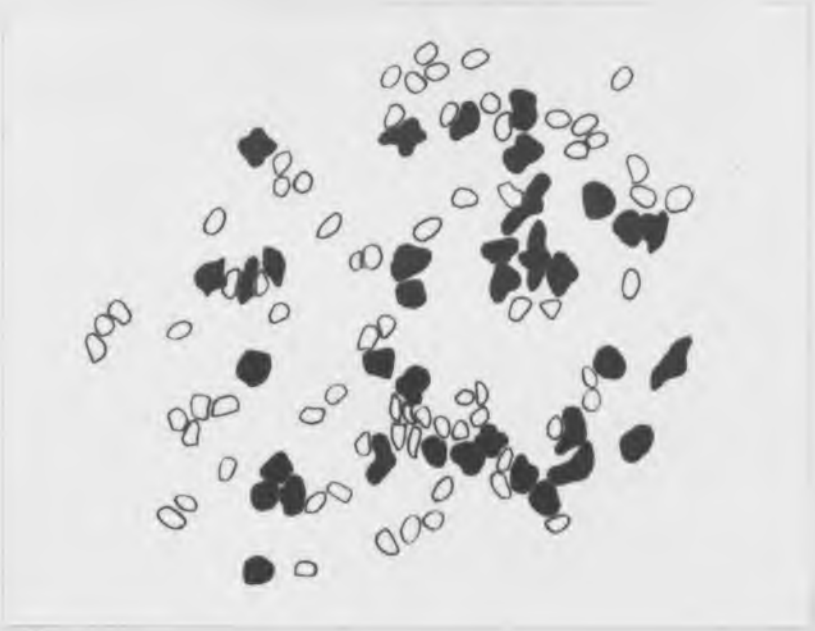


Fig. 37. Diagram of meiosis in tetraploid hybrid A. majoricum 4x (Söller) x A. macedonicum 4x (Markovgrad) showing 36 bivalents (black) and 72 univalents (in outline). Magnification x 2000.

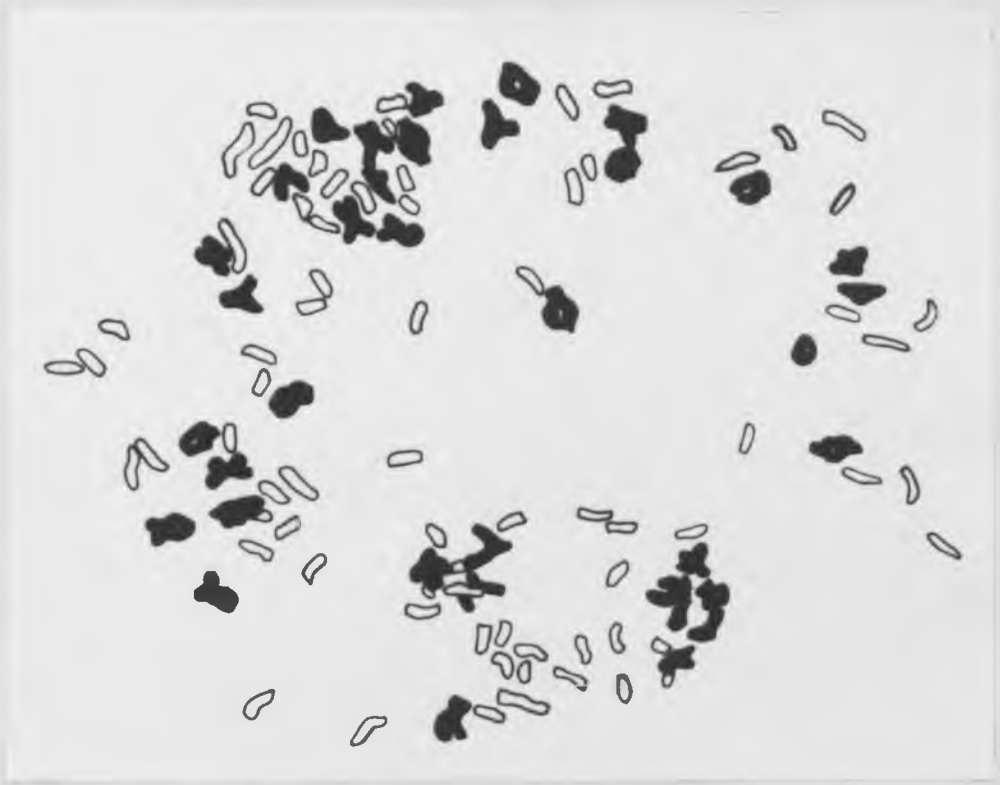


Fig. 38. Diagram of meiosis in tetraploid hybrid A. majoricum 4x (Söller) x A. forisiense 4x (Brissago) showing 36 bivalents (black) and 72 univalents (in outline). Magnification x 2000.

Fig. 39. Frond silhouettes of the synthesised hybrid A. majoricum x A. billotii and it's parents. Natural size.

A. A. majoricum (4x). Sóller, Mallorca. ♀ parent.

B. AS/985. Synthesised hybrid A. majoricum x A. billotii in it's first year of growth.

C. A. billotii (4x). Ronco, Switzerland. ♂ parent.

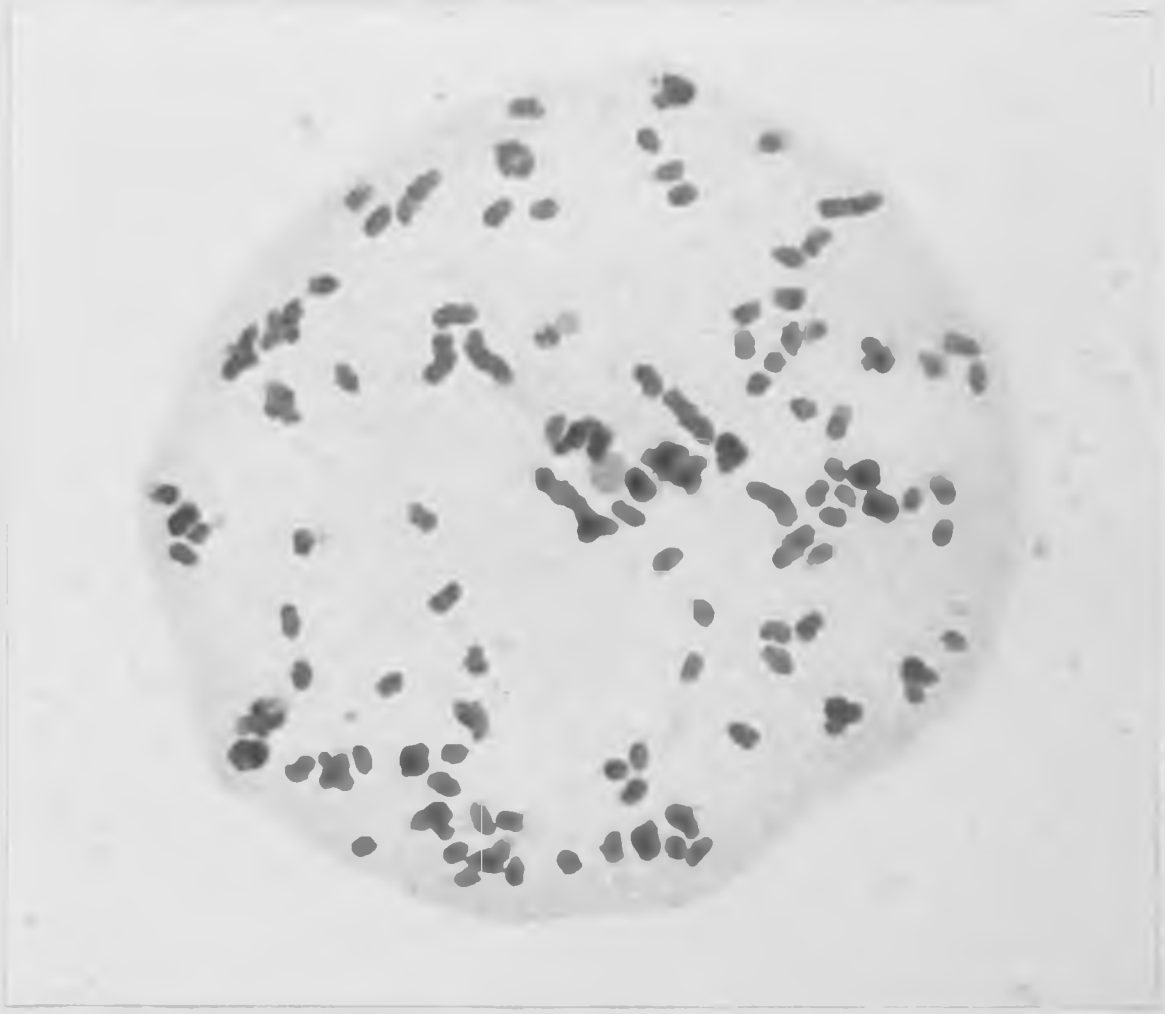


Fig. 39a. Meiosis in tetraploid hybrid A. majoricum 4x (Sóller) x A. billotii 4x (Ronco) showing 36 bivalents and 72 univalents. Magnification x 2000.

3. HYBRIDS INVOLVING A. PETRARCHAE (4x).

Two hybrid combinations have been obtained from repeated attempts to cross this tetraploid species with other Aspleniums. Other, unsuccessful, hybridisation attempts are listed in the appendix on page 231.

16. A. petrarchae (4x) x A. fontanum (2x). Triploid hybrid.

♀				P	Sp.	Se.	D	H	Unc.
	Salon	x	Roche	50	4	3	0	1	0
	Salon	x	Isère	16	0	0	0	0	0
	Salon	x	Ariège	10	0	0	0	0	0
	Karlobak	x	Ariège	18	3	2	1	0	0
R	Roche	x	Salon	12	0	0	0	0	0
R	Ariège	x	Salon	50	24	24	0	0	0
R	Waldenburg	x	Salon	12	0	0	0	0	0
R	Val di Llo	x	Salon	22	0	0	0	0	0
				190	31	29	1	1	0

Percentage success : 0.53%

Analysis of Meiosis. (Fig. 41, p. 117).

One hybrid was obtained, which unfortunately died in the summer of 1963. A photograph of this plant is reproduced on page 118. At meiosis 36 bivalents and 36 univalents were seen.

17. A. billotii (4x) x A. petrarchae (4x). Tetraploid hybrid.

♀				P	Sp.	Se.	D	H	Unc.
	Ronco	x	Salon	36	22	20	0	2	0
R	Salon	x	Ronco	50	5	3	2	0	0
				86	27	23	2	2	0

Percentage success : 2.32%

Analysis of Meiosis.

Two hybrid plants were obtained right at the end of the investigation. They were both of characteristic morphology (see silhouette on page 115) but showed a high proportion of normal-looking, well-filled spores. Upon cytological examination meiotic squashes revealed a high proportion of bivalents, the number varying from 58 to 64. Although it has not been possible to illustrate this result photographically, enough cells were seen to indicate that chromosome pairing of this order is quite typical.

The implications of this result are discussed on page 130.

Fig. 40. Frond silhouettes of the synthesised hybrid A. billotii x A. petrarchae and it's parents. Natural size.

A. A. billotii (4x). Ronco, Switzerland. ♀ parent.

B. AS/977. Synthesised hybrid A. billotii x A. petrarchae in it's first year of growth.

C. A. petrarchae (4x). Salon, France. ♂ parent.

Fig. 41. Frond silhouettes of the synthesised hybrid A. petrarchae x A. fontanum and it s parents. Natural size.

A. A. petrarchae (4x). Salon, France. ♀ parent.

B. AS/262. Synthesised hybrid A. petrarchae x A. fontanum in it s second year of growth.

C. A. fontanum (2x). Roche, Switzerland. ♂ parent.

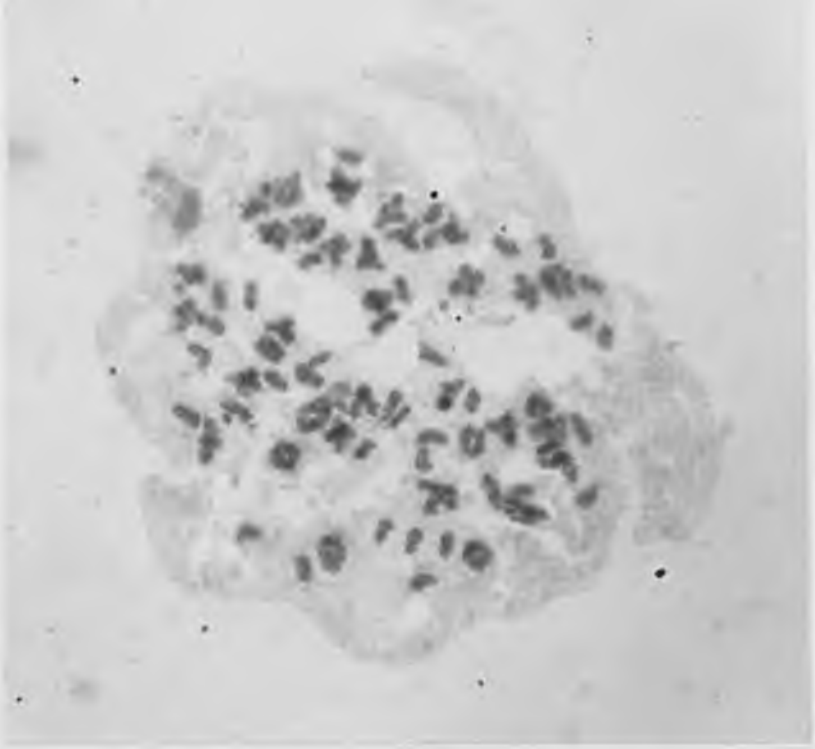


Fig. 41a. Meiosis in triploid hybrid A. petrarchae 4x (Salon) x A. fontanum 2x (Roche). Magnification x 1500.

Fig. 41b. Explanatory diagram to Fig. 41a showing 36 bivalents (black) and 36 univalents (in outline). Magnification x 2000.

Fig. 41c. AS/262. Synthesised triploid hybrid A. petrarchae 4x (Salon) x A. fontanum 2x (Roche) in second year of growth.

4. OTHER HYBRIDS INVOLVING A. BILLOTII.

Four further hybrid combinations have been produced. All involve the tetraploid species A. billotii, and may be recognised by their characteristic morphology and bad spores. These hybrids were all produced at the end of the present investigation, and no meiotic analyses have as yet been made.

18. A. onopteris (2x) x A. billotii (4x). Triploid hybrid.

	♀			P	Sp.	Se.	D	H	Unc.
	Sardinia	x	Ronco	46	15	14	0	0	1
R	Ronco	x	Sardinia	10	0	0	0	0	0
				56	15	14	0	0	1

Percentage success : 1.78%

19. A. obovatum (2x) x A. billotii (4x). Triploid hybrid.

	♀			P	Sp.	Se.	D	H	Unc.
	Sardinia	x	Ronco	31	11	8	1	0	2
R	Ronco	x	Sardinia	64	19	18	0	0	1
				95	30	26	1	0	3

Percentage success : 3.15%

20. A. billotii (4x) x A. forisiense (4x). Tetraploid hybrid.

	♀			P	Sp.	Se.	D	H	Unc.
	Ronco	x	Brissago	20	16	11	1	0	4
R	Brissago	x	Ronco	68	8	5	3	0	0
				88	24	16	4	0	4

Percentage success : 4.54%

21. A. billotii (4x) x A. macedonicum (4x). Tetraploid hybrid.

♀				P	Sp.	Se.	D	H	Unc.
	Bosa	x	Markovgrad	10	0	0	0	0	0
	Ronco	x	Markovgrad	35	10	7	1	0	2
R	Markovgrad	x	Ronco	43	15	3	0	0	12
				88	25	10	1	0	14

Percentage success : 16.0%

CHAPTER V. THE INTERPRETATION OF THE CYTOGENETIC EVIDENCE.

In interpreting the meiotic behaviour of hybrids as evidence of phylogeny it is always necessary to remember that autosyndesis as well as allosyndesis can potentially be involved. Unless the nature of any pairing encountered is clearly indicated by supplementary evidence, conclusions cannot be drawn from a single hybrid with complete certainty.

With this qualification in mind the following interpretations are suggested for the results presented in the foregoing Chapter:

1. THE ASPLENIUM FORISIENSE COMPLEX.

The most obvious fact emerging from the hybridisation programme is the close similarity in behaviour of *A. forisiense* (4x) and *A. macedonicum* (4x) in a parallel series of hybrids (see pages 78 - 81 and 99). The pairing of 36 bivalents and 36 univalents consistently shown by triploid hybrids in a number of combinations between different cultures of these two tetraploids and *A. fontanum* (2x) suggests in the first instance that both these species have one genome in common with *A. fontanum*, and that this diploid is in fact part-parental to both *A. forisiense* and *A. macedonicum*. A similar relationship may also be inferred from the pairing shown by triploid hybrids between *A. obovatum* (2x) and the same two tetraploids (see pages 80, 88 and 90).

If the above is the correct interpretation it follows that *A. forisiense* and *A. macedonicum* have the same cytogenetic origin, both tetraploids having arisen as a result of hybridisation between

A. fontanum and A. obovatum, or other ancestral types with chromosomes homologous with these, followed by chromosome doubling.

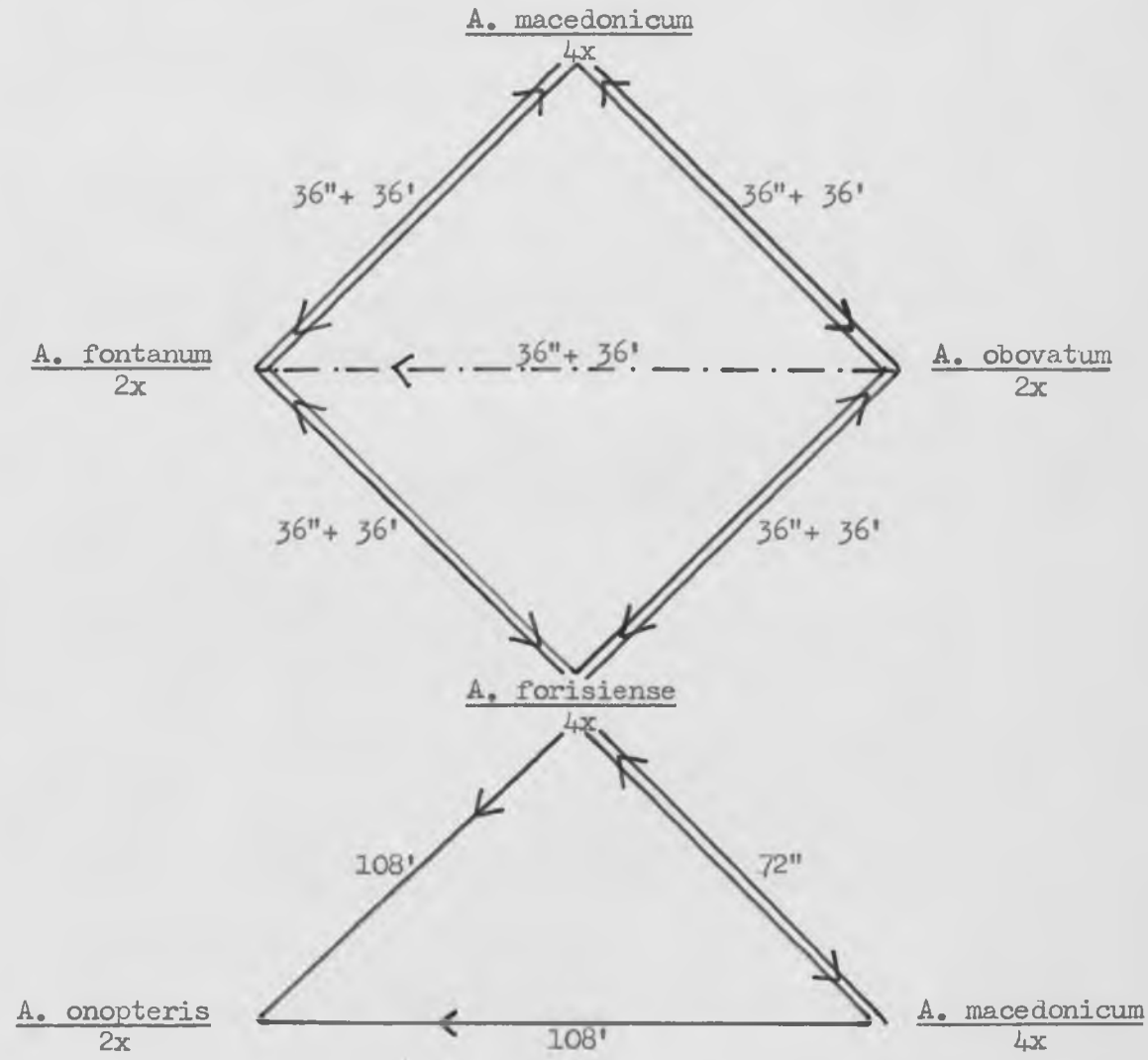
An alternative interpretation of the results from these hybrids is that the pairing is due to autosyndesis within one or both of the tetraploids, and further evidence to distinguish between these hypotheses must be sought. Such information is fortunately available from hybrids between the two tetraploids and an unrelated diploid species, A. onopteris. Both hybrid combinations are triploid, and show complete failure of pairing at meiosis (pages 81, 93 and 94). Identical results were obtained from fixings taken at different seasons and under varying weather conditions. These two results provide strong evidence in favour of an alloploid origin for both A. forisiense and A. macedonicum, the presence of 108 univalent chromosomes in both examples indicating that in each tetraploid the two genomes present are completely unrelated to each other.

With respect to A. macedonicum, further evidence to corroborate it's allotetraploid nature is supplied by a hybrid between Phyllitis hybrida and A. macedonicum synthesised by Dr. Janet Souter (née Emmott) in 1962. (Emmott 1963, 1964). This plant showed complete failure of pairing at meiosis, indicating again that there is no detectable homology between the two constituent genomes of A. macedonicum. It also reduces the possibility that the complete lack of any chromosome pairing in the hybrids between the tetraploids and A. onopteris is due to the presence of some genetical mechanism inhibiting bivalent formation in that particular combination.

Further confirmation of the close cytogenetic affinity between A. forisiense and A. macedonicum is given by the crosses attempted at the tetraploid level (pages 82 and 96). It is difficult to obtain a direct measure of the cytological divergence between these two species by hybridising them together because of the great difficulty in distinguishing hybrids from self-fertilised plants. However, several plants, which from their morphology were thought to be hybrid, showed regular meioses, 72 bivalents being constantly recorded. Thus it would appear likely that these two tetraploids are in fact cytologically equivalent.

Although final confirmation must await the successful re-synthesis of a tetraploid plant from an experimentally produced diploid hybrid between A. fontanum and A. obovatum, all the above evidence points to the conclusion that A. forisiense and A. macedonicum share a common ancestry involving alloploidy from the diploid species A. fontanum and A. obovatum, or ancestral forms with chromosomes homologous with these. The conclusion that both tetraploids investigated have the same cytogenetic origin necessarily involves taxonomic implications, and this will be discussed further on page 136. The results are shown in diagrammatic form on page 124, and the final interpretations are figured on page 135.

FIG. 42. CHROMOSOME PAIRING IN HYBRIDS WITHIN THE A. FORISIENSE COMPLEX.



KEY.

═══ indicates that the reciprocal cross was made and examined.

—.—.—. indicates the suggested formation of a doubled gamete.

←—— Arrow points towards ♀ parent in each case.

2. A. MAJORICUM.

From the theoretical considerations outlined above there are clearly two possible alternative interpretations that might be offered for some of the hybrids involving A. majoricum (4x), and a good example is the pairing of 36 bivalent and 36 univalent chromosomes consistently recorded in the triploid hybrid A. majoricum x A. fontanum (2x) (pages 97 and 102). This result indicates either that:

a) A. majoricum is an allotetraploid species and that the diploid A. fontanum is part-parental to it, or

b) that A. majoricum is effectively autoploid, the pairs recorded being autosyndetic in origin.

The second possibility is in this case discounted by evidence supplied from two further hybrids incorporating two different unrelated diploid species with the tetraploid A. majoricum. The triploid hybrid A. onopteris (2x) x A. majoricum clearly shows (pages 98 and 104) 108 univalent chromosomes at meiosis and the hybrid A. viride (2x) x A. majoricum similarly shows (pages 98 and 106) complete absence of any pairing. These two results demonstrate unequivocally the total lack of any detectable homology between the constituent genomes of A. majoricum, and it may be concluded therefore that this tetraploid is almost certainly alloploid in origin and that A. fontanum (or a form with similar chromosomes) is one of it s parents. Further confirmation of the allotetraploid nature of this plant is available from a hybrid between A. majoricum

and A. adulterinum, a tetraploid of known affinity. This hybrid was synthesised by Dr. J. D. Lovis in 1964 and shows 144 univalent chromosomes at meiosis (Lovis, personal communication). The further relevance of this result to the present investigation is discussed below.

On the basis of the results obtained certain species can be excluded from further consideration as possible second parents of A. majoricum. For example, A. viride, which from it's morphology could possibly have a direct relationship with A. majoricum, is ruled out by the evidence from the hybrid A. viride (2x) x A. majoricum. Similar evidence is available from the tetraploid hybrid A. majoricum x A. adulterinum mentioned above. The complete failure of pairing at meiosis shown by this plant indicates that no detectable homology exists between the genomes of A. majoricum and those of A. viride and A. trichomanes (2x), the two genomes which are known to be present in A. adulterinum (Lovis, 1955a, 1958). The consequent exclusion of A. trichomanes ssp. trichomanes as a possible diploid ancestor of A. majoricum is of interest in view of the fact that wild hybrids described in the literature as A. trichomanes x A. fontanum are considered by Christensen (1913) to be A. majoricum.

Further evidence similarly excluding the genome of diploid A. obovatum from close affinity with A. majoricum is obtained from the three additional hybrids described on pages 98 and 99.
At the tetraploid level A. majoricum has been successfully crossed with both A. forisiense (4x) and A. macedonicum (4x), yielding hybrids

which are morphologically very similar. In both combinations 36 paired and 72 unpaired chromosomes were regularly observed, a result which is to be expected if, as postulated, all three tetraploids have one parent in common, namely A. fontanum. Since it has been shown (see page 123) that both A. forisiense and A. macedonicum share the parentage A. fontanum x A. obovatum the results from these two hybrids therefore give indirect evidence to show that A. obovatum is unrelated to A. majoricum.

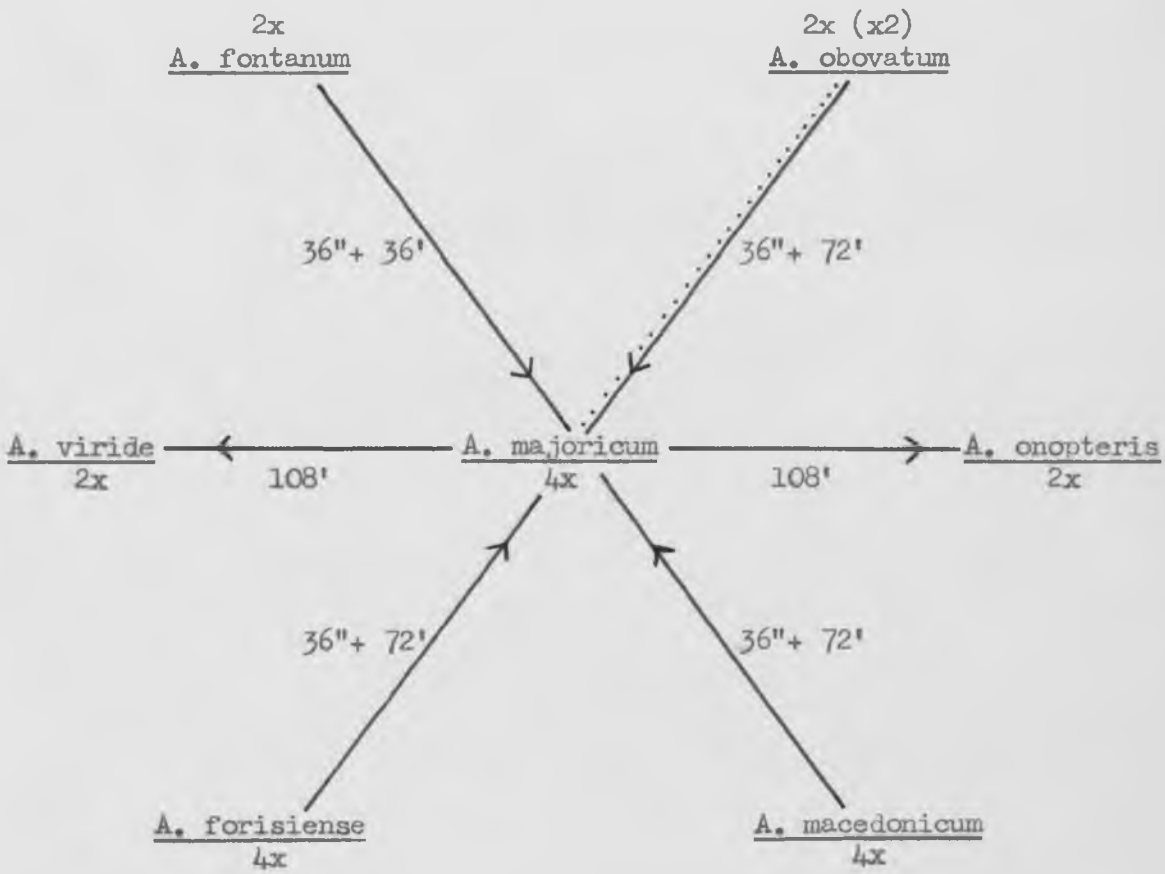
The tetraploid hybrid obtained instead of the expected triploid in a cross involving A. majoricum (4x) and A. obovatum (2x)(see pages 98 and 108) also provides indirect but nevertheless consistent evidence pertinent to this view. The interpretation of this hybrid as the result of the functioning of an unreduced gamete from the diploid parent is compatible with the chromosome pairing found (36 bivalents and 72 univalents) without postulating any cytological affinity between the genomes of A. majoricum and A. obovatum.

There is thus a substantial amount of evidence to show that A. majoricum is yet another allotetraploid Asplenium, and that A. fontanum is one of it's parents. A diagram showing the results obtained is figured on page 129.

There is as yet no evidence as to the nature of the second parent of A. majoricum, although, as stated above, certain species can definitely be excluded from further consideration. In the diagrammatic interpretation of relationships between these Asplenium

species shown on page 135 it is suggested that the second parent could conceivably be the recently discovered diploid form of A. petrarchae. A possible relationship between this species and A. majoricum is inferred on morphological and geographical grounds, although no experimental evidence exists to confirm this hypothesis. The diploid form of A. petrarchae is now in cultivation at Leeds where an investigation of the possible relationship between this species and A. majoricum is at present in progress.

FIG. 43. CHROMOSOME PAIRING IN HYBRIDS INVOLVING A. MAJORICUM.



KEY.

..... indicates the suggested formation of a doubled gamete.

——← Arrow points towards ♀ parent in each case.

3. CYTOGENETIC AFFINITIES OF OTHER TETRAPLOIDS INVESTIGATED WITH EVIDENCE OF AUTOPLOIDY IN TWO EUROPEAN ASPLENIUM SPECIES.

Early in the investigation a triploid hybrid was obtained between A. petrarchae (4x) and A. fontanum (2x), which showed c. 36 paired and c. 36 unpaired chromosomes at meiosis. (see page 117) As with other hybrids showing pairing of the same order there are two possible alternative interpretations, i.e. that A. petrarchae is another allotetraploid having A. fontanum as one parent, or that it is a cryptic autotetraploid, the pairs resulting from autosyndesis between the constituent genomes of A. petrarchae. From the morphology alone it seemed possible that in this case the second hypothesis could be the correct one.

Further evidence pertinent to these alternatives was obtained from two plants of the tetraploid hybrid A. billotii (4x) x A. petrarchae, which were produced late in the summer of 1964. Both plants were intermediate in morphology between their parents (see page115) but showed a high proportion of normal-looking, well-filled spores. These hybrids proved to be tetraploid, as expected, (page 114 and at meiosis a high degree of chromosome pairing was observed, the number of bivalents being of the order of 62 per cell, i.e. less than the maximum number of 72 but substantially more than the 36 which were regularly recorded from a number of other hybrids. The high number of pairs excludes completely an alloploid origin for either A. petrarchae or A. billotii, apart from the most improbable suggestion that both these morphologically dissimilar species are

alloploid on the same two parental diploids. The most likely conclusion is that both these very different tetraploids are effectively autoploid in origin, and that the bivalents observed in hybrids between them are due to autosyndetic pairing within the constituent genomes of each species. An interesting parallel is the meiotic behaviour found by Lovis in A. x murbeckii, the wild hybrid between A. septentrionale and A. ruta-muraria (Lovis, 1963, 1964).

The conclusion that A. billotii shows evidence of autoploidy is a somewhat surprising one, especially in view of previous preliminary statements to be found in the literature (Shivas, 1956, Manton, 1961, Manton & Reichstein, 1962) to the effect that this species is an allotetraploid with A. onopteris as one parent. During the course of the present investigation one hybrid was synthesised which affords evidence pertinent to this view. A. billotii was successfully crossed with A. majoricum, a species which has already been shown to be allotetraploid with A. fontanum (2x) as one parent (see page 125). Meiotic analyses from this tetraploid hybrid clearly showed the presence of 36 bivalent and 72 univalent chromosomes. Since A. majoricum is now known to be unequivocally allotetraploid, and furthermore, to have no affinities with either A. onopteris or A. obovatum, there are only two possible conclusions that can be drawn from the evidence of this hybrid:

a) that A. billotii is a cryptic autotetraploid and that the pairs observed are the result of autosyndesis within the constituent genomes

of this species, or

b) that A. billotii and A. majoricum are allotetraploid and that A. fontanum is part-parental to both. In the light of the facts recorded above A. billotii could then have only one possible constitution, namely A. fontanum x A. onopteris. Any other hypothesis would be inconsistent with previous work (Shivas, 1956). Additional information available in this case fortunately resolves these two alternatives. The evidence supplied by the tetraploid hybrid A. billotii x A. petrarchae discussed above shows clearly that the first possibility considered is in fact the correct imterpretation for the pairing found.

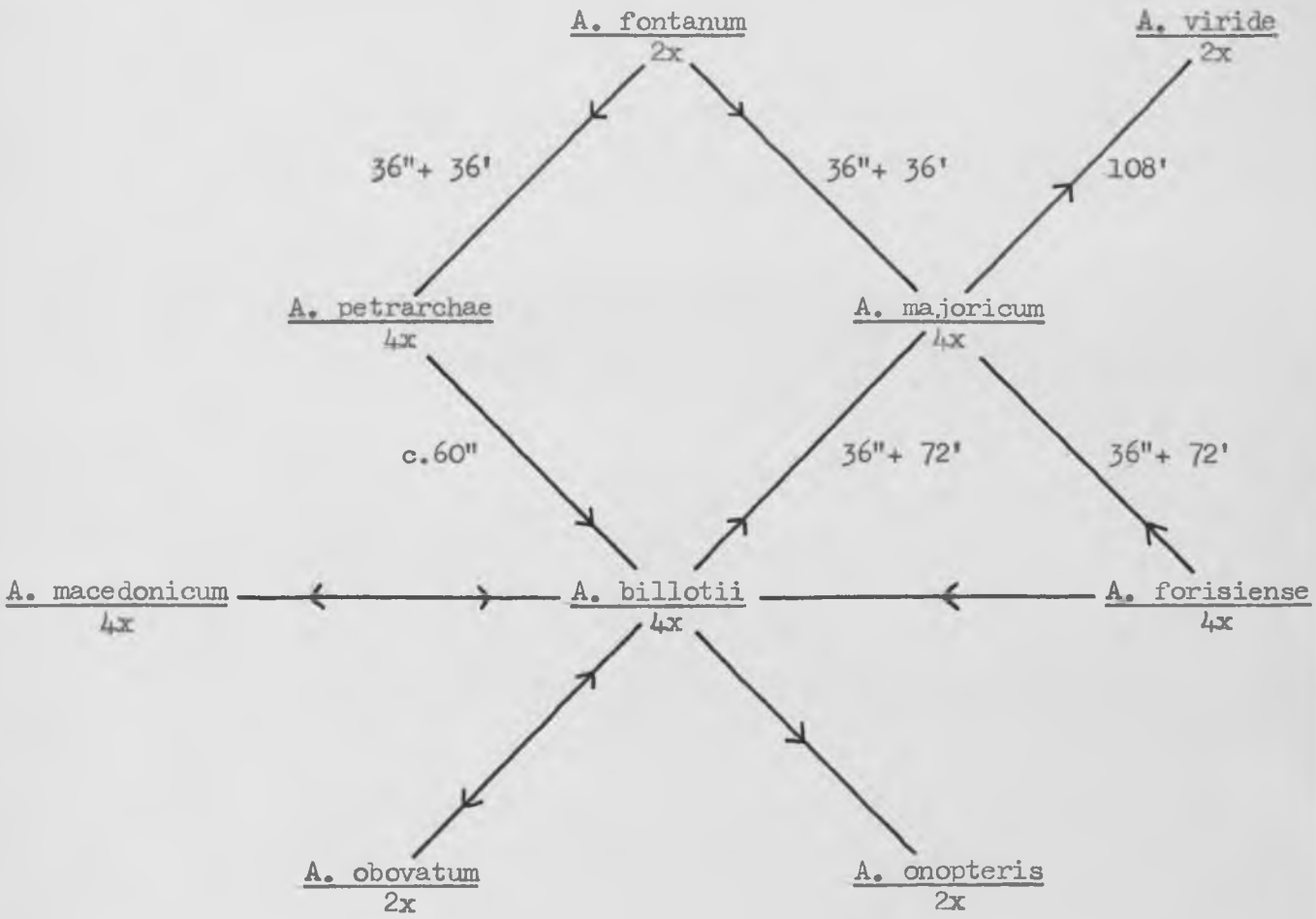
The results obtained are illustrated diagrammatically on page 134. It may be seen that the pairing observed in other hybrids is fully consistent with the conclusion that both A. billotii and A. petrarchae are effectively autoploid in origin but from different diploid ancestors.

These conclusions raise the question of the existence of possible ancestral types, and in connection with the suggested autoploid origin of A. petrarchae it is of considerable interest to note the discovery of a new diploid cytotype of A. petrarchae (published by D. E. Meyer as A. glandulosum Lois. ssp. bivalens D. E. Meyer, from a locality in southern Spain). Plants of this cytotype have now been raised at Leeds, but unfortunately too late to be used in the present investigation.

The most likely candidate as a diploid ancestor to A. billotii

is A. obovatum Viv., a diploid species for many years confused with A. billotii and included with it in the comprehensive taxon A. lanceolatum auct. In the general interpretation of results given on page 135, it is suggested that both these species share the same genome. This is a plausible assumption but not necessarily correct. A. obovatum and A. billotii can be distinguished morphologically and they possess somewhat different distributions (see pages 31, 43 and 44). Thus although it is quite possible that they are in fact related, it may be that a diploid form closer to A. billotii is yet to be discovered somewhere in Europe. The third possibility is that the diploid ancestor of A. billotii has long been extinct. Although a definite decision concerning the ancestry of A. billotii cannot at present be made, these questions may in part be resolved by study of further hybrids produced in the course of this investigation (see page 119) but as yet unexamined. In this connection it is interesting to note that spores of the hybrid A. billotii x A. petrarchae have been collected and sown, and are already showing signs of scattered but even germination. If progeny of this hybrid can be established in cultivation it will be important to try to hybridise them with the diploid species suspected of being of close affinity with the two tetraploids in question.

FIG. 44. CHROMOSOME PAIRING IN HYBRIDS INVOLVING THE TETRAPLOIDS A. MAJORICUM, A. PETRARCHAE AND A. BILLOTII.



Arrow points towards ♀ parent in each case.

FIG. 45. SUGGESTED INTERPRETATION OF RESULTS.

Genomes represented by capital letters.

KEY.

—.—.— indicates the presumed formation of a doubled gamete.

Suggested hybrid genomes are shown in red.

Genomes in brackets (A. petrarchae, A. billotii, A. majoricum) indicates that the suggested constitution can be inferred on morphological or other grounds although there is no experimental evidence to confirm it.

CHAPTER VI. TAXONOMIC IMPLICATIONS.

The only conclusions arrived at so far which raise serious taxonomic implications are those concerning *A. forisiense* and *A. macedonicum*. The cytogenetic evidence presented on pages 121, 122 and 123 shows that these two taxa have a common origin which involves alloploidy from the diploid species *A. fontanum* and *A. obovatum*.

A study of the distribution of these plants and the past history of their present areas suggests that both taxa are part of the same ancient species which previously had a wider and more continuous distribution. Although it is possible that *A. forisiense* and *A. macedonicum* originated independently from morphologically different strains of the same two diploids, it is thought more likely that the plants from France and Macedonia are the surviving relicts of one distinct allotetraploid species. The slight morphological differences between plants from each area are probably due to their geographical isolation and subsequent adaptation over a long period of time.

It is already clear that *A. forisiense* and *A. macedonicum* display the fundamental characteristics of one taxonomic species:

1. They are not reproductively isolated, as hybrids between them show regular meiotic pairing and viable spores;
2. The cytogenetic evidence shows that both taxa have a common ancestry;
3. Their ecological requirements are the same; and

4. They are morphologically very similar (see Table IV), and in fact can only be distinguished with difficulty.

The plants from each region are, however, geographically isolated, and small but constant morphological differences are maintained in cultivation when grown under similar conditions (see page 57.

It thus seems obvious that taxonomic revision is desirable and it is suggested that the two tetraploid taxa should be included within one comprehensive species A. forisiense. Geographically isolated units from western Europe and the Balkan peninsula would then be separated as sub-species forisiense and macedonicum respectively.

Frontispiece: Polystichum braunii (Spenner) Fée growing amongst boulders close to the waterfall at Krimml, Salzburg province, Austria.

PART II. POLYSTICHUM.

CHAPTER VII. INTRODUCTION.

As outlined in the General Introduction, this project began as a cytogenetic investigation of the four European *Polystichum* species, with particular reference to the mode of origin and relationships of the two tetraploids, *P. aculeatum* and *P. braunii*. Material from other parts of the world was included where this was available.

Three species of *Polystichum* are known from Britain, and of these, two (*P. lonchitis* and *P. setiferum*) are diploid, with n = 41 chromosomes, and the third (*P. aculeatum* *) is tetraploid, with n = 82 chromosomes. The remaining European species (*P. braunii*) is also tetraploid. The latter species has been studied on a world scale, but in the limited time available it has proved impossible to extend the study of the British taxa outside Europe. Much confusion exists in the literature, especially with regard to the species *P. setiferum* s.l. and *P. aculeatum* s.l., which are extremely variable and of cosmopolitan distribution. It is apparent from a preliminary study of herbarium material and other records that both morphologically and cytologically distinct units are included within these two comprehensive taxa. A particular example is a plant of the *P. setiferum* complex from Persia, which on cytological examination proved to be tetraploid (see page 173). Although morphologically close to European *P. setiferum*, a more detailed study showed that it could be referred to

* Footnote: The name of *P. aculeatum* (L.) Roth is used here in accordance with current nomenclatural practice (Clapham, Tutin & Warburg, 1962, Valentine, in Flora Europaea, 1964) although the name of *P. lobatum* (Huds.) Chev. is still widely used by continental botanists (see Alston, 1940).

the taxon P. woronowii Fomin. This plant was included in the hybridisation programme, but unfortunately no hybrids have been obtained. P. setiferum and P. aculeatum outside Europe may thus be regarded as species-complexes requiring further investigation.

As the three British species of Polystichum are well-known it has been thought unnecessary to burden the reader with detailed descriptions of their morphology, distribution and ecology, which are given fully elsewhere (e.g. Clapham, Tutin & Warburg, 1962, Manton, 1950, Perring & Walters, 1962). The arrangement of the second half of this thesis has therefore been modified as follows: A brief survey of previous work relating to the two tetraploids, P. aculeatum and P. braunii, is given below, and a detailed account of the ecology and distribution of the less familiar species P. braunii will be found in Chapter 8 , page 149. All experimental data relating to both species and hybrids are given in Chapter IX. page 172.

P. aculeatum (4x).

Manton (1950) observed meiotic pairing in wild triploid hybrids of P. x illyricum (= P. lonchitis x P. aculeatum) and in a synthesised triploid hybrid of P. setiferum x P. aculeatum, and from the results obtained suggested that P. aculeatum was probably an allotetraploid species, and that the two diploids P. lonchitis and P. setiferum were likely to have contributed towards it s parentage. In order to test this hypothesis it is desirable to be able to re-synthesise the tetraploid from it s putative diploid ancestors, and this was one of

the prior aims of the present investigation. Numerous attempts were made to cross P. lonchitis with P. setiferum and several sterile diploid hybrids were successfully obtained (see page 182). When mature, fertile fronds were treated with the spindle-arresting substance colchicine, in an attempt to induce chromosome doubling in the dividing mother cells (see page 20). However, it will be some time before it is known if the successful re-synthesis of P. aculeatum has been achieved.

Chromosome counts have been made on material of P. aculeatum from a number of European and British localities (see page 173). This species has been shown to be uniformly tetraploid, with n = 82. Vida (1963) records a similar result from Hungarian material. The records of a diploid chromosome number (n = 41) for P. aculeatum from northern India (Verma & Loyal, 1960a, Verma in Mehra, 1961) in all probability refer to a distinct taxon, which may or may not be related to our European tetraploid.

The possibility that P. aculeatum might be a cryptic autotetraploid and that the characteristic pairing of n bivalent and n univalent chromosomes shown by triploid hybrids could be due to autosyndesis between the genomes of P. aculeatum cannot be completely excluded. In order to investigate further this possibility two experimental procedures have been used. The first of these is the artificial induction of an apogamously produced plant from the tetraploid under investigation. Such a plant would be diploid and at meiosis any homology between the two constituent haploid genomes could be detected. Complete

failure of pairing would be evidence in favour of an alloploid origin, whereas a high number of pairs would show a considerable amount of homology to be present between the constituent genomes of the tetraploid and thus suggest an autoploid origin. Attempts were also made to produce 'wide' hybrids by crossing the tetraploid with diploid species suspected from their morphology of being totally unrelated to the tetraploid under investigation. In the case of an alloploid species complete failure of pairing would theoretically result. An autoploid species, however, could confidently be expected to show approximately the same number of paired chromosomes in any triploid hybrid with an unrelated diploid species. Fortunately two such species were already in cultivation at the Leeds Botany Department Experimental Garden at the outset of the present investigation in 1961. These were the simply pinnate American diploids P. munitum and P. acrostichoides, which are illustrated on page 168.

P. braunii (4x).

Polystichum braunii is a distinctive fern growing in Central Europe but absent from the British Isles. Outside Europe it is found sparingly in N. America and Asia. That it hybridises readily with the other European species of Polystichum is shown by the records of naturally-occurring wild hybrids which are to be found in the literature.

In 1959 a description of a hybrid Polystichum discovered by Dr. Georg Eberle in the Ebnertal near Luggau, Carinthia, was published by Dr. D. E. Meyer under the name of P. x eberlei and with the postulated origin P. braunii x P. lonchitis. (Meyer, 1959, 1959a).

This particular hybrid combination had not been previously recorded from Europe and Dr. Meyer predicted that the hybrid would prove to be diploid. Although no counts were made from this plant nor from it's putative parents it was assumed that P. braunii was a diploid species. On the basis of this assumption, and the morphological similarity of the above-described hybrid to P. aculeatum, it was suggested (Meyer, 1959b, 1960) that P. aculeatum had arisen by allo-ploidy from P. lonchitis and P. braunii, and not from P. lonchitis and P. setiferum as suggested by Manton (1950).

The above hypothesis was put forward with no experimental evidence to substantiate it, and it was left to Manton & Reichstein (1961) to investigate the cytology of P. braunii. They showed that P. braunii from both Europe (two localities in south Switzerland) and America (Vermont) was uniformly tetraploid, c. 82 regular bivalents being seen at meiosis. This agrees well with earlier reports that the base number for Polystichum is n = 41. Meiosis was also studied in plants of the wild hybrids P. x luerssenii (= P. braunii x P. aculeatum (4x) and P. x wirtgeni (= P. braunii x P. setiferum (2x). As expected from the tetraploid chromosome number found for P. braunii, the hybrid P. x luerssenii proved to be tetraploid, and P. x wirtgeni proved to be triploid. Meiosis in both these hybrids was irregular, and the degree of chromosome pairing observed was very low (of the order of 10 pairs). From these results Manton & Reichstein concluded that P. braunii is an ancient species not closely related to the other European Polystichums.

During the course of the present investigation meiosis has been observed in plants of P. braunii from a number of European localities, America, and from both the north and south islands of Japan. 82 regular bivalents were comsistently observed (see pages 174 and 175) and it would therefore appear that P. braunii is tetraploid throughout it's range. Recent counts by Taylor & Lang (1963) from British Columbia, Canada, and Vida (1963) from Hungary support this.

Attempts were made to synthesise experimentally the hybrids P. braunii x P. aculeatum and P. braunii x P. setiferum, in order to confirm the observations already made on the wild hybrids described above. In spite of the relatively frequent occurrence of both hybrids in nature they proved surprisingly difficult to synthesise, and to date only one cytologically examined plant of the tetraploid hybrid P. braunii x P. aculeatum has been produced.

Attempts were also made to synthesise the controversial hybrid P. braunii x P. lonchitis. This combination too proved difficult to synthesise, and repeated hybridisations using parental stocks from different localities in Europe, America and Japan in a variety of combinations met with no success. Right at the end of the present inquiry, however, a few putative hybrid plants were obtained and these await cytological investigation.

As stated above, P. braunii x P. lonchitis has been claimed from the wild (Eberle, 1959, Meyer 1959), but with no conclusive evidence for the correctness of the diagnosis. However, in the summer of 1964 it was possible to visit the Zillertal Alps in company with

Professor T. Reichstein and Dr. J. D. Lovis. Following information supplied to Professor Reichstein by Dr. Meyer, a search was made for P. braunii x P. lonchitis, and one large plant, suspected from it s morphology of being the correct hybrid, was found. A frond from this plant is figured on page 166. Fixings taken from this hybrid have unfortunately yielded no information. Examination of young sporangia showed that these aborted at an early stage, and no dividing mother cells were seen. However, this plant is now established in cultivation at Basel, and it is hoped that further fixations will yield better results.

A search was made both in the literature and at the National Herbaria for other, possibly diploid, species thought from their morphology to be related to P. braunii. Of particular interest in this connection is P. haleakalense Brack., a species found in the Hawaiian islands and parts of California. It resembles P. braunii in general appearance and for some time Christensen (1906) included it in the taxon P. braunii, citing it as a synonym of this species. Recent information from Dr. W. H. Wagner Jr. (personal communication) reveals that P. haleakalense from both Hawaii and California is diploid with n = 41 chromosomes, and in the light of this knowledge it would be of great interest to be able to include this species in a hybridisation programme.

In addition a collection of Japanese Polystichum species of the P. braunii group were examined cytologically. These were sent to Kew especially for the purposes of this investigation by Professor H. Itô of Tokyo, at the request of Professor T. Reichstein. The majority of

these plants were found to be tetraploid, but one, namely P. ohmurae, proved to be diploid. This species is in appearance rather like a small form of P. braunii; it bears similar soft hairs on the upper surface of the lamina but differs in having the sori in a marginal position. On morphological grounds it seemed possible that this taxon could be related to P. braunii, and it was therefore desirable to incorporate it in the hybridisation programme. Unfortunately the plant at Kew died soon after this result had been obtained and further material of this species was only acquired some time afterwards as a wild spore collection direct from Japan. P. ohmurae is a rare endemic plant in that country, where it is restricted to certain mountains in Central Honshû. Both gametophytes and sporophytes of this species proved particularly difficult to raise under cultural conditions in Leeds, and it was thus unfortunately impossible to incorporate P. ohmurae into the hybridisation programme in the time available. (For other observations on the Japanese material see page 246).

The nature and cytogenetic affinities of P. braunii were further investigated by the methods already outlined above for P. aculeatum. Attempts were made to induce apogamy in this species (see pages 21 & 22) and experimental hybridisations were set up between P. braunii and the two unrelated, simply pinnate American diploids, namely P. munitum and P. acrostichoides. Further aspects of this interesting species are discussed in the following Chapter.

Fig. 46. P. braunii
Silhouettes of

A. P. braunii from Austria. Wild collection.

B. P. braunii from Norway. Cultivated frond.

from Europe and Japan.

fertile fronds.

C. P. braunii from Hokkaidō, Japan. Cultivated frond.

D. The possibly related diploid taxon P. ohmurae from Huzizan, Honshū, Japan. Cultivated frond.

CHAPTER VIII. THE TAXA INVESTIGATED.

SECTION A. P. BRAUNII.

Aspidium braunii was first described by Spenner in Flora Friburgensis (1825) and was transferred to the genus Polystichum by Fée in 1852. Although it has been variously cited as a variety or sub-species of Aspidium aculeatum in a number of European and American floras it is, however, an easily recognised fern and little confusion exists in the literature. The Japanese plant has been given varietal status (var. japonicum (Christ,), likewise material from eastern North America (var. Purshii Fernald, 1928) and Kamtchatka (var. kamtschaticum C.Chr. & Hultén, 1927, subsequently raised to specific rank by Fomin in 1930). Plants from Europe, north America and Japan are, however, remarkably constant in their morphology.

P. braunii may be identified by the following combination of characters:

1. The soft, lax appearance.
2. The presence of soft hairs on the upper surface of the lamina.
3. The pinnae decreasing in size towards the base of the frond.
4. The sub-sessile, serrate pinnules which are rounded in general appearance and only scarcely auricled.

Silhouettes of fronds are illustrated in Figs. 46 A, B and C.

Fig. 47. Map of the Northern Hemisphere showing the circumpolar distribution of Polystichum braunii.

Distribution: P. braunii is restricted to boreal regions of the Northern Hemisphere, where it shows a disrupted circumpolar distribution. It occupies three major areas: Europe (as far as the Caucasus), eastern North America, and the Far East (see map on page 150. These areas are completely isolated from one another as this species does not occur in central North America and is apparently absent from a large part of central Asia.

It is, however, of quite frequent occurrence in north-eastern Asia, being recorded from parts of Siberia, Manchuria, China, Korea, Japan, Sakhalin, the Kuriles and the Kamtchatka peninsula (Owhi, 1965). In Japan it occurs in the central and northern parts of Honshû, and on Hokkaidô (Kurata, 1964). Apart from it's area in eastern North America (see below) it is also found sporadically in the coastal regions of north-western America, where there are records from southern Alaska and from British Columbia (e.g. from Port Simpson, near the Alaskan boundary (Ewan, 1944)). P. braunii is a rare plant on the mainland, but occurs quite frequently in the Queen Charlotte Islands (S. Holland, 1962, personal communication), and has presumably spread to these regions from it's localities in north-eastern Asia. Alaskan material of P. braunii is regrettably only fragmentary. A related plant from the same region has been described as P. alaskense Maxon (1918), which according to Fernald (1928) closely simulates the Kamtchatkan P. braunii (var. kamtschaticum C.Chr. & Hultén) in it s attenuate pinnae and tapering bases of the pinnules.

P. alaskense, however, has simply bipinnate fronds, those of var. kamtschaticum being tripinnatifid. It would be of great interest to compare Alaskan material of either taxon with P. braunii from other regions. Various authors (e.g. Christensen, 1906) have also cited P. braunii as occurring in the Hawaiian Islands, although the two native Polystichum species (P. haleakalense Brack. and P. hillebrandii Carruth.) are morphologically quite distinct (Degener & Degener, 1963).

In eastern North America P. braunii is localised in coastal states to the south of the St. Lawrence River. In Canada it occurs in Newfoundland, Anticosti Island, the Gaspé peninsula of Quebec, westwards as far as Ontario, and south (at low altitudes) to New Brunswick, Nova Scotia and Cape Breton Island. In America it is recorded from the following states, where it is found chiefly in the mountains: Maine, New Hampshire, Vermont (particularly in the Green Mountains), parts of Massachusetts, New York and Pennsylvania, extending as far west as the Thunder Bay district of Michigan.

In Europe P. braunii is a plant of eastern affinities; it occurs in montane forests throughout central Europe and extends across Russia as far as Moscow, Kazan and the Caucasus. It is commonly found in the valleys of Austria and in the Transylvanian and Carpathian mountains. It is recorded from Rumania (Predeal), Poland (where according to Milde (1858) it is abundant in some parts of Silesia), Czechoslovakia (particularly the area of the wooded Moravian hills) and Hungary (Vida, 1963, personal communication). It is also

found in Turkey (Davis, 1965). In Jugoslavia P. braunii is of frequent occurrence in the north, where it is commonly found around the Pohorje, in Slovenia (Mayer, 1962, personal communication). Further south it is of sporadic occurrence, and the only authentic record from Croatia is that of Rossi (1911). Other records (for example, that from Montenegro) are doubtful and need substantiating.

This species is fairly common in the valleys of south Switzerland (chiefly Canton Ticino) but to the north and west of the Alps it is generally of infrequent occurrence. It is recorded from Italy (Fiori, 1943) where it is found mainly in the north. P. braunii is reported from two localities in Germany, the Wehratal in the Black Forest, and at Hinterstein near the Austrian border. A similar situation pertains in France where it can be found only at a station in the Vosges (Malvaux) and in several localities in the Pyrenees. Although rare in western Europe this fern extends locally to Scandinavia, where it is recorded from one locality in south Sweden (Skäralid, Söderåsen, Skåne). It is, however, abundant in Norway where it occurs in scattered stations in the south and extends up the lowland coastal areas of Nordland as far as Brönnöy (c.66°N.). It appears to occur in Denmark also, on the island of Zealand, but it may have spread here from cultivation. P. braunii is absent from arctic Europe, the British Isles, Iceland and Greenland.

Plants are in cultivation from several localities in Austria and Switzerland, and from Norway, Japan and eastern North America (Vermont).

Ecology: The ecological requirements of P. braunii in eastern Europe seem to be shade, humidity and a soil rich in humus. It is very often found close to a stream or river in montane forests on siliceous soils, generally on granite or gneiss. It may also occur on acid soils in limestone districts. It's altitudinal range varies from 450 to 2000m. although in the Alps it is commonly found from 800 - 1200m. In America it occurs in rich woodlands and glades or on shaded talus and scree, at altitudes of 300 - 1525m. (Fernald, 1928). The only information regarding it's ecological requirements in Japan is that in Hokkaidô it grows on the north side of Mt. Moiwa at an altitude of 400m. in dense shade under big trees.

This fern has been collected personally from a number of Austrian localities: Traföss, by Kirchdorf, Kreuzberg and Deutschlandsberg, south of Graz, all in Styria, from Krimml (Salzburg-Pinzgau) and Floitental (Tirol). In the first two localities P. braunii was found in deep shade in mixed woodland consisting mainly of Acer, Fraxinus, Pinus and Picea. In both places it was growing in humus close to a stream in a narrow valley at an altitude of c.600 - 700m. At Deutschlandsberg it was of frequent occurrence in the wooded, steep-sided valley of the River Lassnitz, where it was found in humus on rocks close to the water at an altitude of 400m. and in large numbers on moss-covered walls overlooking the river. Tree cover consisted of Fagus sylvatica L., Fraxinus excelsior L., Carpinus betulus L., Ulmus glabra Huds., Alnus incana (L) Moench., and

Picea abies (L.) Karsten, accompanied by the usual woodland plants and numerous bryophytes. Other ferns present were: Athyrium filix-femina (L.) Roth, Dryopteris filix-mas (L.) Schott, D. borreri Newman, Blechnum spicant (L.) Roth and Polypodium vulgare L.

In the two localities visited in western Austria (Krimml and Floitental) P. braunii was found in open rocky habitats, and this may well be a secondary condition resulting from the clearance of forest. In such places the adult plants are fully exposed to the sun, although shelter and humidity is afforded to the young fronds by the boulders amongst which they are invariably found. In these habitats P. braunii is again found close to water.

At Krimml this fern grew in exposed rocky places close to the waterfall at an altitude of c.1250m. In Floitental many plants were found amongst boulders in open ground near the river, on a N.E. facing slope fully exposed to the sun. The altitude was c.1350 - 1400m., and accompanying P. braunii were P. lonchitis (L.) Roth, P. aculeatum (L.) Roth, P. x illyricum (Borbás) Hahne and several large plants of the hybrid P. x luerssenii (Dörfl.) Hahne. Habitat photographs will be found on pages 156 to 163.

Fig. 48. Floitental, near Ginzling, Zillertal, Tirol, Austria. P. braunii, P. lonchitis, P. aculeatum and the hybrids P. x illyricum and P. x luerssenii occur in abundance both in open ground and under light shade on the lower slopes of the valley. Alt. c.1400m. (See Figs. 49-54).

Fig. 49. The hybrids P. x luerssenii (foreground) and P. x illyricum (behind) growing amongst boulders in open herbaceous vegetation. Floitental, Tirol, Austria.

Fig. 50. A large plant of P. lonchitis growing in an exposed position. Floitental, Tirol, Austria.

Fig. 51. P. x illyricum (Borbás) Hahne (= P. aculeatum x P. lonchitis). Floitental, Tirol, Austria. Note the toothed pinnae which are not deeply cut (compare Fig. 52).

Fig. 52. P. x illyricum (= P. aculeatum x P. lonchitis). Divided form. Floitental, Tirol, Austria.

Fig. 53. P. braunii and P. lonchitis growing together in full sun. Floitental, Tirol, Austria.

Fig. 54. The putative hybrid P. braunii x P. lonchitis growing in an exposed position in Floitental, Tirol, Austria (see pages 145 and 165).
Photo: T. Reichstein.

Fig. 55. The hybrid P. x luerssenii (Dörfl.) Hahne (= P. braunii x P. aculeatum) growing close to the waterfall at Krimml, Salzburg province, Austria.

Fig. 56. The Lassnitzklause, Deutschlandsberg, Styria, Austria. P. braunii, P. aculeatum, P. setiferum and the three corresponding hybrids occur in sheltered places along the wooded banks of the river.

Fig. 57. The Taygetos Mountains, Laconia, Peloponnesos, Greece, locus classicus for the hybrid P. x lonchitiforme Halácsy (= P. lonchitis x P. setiferum).

SECTION B. WILD HYBRIDS.

Members of the genus Polystichum appear to hybridise readily in the wild. All six theoretically possible hybrid combinations between the four European taxa have been reported, and Kurata (1964) lists 21 wild hybrids within the section Metapolystichum which are to be found in Japan. A number of naturally-occurring hybrids are reported from America, ones of particular interest being P. lonchitis x P. acrostichoides (Wagner & Hagenah, 1954), P. braunii x P. acrostichoides (Thompson & Coffin, 1940) and P. braunii var. Purshii x P. lonchitis (Ewan, 1944). Some of the other hybrids reported are listed in Table X. The first two combinations have in fact been synthesised during the course of the present investigation.

In Europe the triploid hybrids P. x illyricum (= P. lonchitis x P. aculeatum) and P. x bicknellii (= P. setiferum x P. aculeatum) are of quite frequent occurrence, and are generally to be found wherever the parents are growing together. The former is the commonest European Polystichum hybrid, and is often found in large numbers. It is of variable morphology, particularly with regard to frond dissection. The two plants shown on page 158 to some extent illustrate this, the hybrid in Fig. 52 approaching P. aculeatum in it s degree of pinnation. P. setiferum and P. aculeatum both hybridise with the second European tetraploid, P. braunii. The triploid hybrid (P. x wirtgeni) is infrequently found. It has been seen at one of it s classic localities, the valley of the river Lassnitz at Deutschlandsberg

in southern Austria, and elsewhere it occurs sparingly in a number of localities in southern Switzerland. P. x luerssenii, the tetraploid hybrid between P. aculeatum and P. braunii, is fairly widely distributed in central Europe, where it often reaches a large size. It is nearly always found where both parents grow together and such places are particularly frequent on the southern slopes of the Alps and in Austria. This fern has been collected personally from a number of localities in Styria and from Krimml and Floitental, both in the Tirol. Two morphological types were found at Floitental, plants growing in full sun being narrow and attenuate in shape, yellowish in colour, coriaceous in texture, often smaller and with a very short petiole. Fronds of both sun and shade forms are illustrated in Fig. 58 overleaf. The appearance of the plants found in exposed places may have led to confusion of such specimens with the putative hybrid P. braunii x P. lonchitis. As stated on page 142, this hybrid was reported from the Ebnertal by Meyer in 1959. Herbarium specimens of this plant however, appear indistinguishable from specimens of P. x illyricum. Further specimens referred to P. braunii x P. lonchitis by Meyer appear to be exposed forms of P. x luerssenii. The hybrid collected from the Zillertal Alps and mentioned on pages 145 and 239 has bad spores and in it's morphology it appears to be intermediate between P. braunii and P. lonchitis (see Figs. 53,54,58 & 77). It cannot be referred to either P. x illyricum or P. x luerssenii, and it is hoped that cytological examination will confirm or refute the correctness of the prediction that this plant is in fact the hybrid P. braunii x P. lonchitis.

Fig. 58. Frond silhouettes of wild hybrids from Floitental, Austria.

A. P. x illyricum (= P. lonchitis x P. aculeatum).

B. The putative hybrid P. braunii x P. lonchitis.

C. P. x luerssenii (= P. braunii x P. aculeatum). Sun form.

D. P. x luerssenii. Shade form.

The sixth European hybrid is P. x lonchitiforme (= P. lonchitis x P. setiferum). This hybrid was described by Halácsy (1904) from a single specimen of Heldreich's from the Peloponnesos (originally collected by Zahn in 1898). The type material, however, appears to be very close to P. x illyricum in it's morphology. A visit was made to the southern Peloponnesos in the summer of 1962 in order to search for this hybrid, which was described from "rupestribus regionis superioris montis Taygeti". The Taygetos proved to be an extensive mountain range (see photograph on page 163) and the hybrid was not found. P. setiferum was however collected from several localities in these mountains, up to an altitude of 4500 feet. P. aculeatum was not seen. Further information concerning the distribution of these two species in the Balkan peninsula is available from a study of records in the literature and of herbarium specimens. Both species are frequent in the north of the region, but in northern Greece P. aculeatum becomes very rare and is not recorded south of Mt. Ghiona (in the eastern Pindus range, north-west of Mt. Parnassus). Elsewhere in northern Greece there are sporadic records from Thessaly, Mt. Olympus and Euboea. P. setiferum, on the other hand, is found throughout the southern part of Greece, being recorded from a number of localities in the mountainous parts of the Peloponnesos. This information therefore suggests that the single record of P. x lonchitiforme from the Taygetos mountains may indeed be referred to the hybrid P. setiferum x P. lonchitis.

Fig. 59. Frond silhouettes of American diploids in cultivation at Leeds.

A. P. acrostichoides. Ithaca. Sterile frond.

B. P. munitum. Oregon.

C. P. acrostichoides. Ithaca. Fertile frond.

SECTION C. AMERICAN SPECIES.

A brief account of the American taxa used in the present investigation is given below. Frond silhouettes of the first two species are figured on page 168. Both are simply pinnate diploids but the latter (*P. acrostichoides*) is characterised by it s markedly dimorphic fronds.

1. *P. munitum* (Kaulf.) Presl.

This fern is endemic to North America, where it has a characteristically western distribution, occurring in Alaska, British Columbia (including Vancouver Island), Washington, Oregon and California, and extending to north-west Montana and northern Idaho. It occurs on damp wooded slopes, and attains it s best development in the Coast Ranges from the Santa Cruz peninsula to Washington.

2. *P. acrostichoides* (Michx.) Schott.

Also endemic to North America, this species is of widespread distribution throughout the central and eastern states. It ranges from eastern Canada (Cape Breton and Prince Edward Isles, Nova Scotia, New Brunswick, south Quebec and south Ontario) through the states bordering the St. Lawrence basin (e.g. New York, Vermont, Michigan) to Wisconsin, Iowa, Kansas and Missouri. In the south it extends as far as northern Florida, Mississippi, Texas and Mexico.

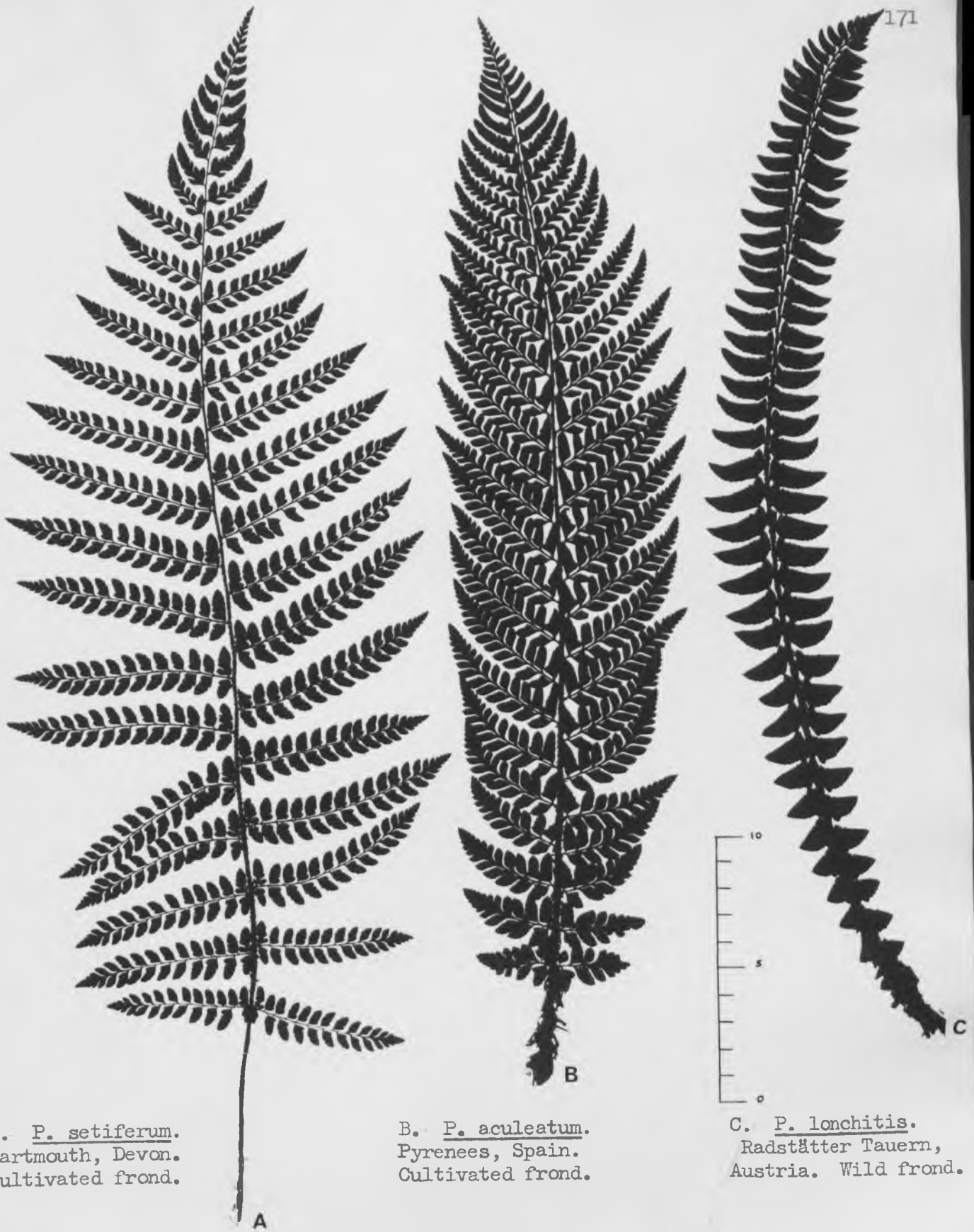
Information concerning it s ecology is given by Steyermark (1963).

It is found on dry or moist, but well-drained wooded slopes, generally on the more acid soils and often associated with sandstone, chert or granitic rocks. It attains it's best development on north-facing slopes, in narrow ravines or at the base of talus slopes in acid soil, and is frequently found along moist mossy banks or rock ledges.

3. P. andersoni Hopkins.

This fern is also endemic to North America and may possibly be related to the taxa investigated. It is tetraploid (n = 82) and material has been successfully used in the hybridisation programme, although preliminary analyses are so far available from only a single hybrid plant (see page 248).

P. andersoni was described from Vancouver Island, British Columbia (Hopkins, 1913). It possesses a coastal distribution in the western states of North America, being recorded from southern Alaska through British Columbia to Montana (Glacier National Park) and Washington (Mt. Rainier). It is foumd on moist rocky slopes, usually in alder thickets.



A. P. setiferum.
Dartmouth, Devon.
Cultivated frond.

B. P. aculeatum.
Pyrenees, Spain.
Cultivated frond.

C. P. lonchitis.
Radstätter Tauern,
Austria. Wild frond.

Fig. 60. The three Polystichum species occurring in Britain.

CHAPTER IX. EXPERIMENTAL DATA.

SECTION A. THE SPECIES : CYTOLOGY.

Meiotic counts were made from the material listed below. These plants, with the exceptions of those marked B or K, are, at the time of writing, in cultivation at Leeds. No abnormalities in the mode of reproduction were observed, all plants sown reproducing normally by sexual means (compare Supplement I, page 246).

TABLE VII.

	Species.	Locality.	n	Ploidy.	
++	P. setiferum	Devon, British Isles.	41	2x	
++	"	Grümpeli, Switzerland.	41	2x	
++	"	Gorge Chauderon, Switzerland.	41	2x	
++	"	Istanbul, Turkey.	41	2x	
	"	Rib Frio, Madeira.	41	2x	
++	"	Quimadas, Madeira.	41	2x	
++	P. lonchitis	Saalbach, Austria.	41	2x	
++	"	Pont-de-Nant, Switzerland.	41	2x	
++	"	Ben Lawers, Scotland.	41	2x	
++	"	Klausenpas, Switzerland.*	-	2x	
++	"	Haggenegg, Switzerland.*	-	2x	
++	"	Val di Bosco, Switzerland.*	-	2x	
++	"	British Columbia, Canada.*	-	2x	
++	P. acrostichoides	Vermont, U.S.A.*	-	2x	
++	"	Ithaca, U.S.A.	41	2x	
++	P. munitum	Oregon, U.S.A.	41	2x	
	"	Victoria, B.C., Canada.	41	2x	
++	P. ohmurae	Huzisan, Honshū, Japan.	41	2x	K'

	Species.	Locality.	n	Ploidy.	
++	P. woronowii	Persia.	82	4x	
++	P. andersoni	Smithers, B.C., Canada.*	-	4x	
	P. aculeatum	Somerset, British Isles.	82	4x	
	"	Scotland, British Isles.	82	4x	
	"	Iceland.	82	4x	
++	"	Auvergne, France	82	4x	
++	"	Bains-de-Tredos, Spain	82	4x	
++	"	Gorge Chauderon, Switzerland.	82	4x	
++	"	Pont-de-Nant, Switzerland.	82	4x	
	P. braunii	Krimml, Austria.	82	4x	
++	"	Hattvik, Norway.	82	4x	
++	"	Alpe di Gem, Switzerland.	82	4x	
++	"	Val Antabbia, Switzerland.	82	4x	
++	"	Val d'Osogna, Switzerland.*	-	4x	
++	"	Vermont, U.S.A.	82	4x	
++	"	Hokkaidô, Japan.	82	4x	B
++	"	Honshû, Japan.	82	4x	B

Collections marked ++ have been used in the hybridisation programme. Young selfed plants of material originating as wild spore collections have been raised at Leeds, and although not yet fertile their ploidy can be inferred from chromosome counts of synthesised hybrids incorporating the same material. These are marked by an asterisk. Stock plants at Kew and Basel respectively are represented by the letters B and K, and ' indicates that the plant referred to is now dead. For further details of the plants listed, and of other collections used in the hybridisation programme, the reader is referred to the appendix on page 235.

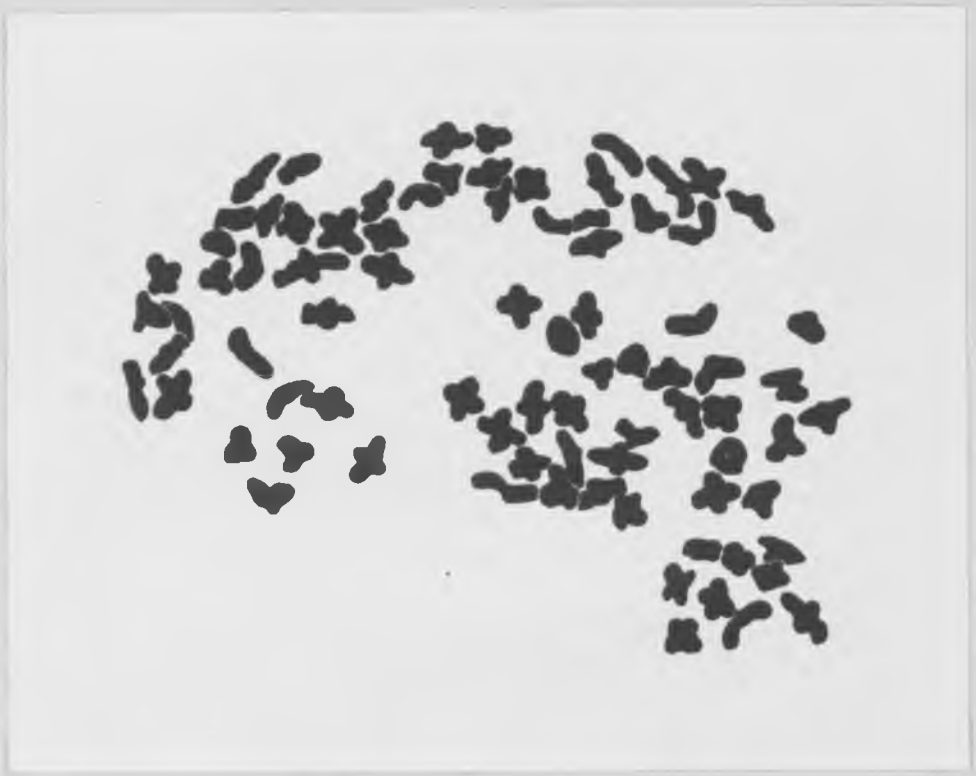


Fig. 61. Diagram of meiosis in Polystichum braunii (Spenner) Fée from Krimml, Austria, showing 82 bivalent chromosomes. Magnification x 2000.

Fig. 62. Diagram of meiosis in Polystichum braunii (Spenner) Fée from Vermont, U.S.A., showing 82 bivalent chromosomes. Magnification x 2000.

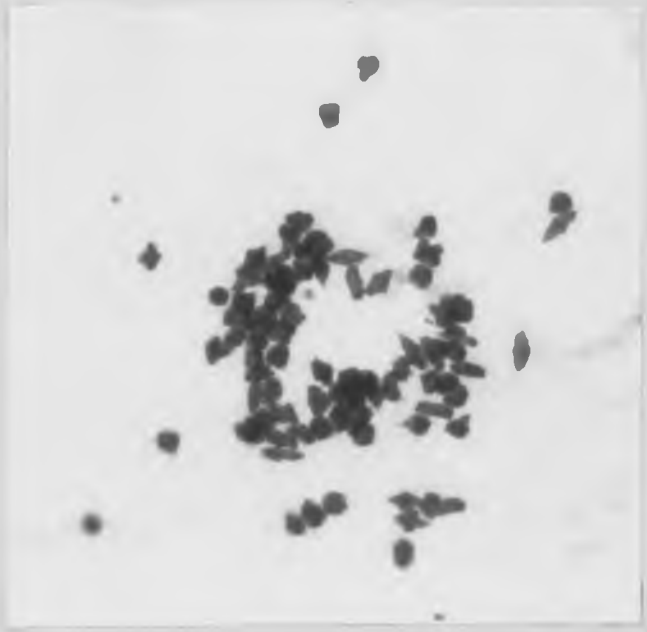


Fig. 63. Meiosis in Polystichum braunii (Spenner) Fée from Hokkaido, Japan (Itô 119). Magnification x 1200.

Fig. 63a. Explanatory diagram to Fig. 63 showing 82 bivalent chromosomes. Magnification x 2000.

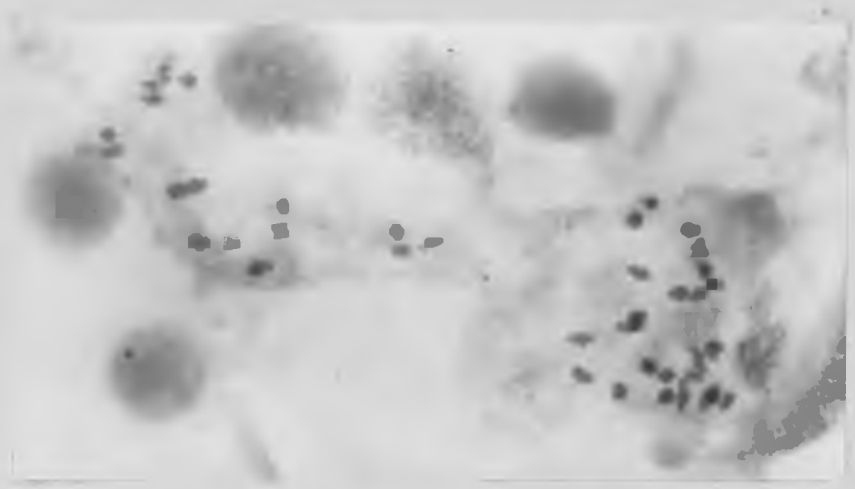


Fig. 64.

Meiosis in Polystichum ohmurae Kurata from Honshû, Japan (Itô 209). Magnification x 1200.

Fig. 64a. Explanatory diagram to Fig. 64 showing 41 bivalent chromosomes. Magnification x 2000.

SECTION B. THE HYBRIDS.

1. THE HYBRIDISATION PROGRAMME.

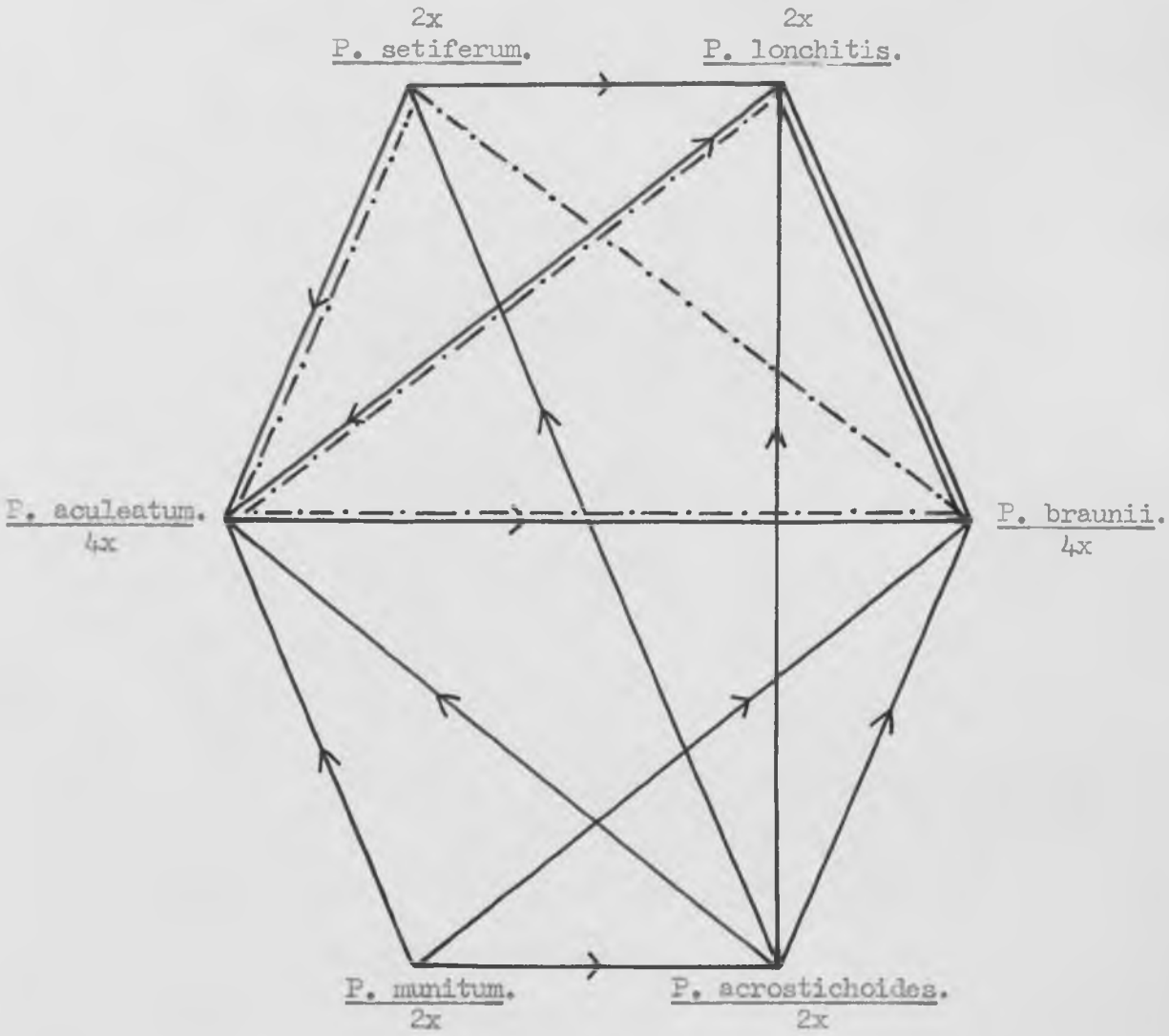
Crosses were attempted in all combinations between the four European Polystichum species and the two American diploids P. munitum and P. acrostichoides. The localities of origin of all cultures used in this programme are listed in Appendix III, page 235, and numerical data relating to each hybridisation attempt are given in Appendix V, page 240. Synthesised hybrids are listed in Table VIII. The hybridisation polygon figured overleaf shows diagrammatically the relationship between the wild hybrids available and those successfully synthesised in the course of the present investigation. The latter are illustrated by silhouettes on pages 180 to 181 and the wild hybrids on pages 166 to 195.

A number of putative hybrid combinations have also been obtained using such species as P. proliferum (4x, Australia), P. andersoni (4x, America) and several Japanese tetraploids. Some of these hybrids produced a few sporangia in the last year of the investigation, and in several cases a preliminary cytological examination has been made. As the results obtained are as yet incomplete, the analyses being made from one or two cells only, these observations are listed as a supplement (see page 248).

In considering the cytology it will be convenient to deal with the synthesised hybrids first in the account which follows. Photographs of cells at meiosis, each accompanied by an explanatory diagram, are illustrated in Figs. 67 to 76, pages 187 to 194.

FIG. 65. HYBRIDISATION POLYGON.

Showing the hybrid combinations available between the two tetraploids, P. braunii and P. aculeatum, and European and American diploids.



KEY.

Synthesised hybrid.

Cytologically examined wild hybrid.

Unexamined wild hybrid.

Arrow points towards ♀ parent in synthesised hybrids.

TABLE VIII. SYNTHESISED HYBRIDS.

PLOIDY.	HYBRID.			NO. OF PLANTS.
2x	P. lonchitis	x	P. setiferum *	4
2x	P. setiferum	x	P. acrostichoides *	9
2x	P. lonchitis	x	P. acrostichoides *	1
2x	P. acrostichoides	x	P. munitum *	3
3x	P. aculeatum	x	P. setiferum *	52
R 3x	P. aculeatum	x	P. lonchitis *	8
3x	P. aculeatum	x	P. acrostichoides *	22
3x	P. aculeatum	x	P. munitum *	9
3x	P. braunii	x	P. munitum *	3
(4x)	P. braunii	x	P. acrostichoides	3
4x	P. braunii	x	P. aculeatum *	1

WILD HYBRIDS.

	P. braunii	x	P. lonchitis
3x	P. braunii	x	P. setiferum *
4x	P. braunii	x	P. aculeatum *
3x	P. lonchitis	x	P. aculeatum *
3x	P. setiferum	x	P. aculeatum *

R indicates that the reciprocal cross was also made.

( ) indicates the presumed formation of a doubled gamete.

All hybrid combinations marked by an asterisk have been examined cytologically.

Fig. 66. Frond silhouettes of synthesised hybrids.

A. AS/712. P. braunii (Switzerland) x P. aculeatum (Switzerland).

B. AS/79. P. aculeatum (Spain) x P. setiferum (England).

C. AS/114. P. aculeatum (Spain) x P. lonchitis (Switzerland).

D. AS/416. P. braunii (Vermont, U.S.A.) x P. munitum (Oregon, U.S.A.).

E. AS/419. P. aculeatum (Spain) x P. munitum (Oregon, U.S.A.).

F. AS/42. P. aculeatum (Spain) x P. acrostichoides (Vermont, U.S.A.).

G. AS/362. P. setiferum (Turkey) x P. acrostichoides (Ithaca, U.S.A.).

2. RESULTS OBTAINED.

CYTOLOGY OF SYNTHESISED HYBRIDS.

In the following section analyses of meiosis are given for each type of hybrid successfully synthesised. As stated previously, these plants are slow-growing and many of the hybrids obtained did not produce sporangia until the last year of the investigation. For this reason it has been possible to make accurate analyses from only a limited number of cells. Results from wild hybrids are listed in Table IX, page 196.

1. P. lonchitis x P. setiferum. Diploid hybrid. Fig. 67.

973 prothalli were inseminated, and four hybrid plants have been obtained. Of these two have produced sporangia and given the following results:

										Average.
Bivalents per cell:	19	8	16	12	6	28	17	17	12	15
Univalents per cell:	44	66	50	58	70	26	48	48	58	52

---

2. P. lonchitis x P. acrostichoides. Diploid hybrid. Fig. 69.

175 prothalli were inseminated and one hybrid plant has been obtained. This hybrid produced a few sori in the last summer of the investigation and from these a single analysis has been made:

Number of bivalents: 25 Number of univalents: 32

---

3. P. setiferum x P. acrostichoides. Diploid hybrid. Fig. 68.

85 prothalli were inseminated and nine putative hybrids have been obtained. One plant only has so far become fertile, and this gave three analyses:

Bivalents per cell:	9	11	18
Univalents per cell:	64	60	46

---

4. P. acrostichoides x P. munitum. Diploid hybrid. Fig. 70.

226 prothalli were inseminated and three putative hybrids have been obtained. A single meiotic analysis has been made from the one plant which has so far produced sporangia.

Number of bivalents: 16 Number of univalents: 50

---

5. P. aculeatum x P. lonchitis. Triploid hybrid. Fig. 71.

181 prothalli were inseminated, and eight morphologically hybrid plants have been obtained. Only a single hybrid synthesised in 1961 has so far become fertile, and analyses from this plant show pairing of the order of 41 bivalent and 41 univalent chromosomes, thus confirming the results obtained from wild hybrids (see page 196).

---

6. P. aculeatum x P. setiferum. Triploid hybrid. Fig. 72.

193 prothalli were inseminated, and 52 hybrids were produced. This hybrid combination generally became fertile within two years of

P. aculeatum x P. setiferum cont.

the hybridisation attempt, and hybrids were detected by an examination of the spores. Meiosis has been observed in a selection of these plants, and the following results were obtained:

Bivalents per cell:	39	39	40	41	41	41	40	40	41	40
Univalents per cell:	45	45	43	41	41	41	43	43	41	43

In these hybrids the female parent came from Spain, and the male parent from three sources: Great Britain, Switzerland and Madeira. It is interesting to note that hybrids with the male parent P. setiferum from Madeira give the same result as those having the male parent from Britain or Switzerland.

---

7. P. aculeatum x P. acrostichoides. Triploid hybrid. Fig. 73.

63 prothalli were inseminated and 22 putative hybrids have been obtained. The plants are large and healthy, and although grown under varying conditions one plant only has become fertile.

Bivalents per cell:	9	13	18	15
Univalents per cell:	105	97	87	93

---

8. P. aculeatum x P. munitum. Triploid hybrid. Fig. 74.

148 prothalli were inseminated and nine putative hybrids have been obtained. So far one plant only has become fertile, and it gave two very clear analyses:

P. aculeatum x P. munitum cont.

Bivalents per cell:	22	19
Univalents per cell:	79	85

Other cells seen indicated pairing of approximately the same order.

---

9. P. braunii x P. lonchitis.

This hybrid combination proved exceptionally difficult to synthesise. 756 prothalli were inseminated, using parental stocks in all possible combinations from a number of different localities (see page 242). It is possible that several small sporelings from crosses made in 1964 may prove to be the correct hybrid.

---

10. P. braunii x P. setiferum.

This hybrid combination also proved extremely difficult to synthesise. 592 prothalli were inseminated (see page 243), but the sporelings raised generally proved to be selfs of P. braunii.

---

11. P. braunii x P. acrostichoides.

230 prothalli were inseminated and three morphologically hybrid plants have been obtained. One of these plants has produced sporangia, but no clear meiotic analyses have as yet been made. The cells seen showed a high number of paired chromosomes (forty or more) and approximate counts showed this plant to be tetraploid, and not triploid, as expected. The male parent, P. acrostichoides from Ithaca, U.S.A.,

P. braunii x P. acrostichoides cont.

is diploid (see page 172) and this suggests that in this case P. acrostichoides has produced an unreduced gamete.

---

12. P. braunii x P. munitum. Triploid hybrid.

260 prothalli were inseminated and three morphologically hybrid plants have been obtained. The following results have been obtained from the one plant which has produced sporangia:

Bivalents per cell:	13	28
Univalents per cell:	97	67

---

13. P. braunii x P. aculeatum. Tetraploid hybrid.

This hybrid combination also proved difficult to synthesise. 146 prothalli were inseminated and one hybrid plant was obtained. This became fertile at the end of the present investigation, and gave the following clear analysis:

Number of bivalents: 11 Number of univalents: 142

Other cells seen indicated a degree of pairing of between 10 and 20 bivalents per cell, and this agrees well with the results obtained from wild hybrids (see page 196).

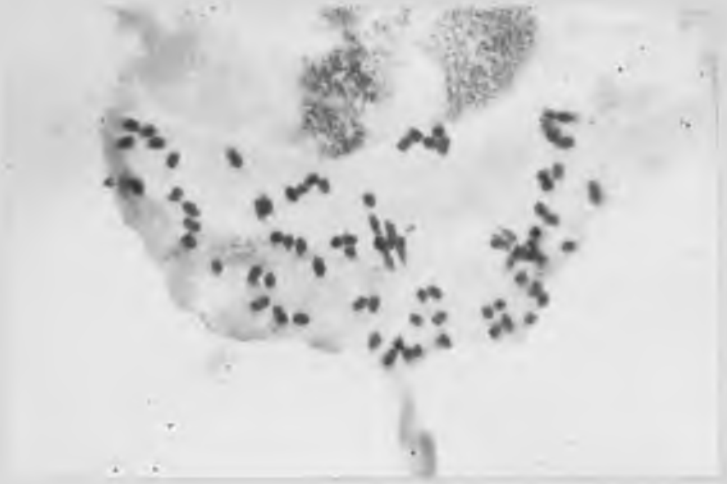


Fig. 67. Meiosis in synthesised diploid hybrid P. lonchitis 2x (Switzerland) x P. setiferum 2x (Switzerland). Magnification x 1200.

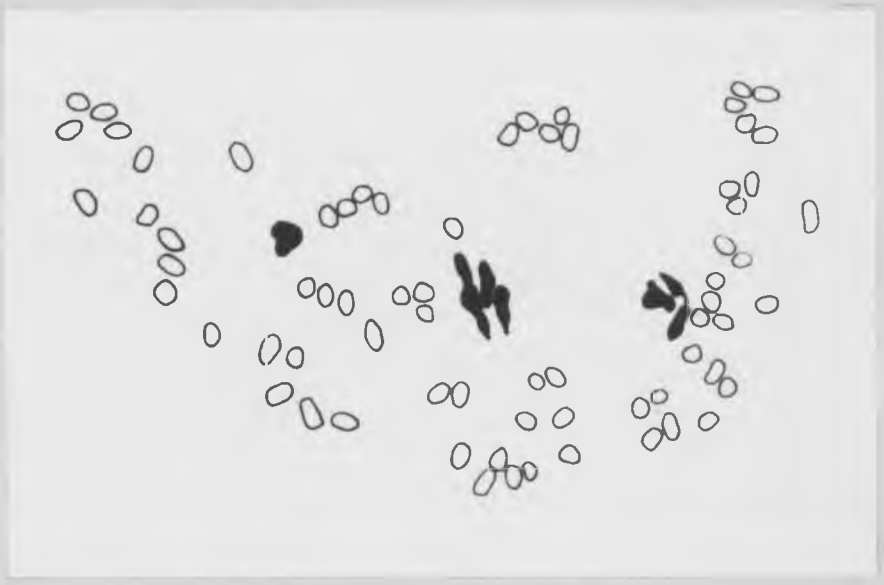


Fig. 67a. Explanatory diagram to Fig 67 showing 6 bivalents (black) and 70 univalents (in outline). Magnification x 2000.

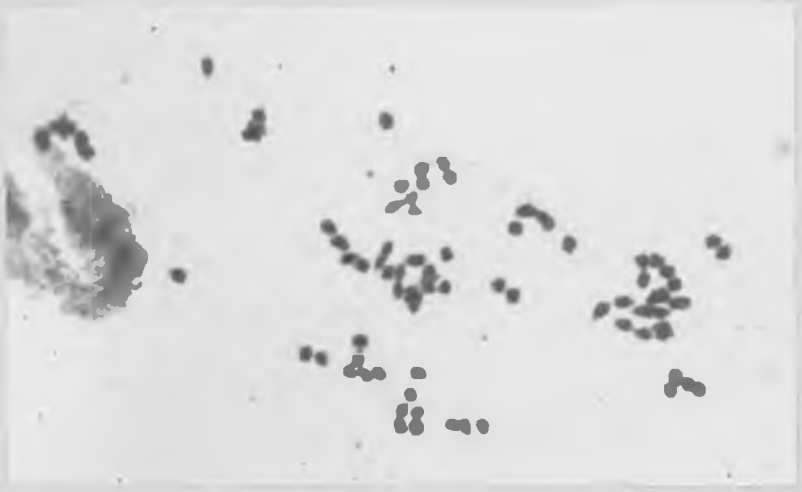


Fig. 68. Meiosis in synthesised diploid hybrid P. setiferum 2x (Turkey) x P. acrostichoides 2x (Ithaca). Magnification x 1200.

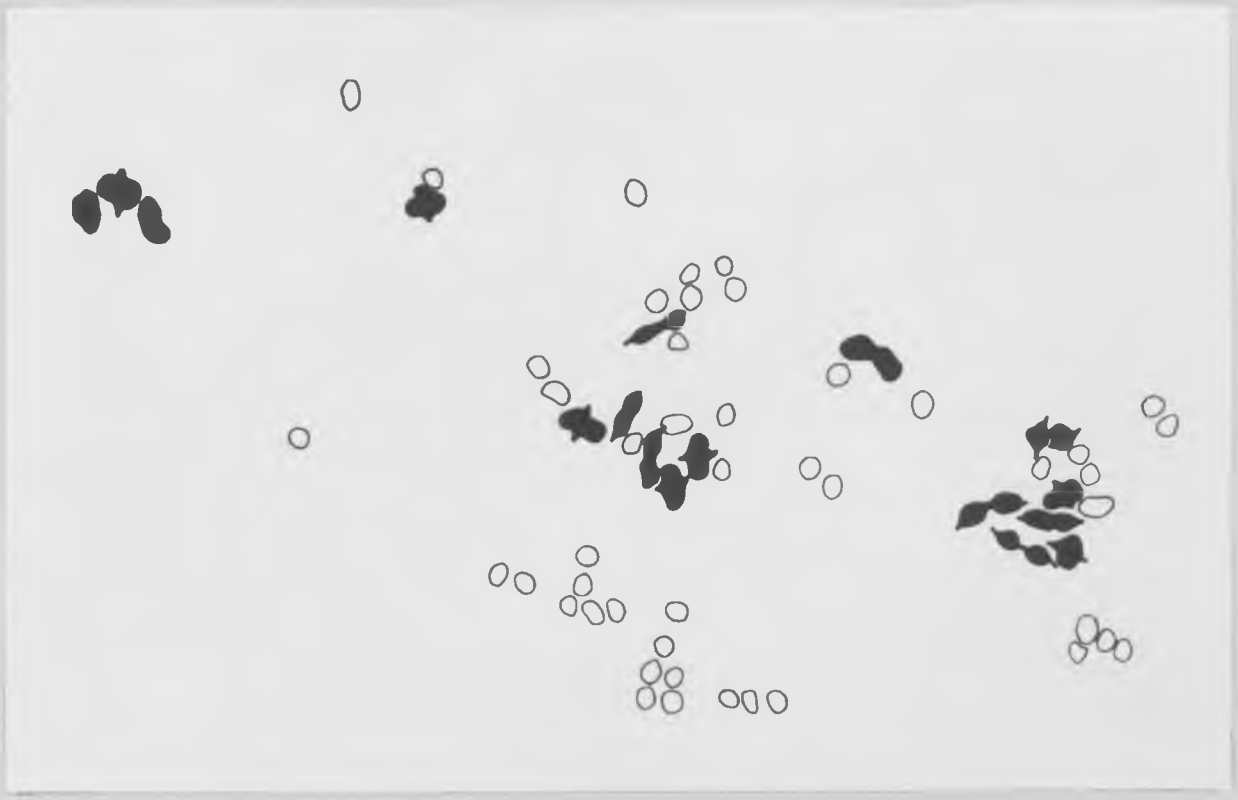


Fig. 68a. Explanatory diagram to Fig. 68 showing 18 bivalents (black) and 46 univalents (in outline). Magnification x 2000.

Fig. 69. Diagram of meiosis in synthesised diploid hybrid P. lonchitis 2x (British Columbia) x P. acrostichoides 2x (Ithaca) showing 25 bivalents (black) and 32 univalents (in outline). Magnification x 2000.

Fig. 70. Diagram of meiosis in synthesised diploid hybrid P. acrostichoides 2x (Ithaca) x P. munitum 2x (Oregon) showing 16 bivalents (black) and 50 univalents (in outline). Magnification x 2000.

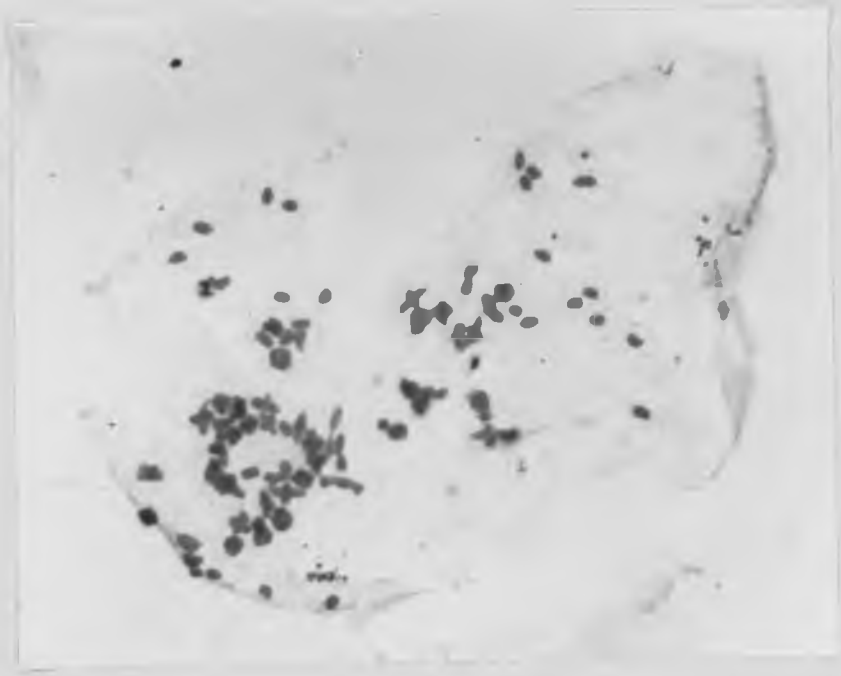


Fig. 71. Meiosis in synthesised triploid hybrid P. aculeatum 4x (Spain) x P. lonchitis 2x (Switzerland). Magnification x 1200.

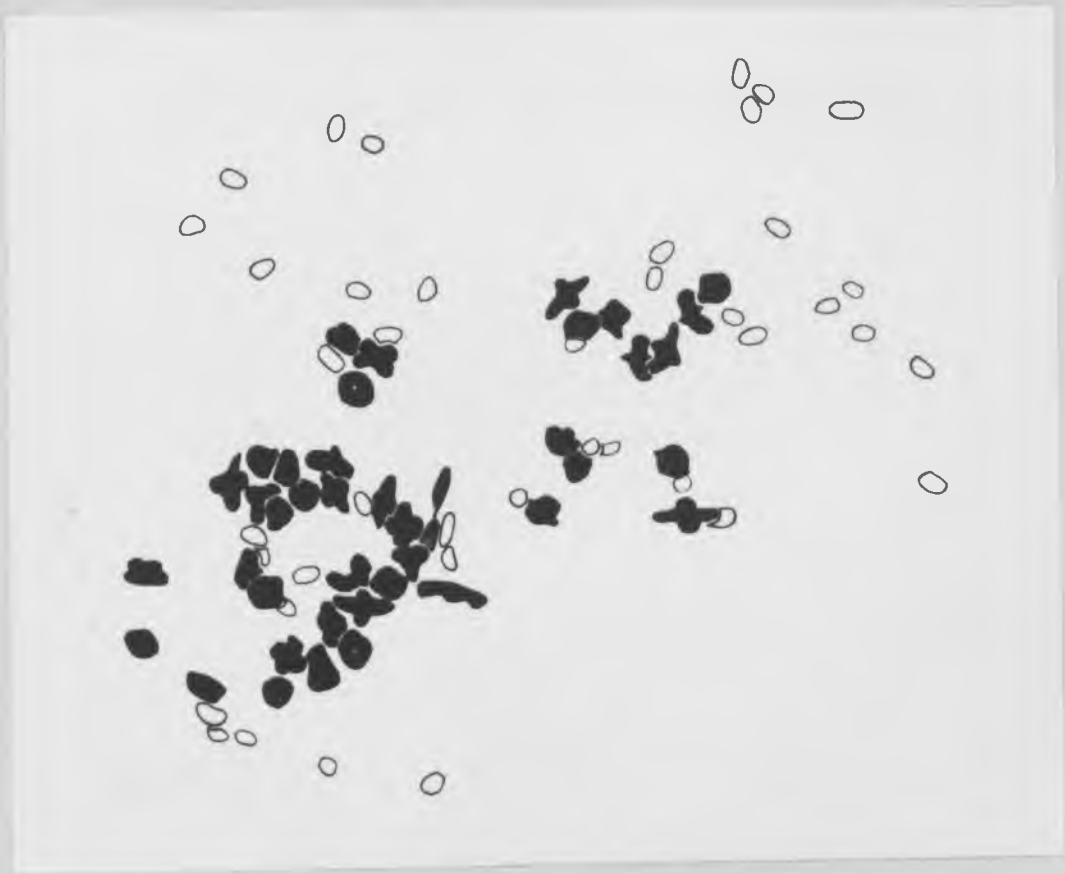


Fig. 71a. Explanatory diagram to Fig. 71 showing 41 bivalents (black) and 41 univalents (in outline). Magnification x 2000.

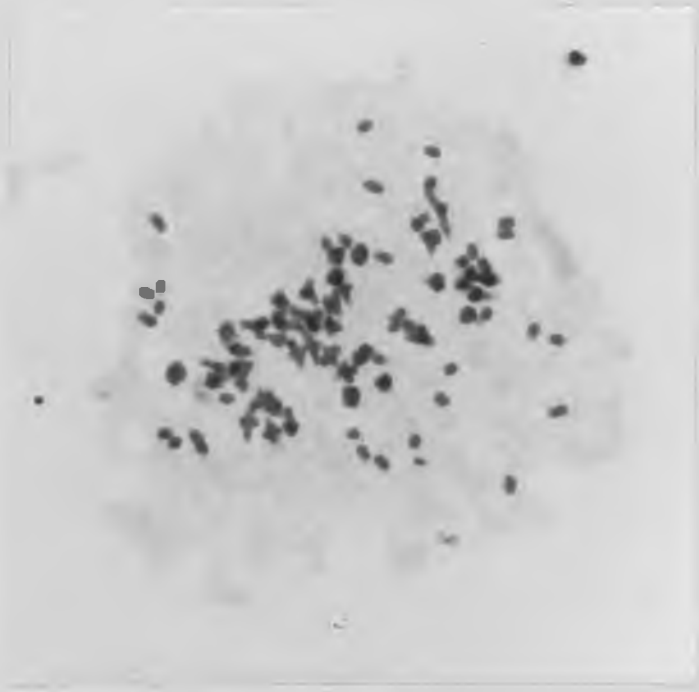


Fig. 72. Meiosis in synthesised triploid hybrid P. aculeatum 4x (Spain) x P. setiferum 2x (England). Magnification x 1200.

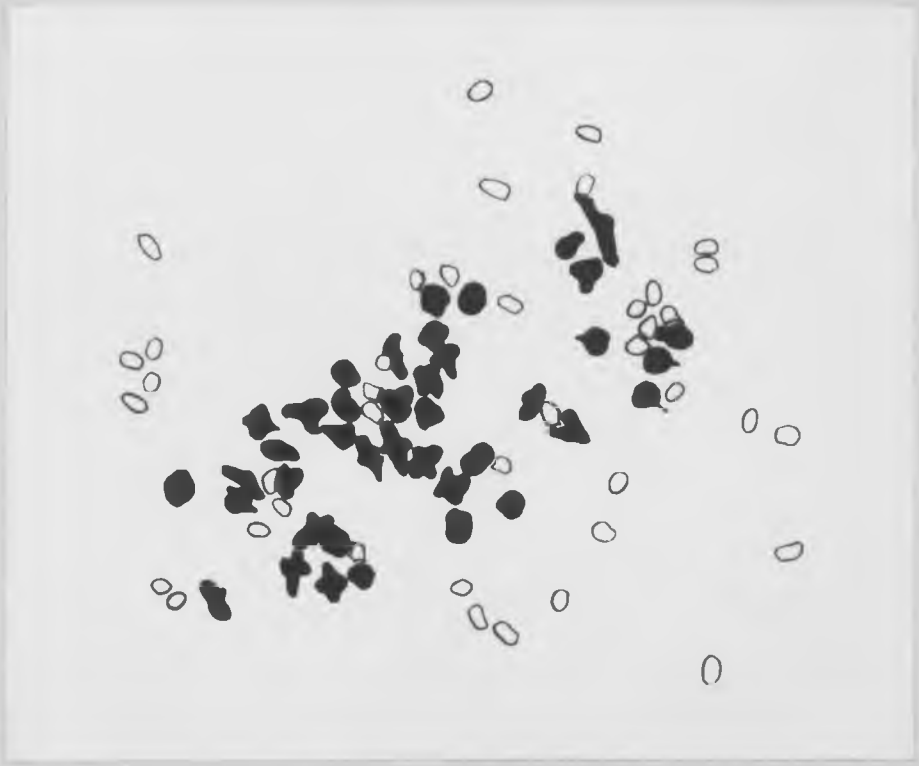


Fig. 72a. Explanatory diagram to Fig. 72 showing 41 bivalents (black) and 41 univalents (in outline). Magnification x 2000.

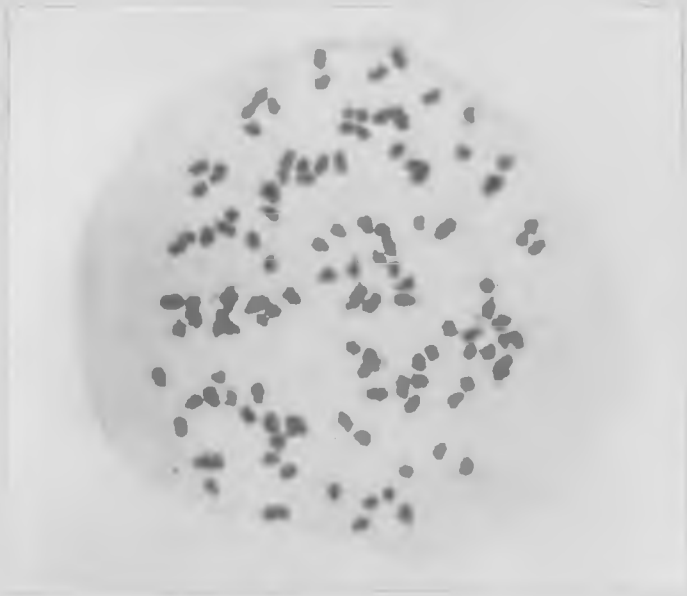


Fig. 73. Meiosis in synthesised triploid hybrid P. aculeatum 4x (Spain) x P. acrostichoides 2x (Vermont). Magnification x 1200.

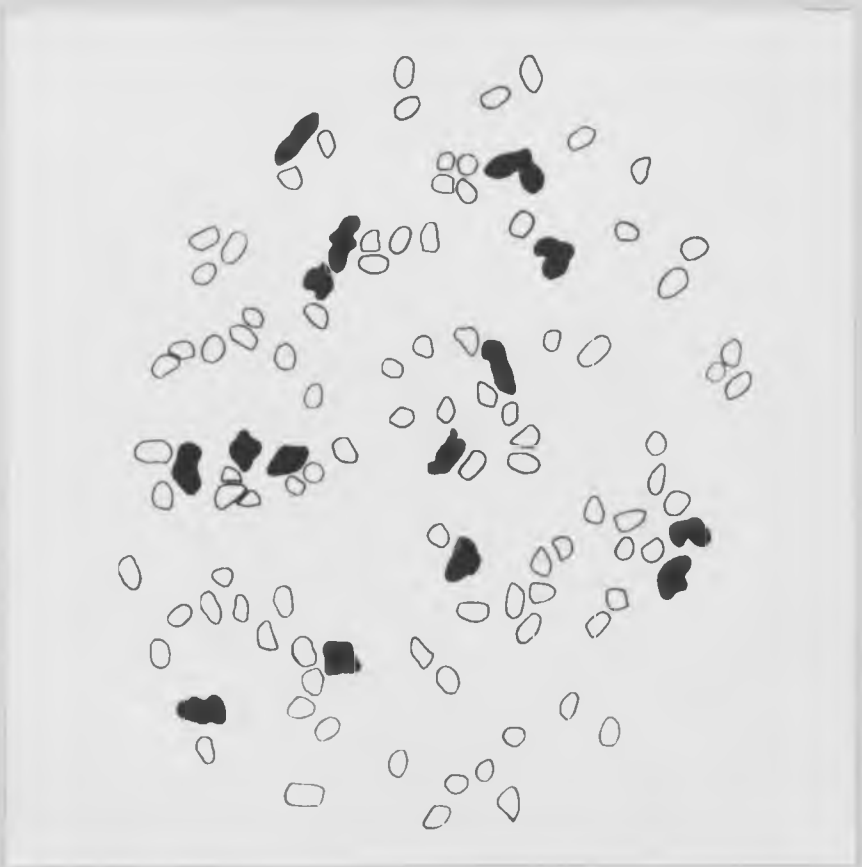


Fig. 73a. Explanatory diagram to Fig. 73 showing 15 bivalents (black) and 93 univalents (in outline). Magnification x 2000.

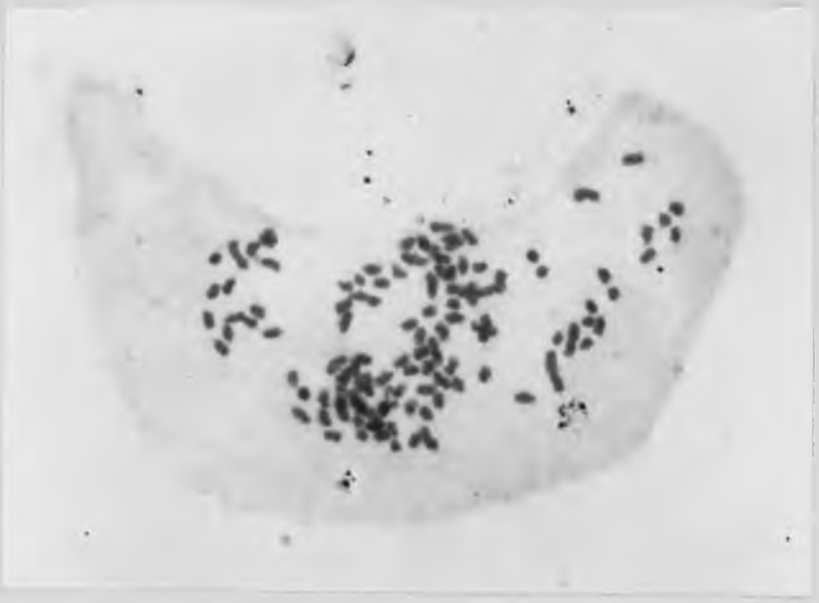


Fig. 74. Meiosis in synthesised triploid hybrid P. aculeatum 4x (Spain) x P. munitum 2x (Oregon). Magnification x 1200.

Fig. 74a. Explanatory diagram to Fig. 74 showing 19 bivalents (black) and 85 univalents (in outline). Magnification x 2000.

Fig. 75. Diagram of meiosis in synthesised triploid hybrid P. braunii 4x (Vermont, U.S.A.) x P. munitum 2x (Oregon, U.S.A.) showing 28 bivalents (black) and 67 univalents (in outline). Magnification x 2000.

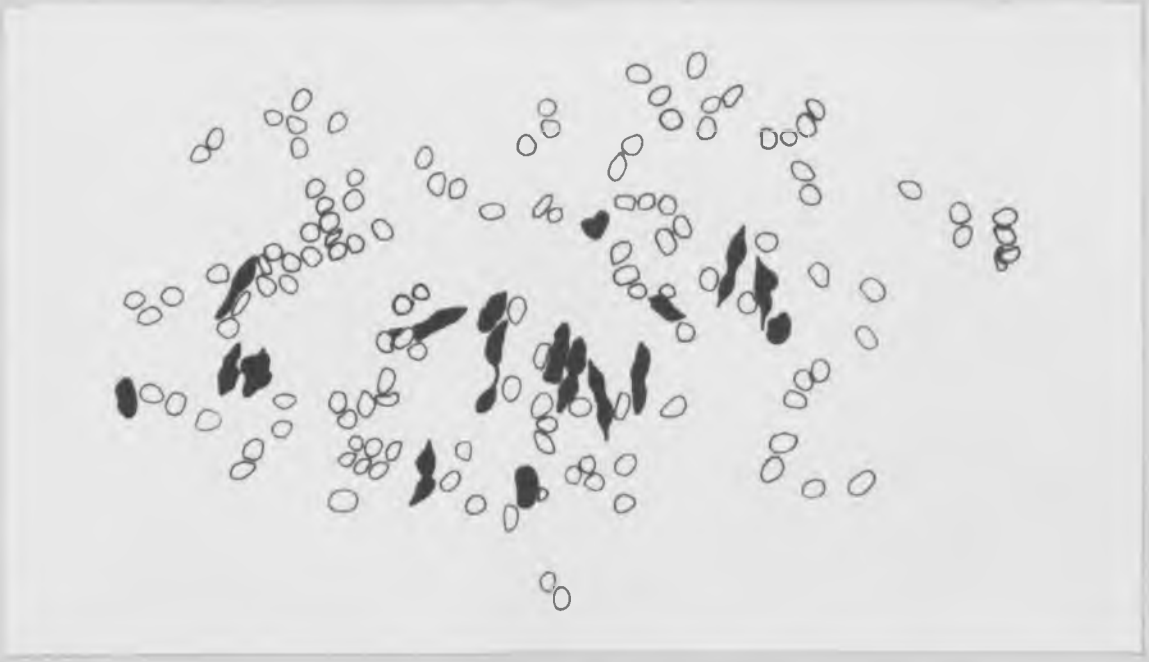
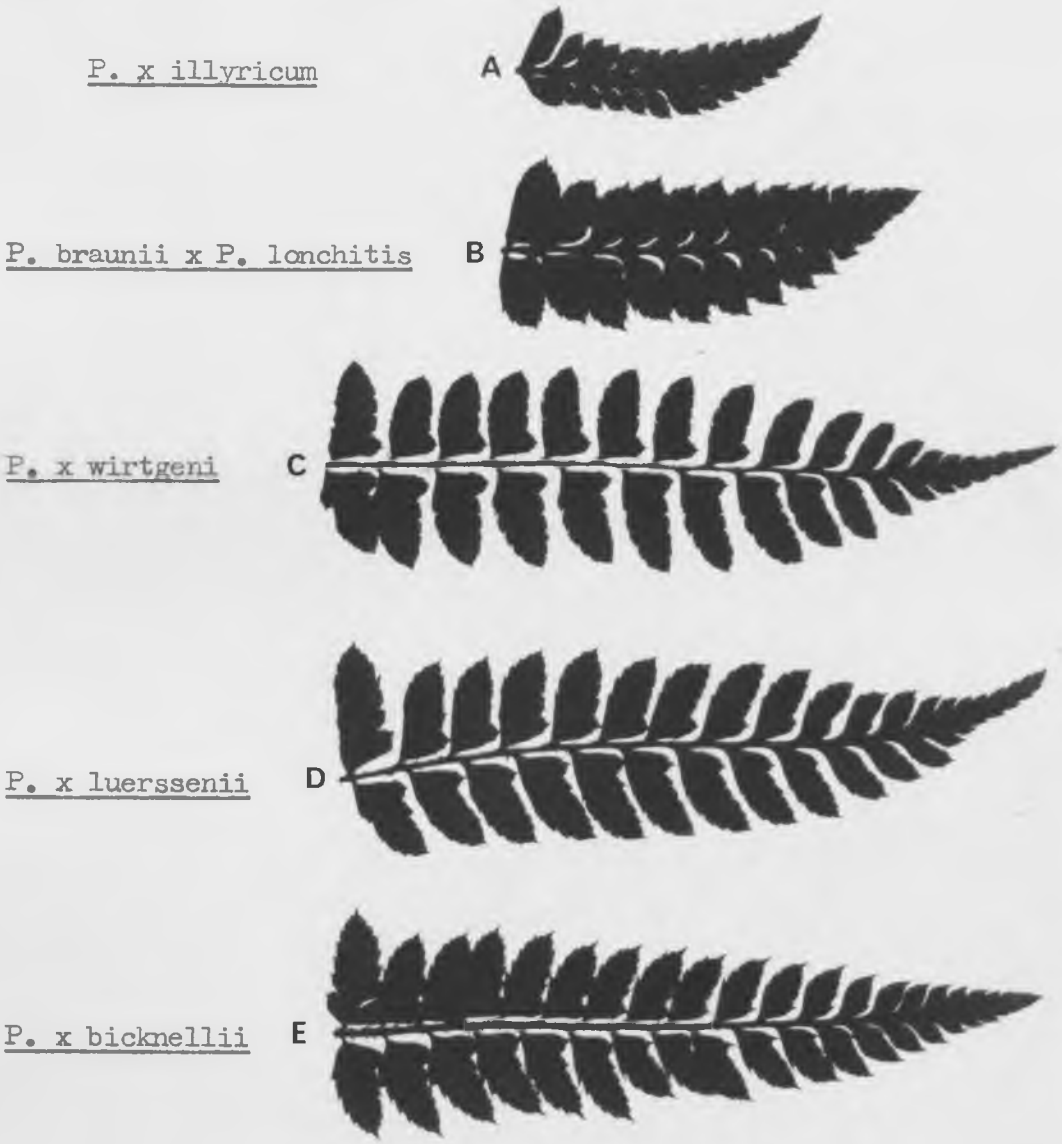


Fig. 76. AS/62/5. Diagram of meiosis in wild tetraploid hybrid P. x luerssenii (= P. braunii (4x) x P. aculeatum (4x)) from Krimml, Austria, showing 18 bivalents (black) and 128 univalents (in outline). Magnification x 2000.

Fig. 77. Individual pinnae of wild hybrids.



A. P. lonchitis x P. aculeatum from Floitental, Tirol, Austria.

B. Putative hybrid P. braunii x P. lonchitis from Floitental, Tirol, Austria.

C. P. braunii x P. setiferum from the Lassnitzklause, Deutschlandsberg, Styria, Austria.

D. P. braunii x P. aculeatum from Krimml, Austria.

E. P. setiferum x P. aculeatum from Grümpeli, Aargau, Switzerland.

TABLE IX. CYTOLOGY OF WILD HYBRIDS.*

1. P. x bicknellii (Christ) Hahne. P.setiferum x P. aculeatum.

Only one plant examined cytologically, this being the stock plant originally collected from Grümpeli, Switzerland. It is triploid, and the pairing shown in several cells was of the order of 41 paired and 41 unpaired chromosomes, thus agreeing with the result obtained from synthesised hybrids.

---

2. P. x illyricum (Borbás) Hahne. P. lonchitis x P. aculeatum.

Three plants were examined cytologically. Two, of my own collection, came from Pont-de-Nant, Switzerland, and the third was from Professor Manton's original collection from the same place. The plants were triploid, and all cells analysed confirmed the result of 41 bivalents and 41 univalents obtained previously by Professor Manton.

---

3. P. x wirtgeni Hahne. P. braunii x P. setiferum.

Only TR 471 examined cytologically. The results are shown below:

Bivalents per cell:	12	12	15	17
Univalents per cell:	99	99	93	89

---

4. P. x luerssenii (Dörfl.) Hahne. P. braunii x P. aculeatum.

Three plants examined cytologically: TR 954, and two stocks collected personally from Krimml, Austria, in 1962.

Bivalents per cell:	10	11	12	13	16	18
Univalents per cell:	144	142	140	138	132	128

---

* For further details of these collections see appendix on page 239.

Fig. 78. AS/485(i). Synthesised diploid hybrid P. lonchitis 2x (Haggenegg, Switzerland) x P. setiferum 2x (Gorge Chauderon, Switzerland) in second year of growth.

Fig. 79. Frond silhouettes of the synthesised diploid hybrid between P. lonchitis and P. setiferum (A and B) showing it's close resemblance to a young frond of P. aculeatum (C).

All silhouettes reproduced at natural size from fronds taken from two year old plants in cultivation at Leeds.

CHAPTER X. DISCUSSION OF THE CYTOLOGICAL RESULTS.

Some of the cytological findings in wild and synthesised hybrids referred to in the previous chapter were expected and are at first sight readily interpretable; others are not. To the first category belong the two triploid hybrids involving *P. aculeatum* with *P. lonchitis* and *P. setiferum* respectively which, as previously reported (Manton, 1950, Manton & Reichstein, 1961), were the reason for initially regarding *P. aculeatum* as a probable allotetraploid with the two diploid species (*P. lonchitis* and *P. setiferum*) representing the modern equivalents of the ancestral types. Previous observations on both wild and synthesised hybrids were repeated, and additional information was obtained from a synthesised hybrid of *P. lonchitis x P. aculeatum*. In all cases the new observations completely confirmed previous results.

As shown by the silhouettes illustrated on page 198 the diploid hybrid between *P. lonchitis* and *P. setiferum* is morphologically very similar to a young plant of *P. aculeatum*. Chromosome pairing is, however, not completely absent from this hybrid (see page 187). Attempts have been made to induce chromosome doubling in synthesised plants of *P. lonchitis x P. setiferum* (see page 20) but so far without success. Whether the pairing found in the diploid is of a kind that would produce multivalent associations in the resulting tetraploid is not yet known. It is possible, however, that it may not, since some capacity for chromosome pairing may exist in a genome but only be expressed in the absence of completely homologous chromosome partners. Even if multivalents are formed immediately following chromosome doubling their presence would

not necessarily invalidate the hypothesis regarding the origin of P. aculeatum as genetical mechanisms able to suppress multivalent formation can be evolved (Riley, 1960). The degree of chromosome pairing found in the diploid hybrid (6 - 28 bivalents per cell), although unexpected, is not therefore necessarily incompatible with the previous conclusion.

The possibility that P. aculeatum might be a cryptic autotetraploid capable of producing 41 pairs by autosyndesis is however eliminated by the behaviour of hybrids involving P. aculeatum with other, more distantly related, species. Wild or synthesised hybrids incorporating P. munitum (diploid), P. acrostichoides (diploid) and P. braunii (tetraploid) have been studied, and all show considerably less than 41 bivalents although chromosome pairing is not entirely absent.

Some pairing had previously been encountered by Manton & Reichstein (1961) in two wild hybrids involving P. braunii. These were the European P. x wirtgeni (= P. setiferum x P. braunii, triploid) and P. x luerssenii (= P. aculeatum x P. braunii, tetraploid). They explained the presence of a few pairs in both these hybrids in terms of autosyndesis within the P. braunii genome potentially produceable from a minor amount of segmental interchange between non-homologous chromosomes, and they did not regard it as giving any evidence of relationship between P. braunii and the other species involved. The latter conclusion still seems to be true, but the explanation for the pairing observed cannot easily be extended to cover the much wider selection of hybrids involving supposedly unrelated species which are now available (see Table X). All show virtually the same degree of pairing (generally of

the order of 10 - 20 bivalents per cell) which is seemingly independent of both the level of ploidy and of gross morphological differences between the species involved. In all the hybrids produced during the course of the present investigation, to which a few records from the literature may be added, there is no known example of complete absence of chromosome pairing in any inter-specific hybrid within the genus Polystichum.

TABLE X. POLYSTICHUM HYBRIDS BETWEEN SUPPOSEDLY UNRELATED SPECIES AND THE CHROMOSOME PAIRING THEY SHOW.

Hybrid.	Ploidy.	Source of Material.	Range of Pairing.	Origin.	Recorded by:
P. lonchitis x P. setiferum.	2x	Europe.	6 - 28"	Synthesised.	A. Sleep.
P. setiferum x P. acrostichoides.	2x	Europe & America.	9 - 18"	Synthesised.	A. Sleep.
P. acrostichoides x P. munitum.	2x	America.	16"	Synthesised.	A. Sleep.
P. munitum x P. fibrilloso-paleaceum.	2x	America & Japan.	19 - 23	Synthesised	A. Sleep. (Supplement).
P. lonchitis x P. acrostichoides.	2x	America.	25"	Synthesised	A. Sleep.
P. acrostichoides x P. lonchitis.	2x	America.	Meiosis irregular	Wild.	W. H. Wagner & K. L. Chen in Löve et Solbrig, 1964a. * 1

* 1 Footnote on following page.

Hybrid.	Ploidy.	Source of Material.	Range of Pairing.	Origin.	Recorded by:
P. mohrioides x P. munitum	2x	America.	Meiosis irregular.	Wild	W. H. Wagner & K. L. Chen in Löve et Solbrig, 1964a.
P. californicum (= P. dudleyi x P. munitum.)	2x	America.	Irregular pairing.	Wild.	W. H. Wagner,*2 1963.
P. andersoni x P. setiferum	3x	America & Europe.	c.20"	Synthesised.	A. Sleep. (Supplement).
P. aculeatum x P. acrostichoides.	3x	Europe & America.	9 - 18"	Synthesised.	A. Sleep.
P. aculeatum x P. munitum.	3x	Europe & America.	10 - 22"	Synthesised.	A. Sleep.

1. Footnote: From the publications of Wagner (Wagner in Fabbri, 1965) it seems likely that some pairing is found also in wild diploid hybrids. A sterile diploid form of P. californicum was found to have 2n = 82 chromosomes, with irregular meiotic pairing (Wagner, 1963), although no number was mentioned. In addition, two wild hybrids, namely P. acrostichoides x P. lonchitis (Wagner & Hagenah, 1954) and P. mohrioides x P. munitum, are both reported as having 2n = 82 and "irregular meioses". No further details are given and it is presumed from this statement that some bivalent formation is present, as the normal method of citation would be to quote n = 41 or n = 82'.

2. Footnote: The occurrence of sterile and fertile forms of P. californicum is of interest. From chromosome pairing in wild triploid hybrids (Wagner (1963) suggested that this species is an allotetraploid derived from the two diploids P. munitum and P. dudleyi (= P. haleakalense Brack.). That the fertile tetraploid form of P. californicum has arisen in the wild in recent times has direct bearing on the question of the origin of P. aculeatum.

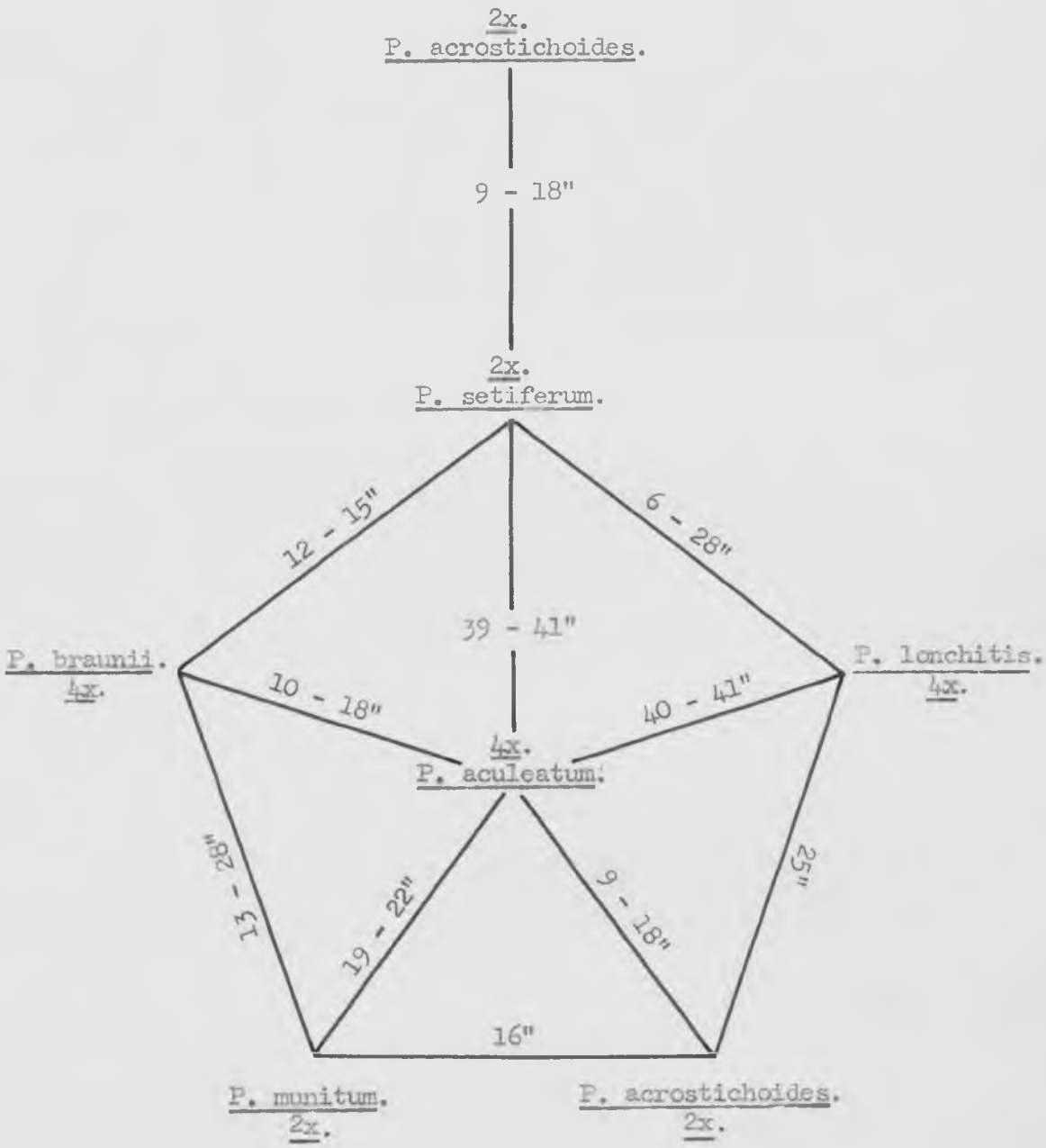
Hybrid.	Ploidy.	Source of Material.	Range of Pairing.	Origin.	Recorded by:
P. braunii x P. munitum.	3x	America.	13 - 28"	Synthesised.	A. Sleep.
P. braunii x P. fibrilloso-paleaceum.	3x	Europe & Japan.	c.20"	Synthesised.	A. Sleep. (Supplement).
P. setiferum x P. braunii	3x	Europe.	c.12"	Wild.	Manton & Reichstein, 1961.
"	"	"	12 - 15"	Wild.	A. Sleep.
P. braunii x P. aculeatum	4x	Europe.	c. 9"	Wild.	Manton & Reichstein, 1961.
"	"	"	10 - 18"	Wild.	A. Sleep.
"	"	"	11"	Synthesised.	A. Sleep.
P. aculeatum x P. proliferum	4x	Australia & Europe.	16 - 24"	Synthesised.	A. Sleep. (Supplement).
P. aculeatum x P. lucidum	4x	Europe & Africa.	17 - 23"	Synthesised.	A. Sleep. (Supplement).

The occurrence of such widespread pairing in a large number of Polystichum hybrids between supposedly unrelated species, in particular those at the diploid level, is a phenomenon not previously encountered in the Pteridophyta. Although detailed analyses are as yet only available from a limited number of cells, the results quoted above and illustrated

diagrammatically in Fig. 80 suggest that this pairing occurs to a similar degree at all the levels of ploidy investigated. It is found in synthesised hybrids involving species from such widely separated areas as Europe, America, Japan, Australia and Africa, and there is evidence that it occurs also in wild hybrids, both in Europe and America. In the next section this phenomenon will be discussed in relation to some of the flowering plant literature. It may be noted here, however, that the occurrence of this widespread pairing adds greatly to the difficulty of drawing phyletic conclusions from hybrids in this genus.

Little can therefore be added to the previous conclusions regarding the two European tetraploids. The resynthesis of P. aculeatum by doubling the chromosomes of the diploid hybrid already discussed should confirm it's suggested parentage. The only conclusion to be drawn concerning P. braunii is that the European diploids P. lonchitis and P. setiferum have no close relationship with it. It is hoped that this will be confirmed by a study of meiosis in the hybrid P. braunii x P. lonchitis, which on the basis of this work may now be expected to show chromosome pairing of the order of 10 - 20 bivalents per cell. On the basis of the above results it also seems unlikely that P. braunii is of autoploid origin, although further work is necessary to confirm this. It may be that diploid ancestors are yet to be found in Japan or elsewhere, and a further extension of the hybridisation programme is necessary to determine whether any direct relationship exists between P. braunii and the Japanese diploid P. ohmurae.

FIG. 80. RANGE OF PAIRING IN POLYSTICHUM HYBRIDS EXAMINED.



Where more than one cell has been analysed the figures given are the range of pairing found. Single figures represent an analysis from one cell only.

GENERAL DISCUSSION.

A few more general comments on the observations discussed in the two previous sections can now usefully be made. Beginning with *Asplenium*, it is clear that among the species studied three different evolutionary patterns have been encountered, namely:

1. Typical allopolyploidy (*A. majoricum* and *A. forisiense*),
2. Autopolyploidy (*A. petrarchae* and *A. billotii*), and
3. Genetical differentiation without gross cytological changes (*A. forisiense* and *A. macedonicum*).

Commenting on item 3 first, it is of interest to note that the facts for *A. forisiense* and *A. macedonicum* (page 122) represent the best example of regional morphological differentiation of one cytogenetic 'species' attributable to geographical isolation that has so far been traced among European Aspleniums (see Distribution Map on page 61).

With regard to autopolyploidy (item 2), the new observations add valuable additional examples to those already placed on record by Lovis (1964). There are now four European *Asplenium* species (*A. septentrionale*, *A. ruta-muraria*, *A. petrarchae* and *A. billotii*) which have been shown to be of effectively autoploid origin, to which may be added two further examples from the neighbouring genus *Phyllitis* * (Emmott, 1964). Collectively these represent one of the most important recent advances in the knowledge of evolutionary

* Footnote: Often included in the genus *Asplenium* (e.g. by Copeland, 1947).

mechanisms in ferns, as hitherto autopolyploidy has been generally believed to be of rare occurrence within the Pteridophyta, nearly all polyploids so far investigated being typical allopolyploids (Manton, 1950, 1961). To what extent the condition of *Asplenium* will be encountered in other fern groups cannot yet be known. It may be that the relatively widespread demonstration of autosyndesis in this genus is due in part to the large number of taxa so far subjected to detailed study, and in this connection it is of interest to note that in a recent investigation of a group of African taxa referable collectively to the *A. aethiopicum* complex (Braithwaite, 1964) evidence of autosyndetic pairing was also found.

A factor which may have some bearing on the incidence of polyploidy in ferns is the frequency with which the functioning of unreduced gametes has been recorded in hybrids. This has been encountered here in both the genera investigated, and other examples can be quoted from the literature. A list of some of the best authenticated instances is assembled below.

TABLE XI. RECORDS OF DOUBLED GAMETE FORMATION.

Hybrid	Ploidy (observed)	No. plants examined	Source of Material	Origin	Recorded by:
A. fontanum (2x) x *A. obovatum* (2x) x 2	3x	2	Europe	Synthesised	A. Sleep
A. majoricum (4x) x *A. obovatum* (2x) x 2	4x	1	Europe	Synthesised	A. Sleep
A. obovatum (2x) x 2 x *P. scolopendrium* var. *americana* (4x)	4x	1	Europe & America	Synthesised	J. I. Emmott.

Hybrid	Ploidy (observed)	No.plants examined	Source of Material	Origin	Recorded by:
P. hemionitis (2x) x2 x *P. scolopendrium* var. *americana* (4x)	4x	1	Europe & America	Synthesised	J. I. Emmott
Polystichum acrostichoides (2x?) x 2 x *P. braunii* (4x). *	4x	1	America	Wild	V. M. Morzenti (1962)
P. braunii (4x) x *P. acrostichoides* (2x) x 2	4x	1	Europe & America	Synthesised	A. Sleep
P. aculeatum (4x) x *P. fibrillosopaleaceum* (2x) x 2	4x	1	Europe & Japan	Synthesised	A. Sleep
Asplenium aethiopicum B 418. Kilimanjaro (4x) x B 448B Aberdare (4x) x 2	6x	1	Africa	Synthesised	A.F.Braithwaite
A. aethiopicum B 263 Cape (8x) x B 418 Kilimanjaro (4x) x 2	8x	1	Africa	Synthesised	A.F.Braithwaite
A. demerkense B 379 (8x) x *A. aethiopicum* B 366C Kilimanjaro (4x) x 2	8x	2	Africa	Synthesised	A.F.Braithwaite
A. aethiopicum Madeira (12x) x B 448B Aberdare (4x) x 2	10x	2	Africa & Madeira	Synthesised	A.F.Braithwaite
A. aethiopicum Madeira (12x) x B 418 Kilimanjaro (4x) x 2	10x	2	Africa & Madeira	Synthesised	A.F.Braithwaite

* Footnote: It is interesting to note that duplication of the normal diploid genome can also occur in the wild. *P. acrostichoides* is a normally diploid species with n = 41 chromosomes, and it is very likely that the tetraploid hybrid of *P. acrostichoides* x *P. braunii* has in fact resulted from the functioning of an unreduced gamete from the diploid parent. The possibility of a tetraploid stock of *P. acrostichoides*, however, cannot be overlooked.

With regard to allopolyploidy it is important to draw attention to the marked difference in meiotic behaviour of hybrids at the diploid level in the genera Polystichum and Asplenium. In European material of the latter genus inter-specific hybrids at the diploid level invariably show complete failure of chromosome pairing. A list of instances encountered by other workers is compiled in Table XII, from which it can be seen that there are a number of diploid taxa of Asplenium extant in Europe at the present time which are well differentiated from each other both morphologically and cytologically. These can potentially provide the raw material for the formation of new amphidiploid species by classical allopolyploidy, and some of the possible combinations have already been found. In Polystichum, on the other hand, although a high degree of morphological differentiation is found among the diploid species investigated, chromosome pairing of some kind is invariably present in inter-specific hybrids. It is therefore apparent that in this genus successful formation of polyploids can occur without complete cytological differentiation of the basic units. In other ferns information concerning cytological differentiation at the diploid level is available only for the genera Dryopteris and Polypodium. No published data exist concerning diploid hybrids in the ~~former~~ latter genus, but the triploid hybrid P. vulgare x P. australe (Shivas, 1961) shows virtually complete failure of chromosome pairing. In Dryopteris similar behaviour has been observed, e.g. in the wild hybrid D. x bootii (Walker, 1961). Further information is available from the following wild diploid hybrids: D. marginalis x D. intermedia

(Manton & Walker, 1953), D. x leedsii (Walker, 1962b) and D. goldiana x D. intermedia (Evans & Wagner, 1964). All three are sterile and show c. 82 unpaired chromosomes at meiosis. Thus in these genera there is as yet no recorded example of the irregular pairing which has been found in Polystichum.

TABLE XII.

MEIOTIC BEHAVIOUR OF DIPLOID HYBRIDS IN EUROPEAN ASPLENIUMS.

Hybrid	Origin	Analysis of Meiosis	Recorded by:
Pleurosorus hispanicus (2x) x A. glandulosum ssp. bivalens (2x)	Synthesised	72'	J. D. Lovis, unpublished result.
A. x adulteriniforme = A. trichomanes ssp. inexpectans (2x) x A. viride (2x)	Wild	72'	Lovis, Melzer & Reichstein. 1965.
A. viride (2x) x A. fontanum (2x)	Synthesised	72'	J. D. Lovis, unpublished result.
A. x woynarianum = A. cuneifolium (2x) x A. viride (2x)	Wild	72'	J. D. Lovis, unpublished result.
A. viride (2x) x A. trichomanes ssp. trichomanes (2x)	Synthesised	72'	J. D. Lovis, unpublished result.
"	Wild	72'	J. D. Lovis, unpublished result.
A. cuneifolium (2x) x A. onopteris (2x)	Synthesised	72'	M. G. Shivas, 1956.
Phyllitis hemionitis (2x) x P. scolopendrium (2x)	Synthesised	2" + 68'	J. I. Emmott, 1963, 1964.

Footnote: It should be noted however that diploid taxa within the A. trichomanes complex show some chromosome pairing in synthesised hybrids (e.g. A. tripteropus (Japan) x A. trichomanes ssp. trichomanes).

Within Flowering Plants the work of Riley and his co-workers on the control of meiotic pairing in wheat seems to have relevant bearing on the situation found in Polystichum. The bread wheat, Triticum aestivum is an allohexaploid with 42 chromosomes. By studying the meiotic pairing in experimental hybrids between wheats of different levels of ploidy and their relatives it can be shown that this complement consists of three distinct diploid chromosome sets which were brought together by hybridisation. Hybrids between pairs of the three diploid species involved in the origin of hexaploid T. aestivum commonly form bivalents, with a mean of 3 - 4 paired chromosomes per cell out of a possible maximum of 7. Riley (1960) uses the term homoeologous to describe the relationship between these chromosomes, and Sears (1953) and Okamoto (1962) have been able to identify the seven probable genetically 'homoeologous' groups, and have shown that each group has one chromosome pair in each of the three genomes ancestral to hexaploid wheat. However, despite the homoeology at the diploid level only regular bivalents are formed in the allohexaploid derivative, where each chromosome pairs only with it's fully homologous partner and there is no pairing between homoeologous chromosomes. The affinity of the homoeologous is clearly suppressed in some way in the hexaploid, and the nature of the controlling influence has been shown by Riley (1960) to a genetic factor or factors carried on chromosome V. Although such a mechanism cannot necessarily be assumed to be present in Polystichum, the concept of homoeologous pairing is obviously relevant to the situation described above. The term 'homoeologous' was first introduced by Huskins (1932) to describe the

pairing of 'similar' but not identical chromosomes. One may therefore accept as normal homologous pairing those instances (pages 183, 184 and 205) in which exactly or approximately n bivalents are formed in hybrids (n in this case being 41). The observations concerning hybrids at the diploid level, which although between parents of distinct morphology, never fail to show some chromosome pairing, may therefore be interpreted as bivalent formation between homoeologous chromosomes. Such an explanation would be fully consistent with the results obtained and also with the postulated origin of P. aculeatum. The occurrence of a similar degree of pairing at both the triploid and tetraploid levels is, on the other hand, an observation for which there is as yet no adequate explanation.

Results such as those of Riley et al. have so far been obtainable only with regard to some of the more intensively studied crop plants, and other Flowering Plant comparisons are therefore unrewarding at this stage. It may be noted, however, that complete failure of pairing in diploid hybrids seems to be a rarer phenomenon in Angiosperms than has so far been observed in the ferns (see Table XII).

While it cannot be assumed that all possible evolutionary mechanisms present in the Pteridophyta have yet been recognised some of the types of speciation discussed above have already been encountered more than once. Examples of autopolyploidy are at present restricted to Asplenium, but allopolyploidy is well-known in some genera (e.g. Dryopteris, Polypodium) and highly probable in many others. Further comparison shows a number of similarities in the distributional patterns of the fern

species investigated. Polystichum braunii (see map on page 150) shares several features of world distribution with Polypodium and Phyllitis. All three are present in Europe, eastern North America and Japan, and are thus circumpolar in total range although they are absent from quite large areas of the Northern Hemisphere. Phyllitis is confined to these areas and shows relict status in two of them (eastern North America and the Far East). P. braunii and Polypodium are also represented in western North America and in some other parts of Asia. One can scarcely doubt that all three are ancient groups now occupying disjunct distributions in the Northern Hemisphere, and that their present discontinuous areas are most probably remnants of a once circumpolar distribution which has become much reduced as a result of severe climatic changes during the Pleistocene glaciations (see Hultén, 1937).

The distribution of some of the Asplenium species studied similarly shows features in common with other genera, and particular reference may be made to the two diploid taxa found in Europe(A. fontanum and A.obovatum), both of which show a restricted distribution in the Mediterranean region (see maps on pages 44 and 61). This familar refuge area harbours many ancient diploids which are ancestral to more temperate polyploids , and examples of other diploids showing a relict distribution within this region are Phyllitis hemionitis, Polypodium australe and Asplenium onopteris. The two latter species do however extend outside the Mediterranean basin and both show a restricted Lusitanian distribution in the British Isles.

SUMMARY.

1. Cytotaxonomic studies have been carried out on six European Asplenium species (described in Chapter III) and on the two tetraploids Polystichum aculeatum and P. braunii. Chromosome pairing at meiosis has been studied in both wild and artificially synthesised hybrids, and within both genera information concerning the phyletic affinities and possible mode of origin of the tetraploid taxa has been obtained.

2. Information is given concerning the cytology, taxonomy, distribution and ecology of the species investigated, together with a comparison of the morphology of the A. forisiense complex.

3. Within Asplenium clear evidence of both autopolyploidy and allopolyploidy has been obtained. Examples of the former are A. petrarchae and A. billotii, both of which show autosyndetic pairing in hybrids. Positive evidence for an allopolyploid origin is available for the taxa A. forisiense, A. macedonicum and A. majoricum. The latter has been shown to have the constitution A. fontanum x unknown, and it is suggested on morphological grounds that the second parent could possibly be the recently discovered diploid cytotype of A. petrarchae. The two tetraploids A. forisiense and A. macedonicum have been found to have the same cytogenetic origin (A. fontanum x A. obovatum). In cultivation slight morphological differences are maintained

between plants of these two taxa and it is suggested that these are the result of genetical divergence following geographical isolation over a long period of time.

4. The conclusion that A. forisiense and A. macedonicum are in all probability the same allotetraploid species necessarily has taxonomic implications, and it is suggested that both should be regarded as sub-species of one taxon, namely A. forisiense.

5. A phenomenon completely new to the ferns is the occurrence of widespread pairing at all levels of ploidy which has been found in hybrids involving species of Polystichum and which can most easily be interpreted as pairing between homoeologous chromosomes. It has invariably been found in all the diploid hybrids investigated (regardless of seemingly gross morphological differences between the parental species) and also in some triploid and tetraploid hybrids. Although the number of bivalents in such hybrids is variable (6 - 28 per cell) it commonly ranges from 12 - 20 bivalents per cell, and the variation appears to be completely independent of the level of ploidy. Only when the number of paired chromosomes approximates to the monoploid number (in this case 41) can homologous pairing be safely inferred. Such evidence has not been encountered in any hybrid involving P. braunii with other European or American species, although it has been found in the two triploid hybrids involving the diploids P. lonchitis and P. setiferum with the European tetraploid

P. aculeatum, where c. 41 bivalents are regularly recorded.

6. The evidence from these two hybrids thus appears to confirm the previous supposition regarding the mode of origin of *P. aculeatum* from *P. lonchitis* and *P. setiferum*, and this hypothesis is consistent with information obtained from other hybrids involving *P. aculeatum*.

7. As the interpretation of chromosome pairing in *Polystichum* hybrids is complicated by the apparent presence of homoeologous as well as homologous pairing, no conclusions can be drawn with certainty regarding the affinities or mode of origin of *P. braunii*. The cytological results do however confirm the distinctness of this species from the other European Polystichums.

8. The marked difference in meiotic behaviour of hybrids at the diploid level in the genera *Polystichum* and *Asplenium* is noted. In the latter a number of diploid species exist in the European flora which are well-differentiated from each other both morphologically and cytologically. This contrasts with the situation observed in *Polystichum*, where some chromosome pairing is invariably present in inter-specific hybrids although the diploid taxa involved are all of distinct morphology.

9. The postulated functioning of doubled gametes has been encountered twice in each of the genera investigated during the course of the

present investigation, and it is suggested that this could possibly have some bearing on the incidence of polyploidy as such.

10. The cytological results are discussed in relation to the present geographical distributions of the various taxa.

11. Chromosome counts of additional Polystichum species (from Japan and Africa) not used in the main investigation are listed as a Supplement, together with preliminary cytological data from synthesised hybrids involving some of the same material.

REFERENCES.

Abrams, L. 1940. Illustrated Flora of the Pacific States. 1. Stanford University Press.

Alston, A.H.G. 1940. The correct application of the name Polystichum aculeatum. J. Bot., Lond. 78, 160-164.

Ascherson, P.F.A. & Graebner, K.O.P.P. 1912. Synopsis der mitteleuropäischen Flora. 1. Ed.2. Leipzig.

Becherer, A. 1929. Asplenium lanceolatum Hudson, ein zu verwerfender name. Ber. schweiz. bot. Ges. 38, 26-29.

---------- 1935. Note sur deux espèces d'Asplenium. Candollea 6, 22-24.

Bernhardi, J.J. 1799. In Schrader, H.A. Journal für die Botanik. 1, 314. Göttingen.

Bornmüller, J. 1928. Beiträge zur Flora Mazedoniens III. Bot. Jb. 61, Beiblatt 140, 188-189.

Braithwaite, A.F. 1964. A cytotaxonomic investigation on the Asplenium aethiopicum complex in Africa. Ph.D. thesis, University of Leeds.

Briquet, J.I. 1910. Prodrome de la Flore corse. 1. Geneve, Bâle & Lyon.

Chambers, T.C. 1955. The use of snail stomach cytase in plant cytology. Nature, Lond. 175, 215.

Chiarugi, A. 1960. Tavole cromosomiche delle Pteridophyta. Caryologia 13 (1), 27-150.

Christ, H. 1900. Die Farnkräuter der Schweiz. Beiträge zur Kryptogamenflora der Schweiz. 1 (2). Bern.

--------- 1902. Die Farnflora der östlichen Riviera. Allg. bot. Z. 8 (9/10), 141-147.

Christensen, C. 1906. Index Filicum, and Supplements I (1913), II (1917) and III (1934). Hafniae.

Clapham, A.R., Tutin, T.G. & Warburg, E.F. 1962. Flora of the British Isles. Ed.2. Cambridge.

Clausen, J., Keck, D. & Hiesey, W.M. 1945. Experimental studies on the nature of species II. Carnegie Institution, Washington. Publ. No.564.

Copeland, E.B. 1947. Genera Filicum. Waltham, Massachusetts, U.S.A.

Crabbe, J.A., Jermy, A.C. & Lovis, J.D. 1964. Asplenium. In Flora Europaea (Tutin, T.G., et al. Ed.). 1, 14-17. Cambridge.

Darby, H.C. (Ed.) 1944. Jugoslavia. 1: Physical Geography. Geographical Handbook Series. No. B.R.493. Naval Intelligence Division.

Davis, P.H. 1965. Flora of Turkey. 1. Edinburgh, at the University Press.

Degener, O. & Degener, I. 1963. Flora Hawaiiensis or New Illustrated Flora of the Hawaiian Islands. Book 6. Family 17b.

Eberle, G. 1959. Unsere mitteleuropäischen Schildfarne (Polystichum) in lichte neuer Erkenntnisse. Natur Volk. 89, 407-414.

Emmott, J.I. 1963. A cytotaxonomical investigation of the north temperate species of the genus Phyllitis. Ph.D. thesis, University of Leeds.

---------- 1964. A cytogenetic investigation in a Phyllitis - Asplenium complex. New Phytol. 63, 306-318.

Evans, A.M. & Wagner, W.H. Jr. 1964. Dryopteris goldiana x intermedia - A natural woodfern cross of noteworthy morphology. Rhodora 66, 255-266.

Ewan, J. 1944. Annotations on west American ferns III. Am. Fern J. 34 (4), 107-120.

Fabbri, F. 1963, 1965. Supplements to Tavole cromosomiche delle Pteridophyta di Alberto Chiarugi. I (1963) Caryologia 16 (2), 237-335. II (1965) Caryologia 18 (4), 675-731.

Fée, A.L.A. 1852. Genera Filicum. (Mém. Fam. Foug. 5). Strasbourg.

Fernald, M.L. 1928. The eastern American variety of Polystichum braunii. Rhodora 30, 28-31.

---------- 1950. Gray's Manual of Botany. Ed.8. American Book Company.

Fernandes, R. 1960. Notas sobra a Flora de Portugal VIII. Bolm Soc. broteriana. (2) 34, 99-165.

Fiori, A. 1943. Flora Italica Cryptogama. 5. Firenze.

Fomin, A.B. 1911. A note on new ferns from the Caucasus. Vêst. tiflis. bot. Sada. 18, 21-22.

---------- 1930. Flora Sibiriae et Orientis Extremi. 5. Leningrad.

Forskål, P. 1775. Flora Aegyptiaco-Arabica etc. Hauniae.

Guérin, J. 1804, 1813. Description de la fontaine de Vaucluse. Ed.1. (1804). Ed.2. (1813). Avignon.

Halácsy, E. von 1901. Conspectus Florae Graecae. 1. Leipzig.

---------- 1904. Aspidium aculeatum x lonchitis nov. hybr. Verh. zool.-bot. Ges. Wien. 54, 129-132.

Hayek, A. von 1924. Prodromus florae peninsulae Balcanicae. In Feddes Repert. Beih. 30. 1.

Hopkins, L.S. 1913. A new Polystichum from British Columbia. Am. Fern J. 3 (4), 116-118.

Hudson, W. 1778. Flora Anglica. Ed.2. London.

Hultén, E. 1927. Flora of Kamtschatka and the adjacent islands I. K. svenska Vetensk-Akad. Handl. (3) 5 (1), 38-39. (Pteridophyta determined by C. Christensen).

---------- 1937. Outline of the history of arctic and boreal biota during the Quaternary period. Stockholm.

---------- 1950. Atlas of the distribution of vascular plants in north-west Europe. Stockholm.

Huskins, C.L. 1932. A cytological study of vilmorin unfixable dwarf wheat. J. Genet. 25, 113-124.

Jermy, A.C. & Lovis J.D. 1964. Asplenium majoricum Litardière. Br. Fern Gaz. 9 (5), 163-167.

Kossinksky, C. 1922. Asplenium pseudofontanum sp.n. Bot. Mater. Gerb. glavn. bot. Sada 3, 121-124.

Kümmerle, J.B. 1916. Adatok a Balkánfélsziget Pteridophtáinak ismeretéhez. Bot. Közl. 15, 143-148.

---------- 1921. Asplenium Bornmülleri Kümm. spec. nova. Bot. Közl. 19, 81-83. (German summary, with additions, on pp. (13) & (14)).

Kurata, S. 1956. Notes on Japanese Ferns IX. Hokuriku J. Bot. 5 (3), 77-80.

---------- 1961. Notes on Japanese Ferns XXI. J. Geobot., Kanzawa 9 (3/4), 95-100.

---------- 1964. On the Japanese ferns belonging to the Polystichum polyblepharum group. Sci. Rep. Yokosuka Cy Mus. 10, 17-41.

Lamarck, J.B.A.P.M. de & de Candolle, A.P. 1815. Flore française, 238. 1. Ed.3. Paris.

Lanjouw, J. et al. (Ed.) 1961. International Code of Botanical Nomenclature, adopted by the 9th International Botanical Congress. Regnum Vegetabile 23. Utrecht.

Le Grand, A. 1869. Asplenium Halleri DC. Bull. Soc. bot. Fr. 16, 61.

---------- 1873. Statistique botanique du Forez. St Etienne. 252.

Linnaeus, C. 1753. Species Plantarum. Holmiae.

Litardière, R. de 1911. Contribution à l'étude de la Flore ptéridologique de la pénisule ibérique. Bull. Geogr. bot. 21, 28-29.

Loiseleur-Deslongchamps, J.L.A. 1810. Notice sur les Plantes à ajouter à la Flore de France (Flora Gallica) etc. Paris. 145.

Lovë, A. & Solbrig, O.T. 1964a. IOPB chromosome number reports I. Taxon 13 (3), 99-110.

Lovis, J.D. 1955b. Asplenium adulterinum and it's probable parents. Proc. bot. Soc. Br. Isl. 1, 388-390.

---------- 1958. An evolutionary study of the fern Asplenium trichomanes. Ph.D. thesis, University of Leeds.

---------- 1963. Meiosis in Asplenium x murbeckii. Br. Fern Gaz. 9 (4), 110-113.

Lovis, J.D. 1964. Autopolyploidy in Asplenium. Nature, Lond. 203, 324-325.

Lovis, J.D., Melzer, H. & Reichstein, T. 1965. Asplenium x adulteriniforme hybr.nov. = diploides Asplenium trichomanes L. x A. viride Hudson. Bauhinia 2 (3), 231-237 and 315-321.

Luerssen, C. 1889. Die Farnpflanzen. In Rabenhorst, L. Kryptogamen-Flora. 3. Leipzig.

Manton, I. 1950. Problems of cytology and evolution in the Pteridophyta. Cambridge.

---------- 1961. Evolution in the Pteridophyta. In 'A Darwin Centenary' (Ed. Wanstall, P.J.), 105-119. B.S.B.I. Conference Report, Arbroath.

Manton, I. & Reichstein, T. 1961. Zur cytologie von Polystichum braunii (Spenner) Fée und seiner hybriden. Ber. schweiz. bot. Ges. 71, 370-383.

---------- 1962. Diploides Asplenium obovatum Viv. Bauhinia 2, (1), 79-91.

Manton, I. & Walker, S. 1953. Cytology of the Dryopteris spinulosa complex in eastern North America. Nature, Lond. 171, 1116-1117.

---------- 1954. Induced apogamy in Dryopteris dilatata (Hoffm.) A. Gray and D. filix-mas (L.) Schott emend. and it's significance for the interpretation of the two species. Ann. Bot. n.s. 18, 377-383.

Maxon, W.R. 1918. Polystichum andersoni and related species. Am. Fern J. 8 (2), 33-37.

Mayer, E. 1963. Pregled Pteridofitov Jugoslavije. Raspr. slov. Akad. Znan. Umet. 7, 47-73.

McClintock, D. 1961. The ~~small~~ ferns of the Channel Islands. Br. Fern Gaz. 9 (2), 34-37.

Meyer, D.E. 1952. Untersuchungen uber Bastardierung in der Gattung Asplenium. Biblthca bot. 30 (123).

---------- 1959. Über einen neuen farnbastard aus Kärnten. Ber. dt. bot. Ges. 72 (11), 15.

Meyer, D.E. 1959a. Ein neuer Farnbastard gefunden Polystichum braunii x lonchitis. Naturwissenschaften 46, 237-238.

---------- 1959b. Polystichum x eberlei (P. braunii x lonchitis) ein neuer Farnbastard. Nova Hedwigia 1, 105-114.

---------- 1960. Zur Gattung Polystichum in Mitteleuropa. Willdenowia 2 (3), 336-342.

---------- 1964. Über neue und seltene Asplenien Europas III. Ber. dt. bot. Ges. 77, 3-13.

Milde, J. 1858. Ueber Aspidium lobatum, aculeatum und braunii. Öst. bot. Z. 8, 184-190.

Morzenti, V.M. 1962. A first report on pseudomeiotic sporogenesis, a type of spore reproduction by which 'sterile' ferns produce gametophytes. Am. Fern J. 52 (2), 69-78.

Okamoto, M. 1962. Identification of the chromosomes of common wheat belonging to the A and B genomes. Can. J. Genet. Cytol. 4, 31-37.

Ohwi, J. 1965. A Flora of Japan. Smithsonian Institution.

Perring, F.H. & Walters, S.M. 1962. Atlas of the British Flora. Published by Nelson for the Botanical Society of the British Isles.

Pichi-Sermolli, R.E.G. et al. (Ed.). 1965. Index Filicum Supplementum quartum pro annis 1934-1960. International Bureau for Plant Taxonomy and Nomenclature, Utrecht.

Pinto da Silva, A.R. 1959. Plantas novas e novas areas para a flora de Portugal V. Agronomia lusit. 20, 217-247.

Pinto da Silva, A.R. & Sobrinho, G.L. 1950. Flora vascular da serra do Gerês. Agronomia lusit. 12, 233-402.

Rechinger, K.H. 1943. Flora Aegaea. Denkschr. Akad. Wiss., Wien. Math.-nat. Kl. 105 (1)

Riley, R. 1960. The diploidisation of polyploid wheat. Heredity 15, 407-429.

Rossi, L. 1911. Beiträge zur Kenntnis der Pteridophyta Süd-Kroatiens. Magy. bot. Lap. 10, 22-38.

Roth, A.W. 1799. Tentamen Florae Germanicae. 3. Lipsiae.

Rouy, G. 1913. Flore de la France. 14. Paris.

Schulze, F.W. 1845. Einige neue Pflanzenarten. Flora (Regensb.) 28, 738.

Sears, E.R. 1953. Nullisomic analysis in common wheat. Am. Nat. 87, 245-252.

Shivas, M.G. 1956. Some problems in cytology and taxonomy in the genera Polypodium and Asplenium. Ph.D. thesis, University of Leeds.

---------- 1961. Contributions to the cytology and taxonomy of species of Polypodium in Europe and America. I. Cytology. J. Linn. Soc. (Bot.) 58, 13-25.

Spenner, F.C.L. 1825. Flora Friburgensis et regionem proxime adjacentium. Friburgi Brisgoviae. 1, 9.

Stebbins, G.L. Jr. 1947. Types of polyploids: their classification and significance. Adv. Genet. 1, 403-429.

---------- 1950. Variation and evolution in plants. London, Oxford University Press.

Steyermark, J.A. 1963. Flora of Missouri. The Iowa State University Press.

Sudre, M.H. 1894. Notes sur quelques plantes critiques de la flore du Tarn. Revue Bot., Courrensan 12, 29-30.

Tagawa, M. 1940. Polystichum of Japan, Korea and Formosa I. Acta phytotax. geobot., Kyoto 9 (3), 119-138.

---------- 1962. Coloured illustrations of the Japanese Pteridophyta. Osaka.

Taylor, T.M.C & Lang, F. 1963. Chromosome counts in some British Columbia Ferns. Am. Fern J. 53 (3), 123-126.

Thompson, R.H. & Coffin, R.L. 1940. A natural hybrid between Polystichum braunii (Spenner) Fée and P. acrostichoides (Michx.) Schott. Am. Fern J. 30 (3), 81-88.

Tjio, J.H. & Levan, A. 1950. The use of oxyquinoline in chromosome analysis. An. Estac. exp. Aula Dei 2 (1), 21.

Turrill, W.B. 1929. The Plant-Life of the Balkan Peninsula. Oxford. At the Clarendon Press.

Valentine, D.H. 1964. Polystichum. In Flora Europaea (Tutin, T.G., et al. Ed.). 1, 20. Cambridge.

Verma, S.C. 1961. In Mehra, P.N. 1961. Chromosome numbers in Himalayan ferns. Res. Bull. Panjab. Univ. Sci. n.s. 12, 139-164.

Verma, S.C. & Loyal, D.S. 1960a. Chromosome counts in some ferns from Naini Tal. Curr. Sci. 29, 69-70.

Vida, G. 1963. A Dryopteris nemzetség (sensu lato) szisztematikaja. Bot. Közl. 50 (3), 125-133.

Viviani, D. 1824. Florae libycae specimen etc. Genuae. 68.

---------- 1824. Florae Corsicae Specierum novarum diagnosis etc. Genuae.

Wagner, W.H. Jr. 1963. A biosystematic survey of United States ferns - preliminary abstract. Am. Fern J. 53 (1), 1-16.

Wagner, W.H. Jr. & Hagenah, D.J. 1954. A natural hybrid of Polystichum lonchitis and P. acrostichoides from the Bruce peninsula. Rhodora 56, 1-6.

Walker, S. 1953. A cytogenetic and taxonomic study of the Dryopteris spinulosa complex in Europe and eastern North America. Ph.D. thesis, University of Leeds.

---------- 1961. Cytogenetic studies in the Dryopteris spinulosa complex II. Am. J. Bot. 48 (7), 607-614.

---------- 1962b The problem of Dryopteris leedsii. Am. J. Bot. 49 (9), 971-974.

Walker, T.G. 1956. Cytotaxonomic studies in the tropical fern genus Pteris. Ph.D. thesis, University of Leeds.

Whittier, D.P. & Steeves, T.A. 1960. The induction of apogamy in the Bracken fern. Can. J. Bot. 38, 925-930.

Willdenow, C.L. 1810. Ed.4 of Linnaeus, C. Species Plantarum. 5. Berolini.

APPENDIX I.

ADDITIONAL LOCALITY DETAILS OF ASPLENIUM SPECIES SUCCESSFULLY INCORPORATED INTO HYBRIDS. (numbers as in list on page 26).

1. A. fontanum (L.) Bernh. AS/24; AS/25.

From steep woodland by road-side near Roche. Growing in crevices of shaded, west-facing, calcareous rocks with Polypodium australe Fée. Altitude c.400 metres.

Between Villneuve and Aigle, Canton Vaudois, Switzerland.

Legit: A. Sleep. 28.7.61.

2 plants cultivated in Leeds.

2. A. fontanum (L.) Bernh.

Exsc. Walter-Callé. 3me. fasc. 1946. No. 180.

Pyrénées françaises: fissures de rocs calcaires au-dessus de l'entree de la grotte de Niaux. Dept. Ariège, France.

Legit: Cl. Leredde. 31.8.46.

2 plants in cultivation at Leeds, raised originally from spores taken from a herbarium sheet at the British Museum (Natural History).

3. A. fontanum (L.) Bernh.

Pas au Col Vert, near Villard de Lans, Dept. Isère, France.

Legit: A. H. G. Alston. 29.9.49.

2 plants in cultivation at Leeds, raised originally from spores taken from a herbarium sheet at the British Museum (Natural History).

5. A. fontanum (L.) Bernh. TR 16.

Val di Llo, south-east of Saillagouse, Pyrénées-Orientales, France.

Altitude c.1500m.

Legit: H. Kunz and T. Reichstein. 12.8.57.

Cultivated in Basel.

6. A. fontanum (L.) Bernh. TR 69.

Limestone rocks by road-side, partly under trees. Fort d'Ecluse, near Collonges, south-west of Geneva in the valley of the river Rhône, Haute-Savoie, France.

Altitude c.450m.

Legit: H. Kunz and T. Reichstein. 9.4.59.

Cultivated in Basel.

9. A. obovatum Viv. TR 621.

On silicate rocks, altitude c.150 metres, growing with A. billotii F. Schulze. Trinité near Bonifacio, south-west Corscia.

Legit: H. Kunz and T. Reichstein. 16.4.62.

Cultivated in Basel.

10. A. obovatum Viv. TR 123.

Altitude c.50 metres., on north-facing granite rocks. Growing together with A. billotii F.Schulze; A. marinum L. and Arenaria balearica L. nearby. Capo di Testa is the most northerly promontory of Sardinia, abutting the Straits of Bonifacio.

Legit: H. L and T. Reichstein. 6.10.59.

Cultivated in Basel.

11. A. forisiense Le Grand. TR 34

On silicate rocks, and in crevices of dry stone walls by vineyards, along the path Brissago - Incella. West side of Lake Maggiore, south of Locarno, Canton Ticino, Switzerland.

Altitude c.300 metres.

Legit: T. Reichstein. 18.5.59.

Cultivated in Basel.

12. A. forisiense Le Grand.

Crevices of granite rocks at c.550 metres, Val Vizézy, west of Montbrison in Auvergne, Dept. Loire, France.

Legit: T. Reichstein. 30.5.59.

Cultivated in Basel.

13. A. forisiense Le Grand.

Eastern slope of the Col de Maz de l'Air, between Villefort and Les Vans, Cevennes. Border of Depts. Lozère and Ardèche, France.

Legit: T. Reichstein.

Cultivated in Basel.

15. A. macedonicum Kümmerle. Alston 17776.

Crevices of granite rocks, Markovgrad, NW. of Prilep, Macedonia.

Legit: A. H. G. Alston. 30.8.57.

All plants at Leeds were raised from spores from one frond taken from a stock plant at Kew in March 1959. This plant was one of several

raised at Kew from spores of Alston's original collection.

Specimen in Herb. B.M.

16. A. macedonicum Kümmerle. AS/62/32 .

Sheltered crevices in siliceous rocks on the east side of the Treskavec planina at an altitude of c.1000 metres. General habitat very exposed. NE. of Prilep, on west side of road No. 500, to Titov Veles, South Macedonia.

Legit: A. Sleep. 7.8.62.

6 plants (A - F) in cultivation at Leeds.

17. A. petrarchae (Guérin) DC. TR 358.

Crevices of limestone rocks to the east of the road between Pélisanne and Aurons, near Salon, west of Aix-en-Provence, Bouches-du-Rhône, France.

Altitude c.150 metres.

Legit: T. Reichstein.

Cultivated in Basel.

19. A. majoricum Litard.

Crevices on the north-facing side of dry limestone walls forming the terraces of olive groves. With A. trichomanes L., Ceterach officinarum DC., Polypodium australe Fée, Selaginella denticulata (L.) Link and Sedum dasyphyllum L.

Altitude c.250 metres.

C'as Patro Lau, Barranc. Beyond Biniaralx, ESE. of the town of Söller, Mallorca.

Legit: J. Orell Casasnovas. 1959.

This gathering was originally represented by a specimen in the Herbarium of the Department of Botany at Liverpool University, and by another specimen in cultivation in the Botanic Garden of the same University. Subsequently, a number of plants were raised at Leeds from spores obtained (through Dr. P. W. Ball) from the original plant in cultivation at Liverpool and specimens from these are now in Herb. B.M.

21. A. billotii F.Schulze. TR 276.

Crevices of dry silicate wall at about 300 metres. Near Ronco, south of Ascona, Ticino, Switzerland. A. forisiense Le Grand. grows nearby.

Legit: T. Reichstein. 9.9.61.

Cultivated in Basel.

22. A. onopteris L. TR 114.

Crevices of silicate rock at 900 metres. Aritzo, Sardinia, Italy

Legit: T. Reichstein. 26.9.59.

Cultivated in Basel.

29. A. viride Huds.

Cracks in exposed limestone pavement.

Hutton Roof, Westmorland, Great Britain.

Legit: A. Sleep. 29.6.63.

Originally a wild spore collection, but plants now in cultivation at Leeds.

APPENDIX II.

HYBRIDISATION ATTEMPTS WITHIN ASPLENIUM: THE NUMERICAL DATA.

DIPLOID CROSSES.

	P	Sp.	Se.	D	H	Unc.
♀ A. fontanum x A. obovatum	384	57	54	1	2	0
Reciprocal cross.	315	101	99	2	0	0
♀ A. fontanum x A. onopteris	12	0	0	0	0	0
Reciprocal cross.	51	5	4	1	0	0
♀ A. fontanum x A. cuneifolium	16	1	1	0	0	0
Reciprocal cross.	28	2	0	2	0	0
♀ A. fontanum x A. trichomanes	233	49	49	0	0	0
Reciprocal cross.	73	8	8	0	0	0
♀ A. jahandiezii x A. fontanum	10	0	0	0	0	0
♀ A. viride x A. fontanum	263	7	3	4	0	0
♀ A. onopteris x A. obovatum	60	14	14	0	0	0

TRIPLOID CROSSES.

♀ A. jahandiezii x A. macedonicum (Alston)	78	3	3	0	0	0
♀ A. jahandiezii x A. forisiense	52	2	1	1	0	0
♀ A. jahandiezii x A. billotii	33	1	0	1	0	0
♀ A. majoricum x A. jahandiezii	10	2	2	0	0	0
♀ A. trichomanes x A. macedonicum (Alston)	14	8	8	0	0	0
Reciprocal cross.	57	7	7	0	0	0
♀ A. trichomanes x A. forisiense	12	2	2	0	0	0
Reciprocal cross.	41	1	1	0	0	0
♀ A. trichomanes x A. majoricum	23	11	11	0	0	0
Reciprocal cross.	162	8	6	2	0	0

	P	Sp.	Se.	D	H	Unc.
♀ A. trichomanes x A. petrarchae	73	33	33	0	0	0
♀ A. viride x A. petrarchae	196	0	0	0	0	0
♀ A. viride x A. majoricum	265	9	5	3	1	0
♀ A. viride x A. forisiense	104	1	1	0	0	0
♀ A. viride x A. macedonicum (Alston)	180	1	0	1	0	0
♀ A. viride x A. macedonicum (A.S.)	12	3	3	0	0	0
♀ A. cuneifolium x A. macedonicum (Alston)	12	0	0	0	0	0
Reciprocal cross.	31	4	4	0	0	0
♀ A. cuneifolium x A. macedonicum (A.S.)	19	1	1	0	0	0
♀ A. cuneifolium x A. forisiense	11	0	0	0	0	0
Reciprocal cross.	27	3	2	1	0	0
♀ A. petrarchae x A. cuneifolium	38	3	2	1	0	0
♀ A. onopteris x A. macedonicum (Alston)	76	15	13	0	1	1
♀ A. onopteris x A. macedonicum (A.S.)	31	5	4	1	0	0
♀ A. onopteris x A. forisiense	158	36	7	7	22	0
Reciprocal cross.	10	0	0	0	0	0
♀ A. onopteris x A. majoricum	26	9	7	0	1	1
Reciprocal cross.	44	4	4	0	0	0
♀ A. onopteris x A. petrarchae	202	36	29	7	0	0
♀ A. onopteris x A. billotii	46	15	14	0	0	1
Reciprocal cross.	10	0	0	0	0	0
♀ A. obovatum x A. billotii	31	11	8	1	0	2
Reciprocal cross.	64	19	18	0	0	1
♀ A. obovatum x A. petrarchae	40	8	8	0	0	0
Reciprocal cross.	113	12	12	0	0	0
♀ A. majoricum x A. obovatum	414	13	10	2	1	0

	P	Sp.	Se.	D	H	Unc.
♀ A. macedonicum (A.S.) x A. obovatum	56	8	5	1	2	0
♀ A. obovatum x A. macedonicum (Alston)	237	129	89	6	34	0
Reciprocal cross.	140	18	12	4	2	0
♀ A. obovatum x A. forisiense	112	68	31	0	37	0
Reciprocal cross.	162	22	16	4	2	0
♀ A. fontanum x A. forisiense	150	39	23	1	15	0
Reciprocal cross.	108	25	16	0	9	0
♀ A. fontanum x A. macedonicum (Alston)	206	43	20	0	23	0
Reciprocal cross.	161	52	27	0	25	0
♀ A. fontanum x A. macedonicum (A.S.)	64	28	8	2	18	0
Reciprocal cross.	20	8	2	2	4	0
♀ A. majoricum x A. fontanum	109	29	10	12	7	0
♀ A. petrarchae x A. fontanum	94	7	5	1	1	0
Reciprocal cross.	96	24	24	0	0	0
♀ A. fontanum x A. billotii	43	2	1	1	0	0
Reciprocal cross.	77	20	20	0	0	0
TETRAPLOID CROSSES.						
♀ A. forisiense x A. macedonicum (Alston)	18	7	0	3	4	0
Reciprocal cross.	20	7	2	3	2	0
♀ A. majoricum x A macedonicum (Alston)	123	16	7	0	9	0
♀ A. majoricum x A. forisiense	9	2	0	0	2	0
♀ A. majoricum x A. billotii	363	10	7	2	1	0
Reciprocal cross.	37	24	24	0	0	0
♀ A. majoricum x A. petrarchae	27	6	5	1	0	0
♀ A. petrarchae x A. forisiense	13	0	0	0	0	0
Reciprocal cross.	53	9	9	0	0	0

	P	Sp.	Se.	D	H	Unc.
♀ A. petrarchae x A. macedonicum (Alston)	33	6	6	0	0	0
Reciprocal cross.	21	8	6	2	0	0
♀ A. petrarchae x A. macedonicum (A.S)	35	6	6	0	0	0
Reciprocal cross.	14	4	1	3	0	0
♀ A. billotii x A. petrarchae	36	22	20	0	2	0
Reciprocal cross.	50	5	3	2	0	0
♀ A. billotii x A. forisiense	20	16	11	1	0	4
Reciprocal cross.	68	8	5	3	0	0
♀ A. billotii x A. macedonicum (Alston)	45	10	7	1	0	2
Reciprocal cross.	43	15	3	0	0	12
OTHER CROSSES.						
♀ A. pseudofontanum x A. fontanum	27	0	0	0	0	0
♀ A. pseudofontanum x A. macedonicum (Alston)	11	0	0	0	0	0
♀ A. pseudofontanum x forisiense	25	0	0	0	0	0

APPENDIX III.

PROVENANCE OF ALL POLYSTICHUM SPECIES USED IN THE PRESENT INVESTIGATION.

Listed below are all the species of Polystichum which were used in the hybridisation programme, together with their localities of origin and other relevant details. The letter H indicates that a collection has been successfully incorporated in a hybrid combination. The majority of the plants listed have been examined cytologically; a list of chromosome counts will be found in Table VII, page 172. Other cytologically examined plants in cultivation at Leeds which were not used in the hybridisation programme are marked by an asterisk.

SPECIES.	LOCALITY OF ORIGIN.	SOURCE OF SPORES.	COLLECTOR.	SUCCESS.
P. setiferum	Dartmouth, Devon, England.	Stock plants. University of Leeds Botanic Garden.	Professor Manton.	H
P. setiferum	Grümpeli, nr. Rheinfelden, Aargau, Switzerland.	Plant cultivated in Basel.	Professor Reichstein.	H
P. setiferum	Gorge Chauderon, nr. Montreux, Vaud, Switzerland.	Stock plant at Leeds. AS/16.	A. Sleep.	H
P. setiferum	Pennant Wood, Caernarvonshire, Wales, G.B.	Wild collection.	A. Sleep.	
P. setiferum	Quimadas, Madeira.	Stock plant at Leeds.	Professor Manton.	H
P. setiferum *	Rib Frio, Madeira.	Stock plant at Leeds.	Professor Manton.	
P. setiferum	Istanbul, Turkey.	Stock plant at Leeds.	-	H

SPECIES.	LOCALITY OF ORIGIN.	SOURCE OF SPORES.	COLLECTOR.	SUCCESS.
P. lonchitis	Granite above Bains de Tredos, Pyrenees, Spain.	Stock plant at Leeds. 52/59.	D. Bartley.	
P. lonchitis	Spielbergerhorn, nr. Saalbach, Salzburg, Austria.	Stock plant at Leeds. AS/5.	A. Sleep.	
P. lonchitis	Pont-de-Nant, Vaud, Switzerland.	Stock plant at Leeds. AS/62.	A. Sleep.	
P. lonchitis	Klausenpas, Uri, Switzerland.	Wild collection.	Professor Reichstein.	H
P. lonchitis	Haggenegg, Mythen, Schywz, Switzerland.	Wild collection.	Professor Reichstein.	H
P. lonchitis	Val di Bosco, Ticino, Switzerland.	Wild collection.	Professor Reichstein.	H
P. lonchitis	M-tele Piatra Craiului, Rumania.	Wild collection.	Botanic Garden.	
P. lonchitis	Meall nan Tarmachan, nr. Ben Lawers, Perthshire, Scotland.	Stock plant at Leeds. AS/62/43.	A. Sleep.	
P. lonchitis	Corrie above Fee Burn, Clova Mountains, Angus, Scotland.	Wild collection.	A. Sleep.	
P. lonchitis	British Columbia, Canada.	Wild collection.	Stuart Holland.	H
P. munitum *	Victoria, British Columbia, Canada.	Stock plant at Leeds.	A. Wesley.	
P. munitum	North of Mohles, Oregon, U.S.A.	Stock plant at Leeds.	-	H
P. munitum	Reedwood above Searsville Lake, California, U.S.A.	Stock plant at Leeds.	H.G.Baker	
P. acrostichoides	Vermont, U.S.A.	Wild collection	-	H
P. acrostichoides	McLean Bog, nr. Ithaca, U.S.A.	Stock plant at Leeds.	H.G.Baker	H

SPECIES.	LOCALITY OF ORIGIN.	SOURCE OF SPORES.	COLLECTOR.	SUCCESS.
P. ohmurae	Huzizan, (Mt. Fuji), Yamanasi Pref., Honshû, Japan.	Stock plants at Leeds & Basel. Itô 209; 210.	Professor H. Itô.	
P. ohmurae	Huzizan, (Mt. Fuji), Yamanasi Pref., Honshû, Japan.	Wild collection.	Y. Shimura, Ex Prof. H Itô.	
P. aculeatum	Granite scree above Bains de Tredos, Pyrenees, Spain.	Stock plant at Leeds. 52/59.	D. Bartley.	H
P. aculeatum	Hattvik, Norway.	Stock plant at Leeds. 100/61.	Professor Manton.	
P. aculeatum	Auvergne, France.	Stock plant at Leeds.	-	
P. aculeatum	Gorge Chauderon, nr. Montreux, Vaud, Switzerland.	Stock plants at Leeds. AS/15; AS/28.	A. Sleep.	H
P. aculeatum	Pont-de-Nant, Vaud, Switzerland.	Stock plant at Leeds. AS/57.	A. Sleep.	
P. aculeatum	Cwm Glas Crafnant, Conway valley, N. Wales.	Wild collection.	A. Sleep.	
P. aculeatum*	Asham Wood, Somerset, Great Britain.	Stock plant at Leeds.	A. Sleep.	
P. aculeatum*	Birks O' Aberfeldy, Perthshire, Scotland.	Stock plant at Leeds.	A. Sleep.	
P. aculeatum*	Iceland.	Stock plant at Leeds.	-	
P. braunii	Val d'Osogna, N. of Bellinzona, Ticino, Switzerland.	Wild collection. Plant now cultivated in Basel.	Professor Reichstein.	H
P. braunii	Val Cresciano, N. of Bellinzona, Ticino, Switzerland.	Wild collection.	Professor Reichstein.	
P. braunii	Val di Bosco, NW of Locarno, Ticino, Switzerland.	Wild collection	Professor Reichstein.	
P. braunii	Alpe di Gem, NW of Lugano, Ticino, Switzerland.	Wild collection.	Professor Reichstein.	

SPECIES.	LOCALITY OF ORIGIN.	SOURCE OF SPORES.	COLLECTOR.	SUCCESS.
P. braunii	Val Antabbia, NW of Locarno, Ticino, Switzerland.	Wild collection. Plant now cultivated in Basel.	Professor Reichstein.	
P. braunii	Val Antabbia, Ticino, Switzerland.	Stock plant at Leeds. 40/60.	Professor Reichstein.	H
P. braunii *	Krimml water-fall, Salzburg, Austria.	Stock plant at Leeds.	A. Sleep.	
P. braunii	Hattvik, Hordaland, Norway.	Stock plant at Leeds. 100/61.	Professor Manton.	
P. braunii	Tysse, Kvitingsdalen, Hordaland, Norway.	Wild collection.	Professor Manton.	
P. braunii v. Purshii	Green Mountains, Vermont, east U.S.A.	Stock plant at Leeds.	-	H
P. braunii v. Purshii	Cascade Mountains, Oregon, U.S.A.	Wild collection.	Botanic Garden.	
P. braunii v. Purshii	Queen Charlotte Islands, British Columbia, Canada.	Wild collection. Plant now in cultivation at Victoria, B.C.	Stuart Holland.	
P. braunii v. Purshii	E. Canada.	Wild collection	Montreal Botanic Garden.	
P. braunii	Mt. Moiwa, Sapporo, Hokkaidô, Japan.	Stock plant at Basel. TR 491.	Professor H. Mitsuhashi.	
P. braunii	Titibu Mountains, Saitama Pref., Honshû, Japan.	Stock plant at Basel. Itô 119.	Professor H. Itô.	
P. andersoni	Smithers, British Columbia, Canada.	Wild collection. Plant cultivated in Victoria, B.C.	Stuart Holland.	H
P. andersoni	Cascade Mountains, Oregon, U.S.A.	Wild collection.	Botanic Garden.	
P. woronowii	Elburz Mountains, S.End of Caspian Sea, Persia.	Stock plant at Leeds.	F. Merton.	

APPENDIX IV.

CYTOLOGICALLY-EXAMINED WILD HYBRIDS IN CULTIVATION.

HYBRIDS.	LOCALITY OF ORIGIN.	COLLECTOR.
P. x bicknellii (Christ) Hahne.	Grümpeli, nr. Rheinfelden, Aargau, Switzerland. No. 206. Plant in cultivation at Leeds.	Professor T. Reichstein.
P. x illyricum (Borbás) Hahne.	Pont-de-Nant, nr. Les Plans/Bex, Vaud, Switzerland. Plant in cultivation at Leeds.	Professor I. Manton.
P. x illyricum (Borbás) Hahne.	Pont-de-Nant, nr. Les Plans/Bex, Vaud, Switzerland. AS/59; AS/60. 2 plants in cultivation at Leeds.	A. Sleep.
P. x wirtgeni Hahne.	Val dei Molini, northern side of the Grigna, N. Italy. TR 471. Plant in cultivation at Leeds.	Professor T. Reichstein.
P. x luerssenii (Dörfl.) Hahne.	Krimml waterfall, Salzburg, Austria. AS/62/5. 5 plants in cultivation at Leeds.	A. Sleep.
P. x luerssenii (Dörfl.) Hahne.	Val Antabbia, NW of Locarno, Ticino, Switzerland. TR 954. Plant in cultivation at Basel.	J. D. Lovis & Professor T. Reichstein.
P. braunii x P. lonchitis *	Floitengrund, SE of Ginzling, Zillertal, Tyrol, Austria. Open slope, on scree with big boulders and few trees. Together with the parents and the two other hybrids involving P. aculeatum. c. 1400m. TR 1376. Plant in cultivation at Basel.	J. D. Lovis, A. Sleep & Professor T. Reichstein. 15.9.1964.

* No accurate meiotic analyses have as yet been made from this plant (see page 145).

APPENDIX V.

NUMERICAL DATA RELATING TO THE HYBRIDISATION ATTEMPTS.

In this genus hybridity cannot be determined by an examination of the spores as synthesised hybrids generally take two to three years, perhaps longer, before producing sporangia. In addition, the juvenile fronds produced during the first two years are often very different from the mature ones and therefore not a good guide to suspected hybridity. The later produced fronds are however often of distinctive appearance and give a clear indication of suspected parentage, which can later be confirmed by an examination of meiosis. In the following tables hybrids synthesised during the first two years of the investigation are listed in column 5 (H). Some of these are confirmed hybrids and the others, still infertile, are so distinctive in appearance that their hybrid nature is unquestionable. Putative hybrids produced in 1963 and later are listed in column 6 (unconfirmed) as for the reasons stated above their morphology is not necessarily indicative of hybridity. An explanation of the symbols used will be found on page 76.

I. DIPLOID : DIPLOID CROSSES.

1. P. setiferum (2x) x P. lonchitis (2x). Diploid hybrid.

♀				P	Sp.	Se.	D	H	Unc.
	England	x	Switzerland	56	0	0	0	0	0
R	Switzerland	x	England	16	2	2	0	0	0
	Switzerland	x	Switzerland	182	12	6	2	4	0
	Wales	x	Spain	14	0	0	0	0	0

P. setiferum x P. lonchitis - cont.

	♀			P	Sp.	Se.	D	H	Unc.
R	Spain	x	Wales	26	0	0	0	0	0
	Switzerland	x	Wales	11	0	0	0	0	0
	Switzerland	x	Madeira	48	1	0	1	0	0
	Scotland	x	England	54	29	1	18	0	10
	Spain	x	England	48	0	0	0	0	0
	Austria	x	England	77	0	0	0	0	0
	Rumania	x	England	189	24	0	14	0	10
	B.C., Canada	x	England	252	47	11	21	0	15
				973	115	20	56	4	35

Percentage success: 0.40%

2. P. lonchitis (2x) x P. acrostichoides (2x). Diploid hybrid.

♀				P	Sp.	Se.	D	H	Unc.
	Switzerland	x	Vermont	8	0	0	0	0	0
	Spain	x	Vermont	16	1	1	0	0	0
	Switzerland	x	Ithaca	53	2	1	1	0	0
	Spain	x	Ithaca	11	0	0	0	0	0
	Austria	x	Ithaca	18	0	0	0	0	0
	Rumania	x	Ithaca	30	0	0	0	0	0
	B.C., Canada	x	Ithaca	39	4	3	0	1	0
				175	7	5	1	1	0

Percentage success: 0,57%

3. P. setiferum (2x) x P. munitum (2x). Diploid hybrid.

♀				P	Sp.	Se.	D	H	Unc.
	Switzerland	x	Oregon	24	3	3	0	0	0
R	Oregon	x	Switzerland	8	0	0	0	0	0
	England	x	Oregon	31	1	1	0	0	0
R	Oregon	x	England	16	0	0	0	0	0
				79	4	4	0	0	0

Percentage success: 0.

4. P. setiferum (2x) x P. acrostichoides (2x). Diploid hybrid.

♀				P	Sp.	Se.	D	H	Unc.
	Turkey	x	Ithaca	18	7	0	1	6	0
	England	x	Ithaca	25	0	0	0	0	0
	Switzerland	x	Vermont	32	7	3	1	3	0
R	Vermont	x	Switzerland	10	0	0	0	0	0
				85	14	3	2	9	0

Percentage success: 10.60%

5. P. lonchitis (2x) x P. munitum (2x). Diploid hybrid.

♀				P	Sp.	Se.	D	H	Unc.
	B.C., Canada	x	Oregon	20	0	0	0	0	0
	Switzerland	x	Oregon	55	2	2	0	0	0
R	Oregon	x	Switzerland	16	1	1	0	0	0
				91	3	3	0	0	0

Percentage success: 0.

6. P. munitum (2x) x P. acrostichoides (2x). Diploid hybrid.

♀				P	Sp.	Se.	D	H	Unc.
	California	x	Ithaca	65	2	1	1	0	0
R	Ithaca	x	California	8	2	2	0	0	0
	Oregon	x	Ithaca	39	1	1	0	0	0
R	Ithaca	x	Oregon	61	4	0	1	3	0
	Oregon	x	Vermont	16	0	0	0	0	0
R	Vermont	x	Oregon	37	1	1	0	0	0
				226	10	5	2	3	0

Percentage success: 1.32%

II. DIPLOID : TETRAPLOID CROSSES.

7. P. braunii (4x) x P. lonchitis (2x). Triploid hybrid.

♀				P	Sp.	Se.	D	H	Unc.
	Switzerland	x	Switzerland	237	4	4	0	0	0

P. braunii x P. lonchitis - cont.

♀				P	Sp.	Se.	D	H	Unc.
	Switzerland	x	Austria	5	0	0	0	0	0
	Switzerland	x	Scotland	69	0	0	0	0	0
R	Scotland	x	Switzerland	22	17	15	2	0	0
	Switzerland	x	Spain	24	0	0	0	0	0
R	Spain	x	Switzerland	76	2	1	1	0	0
	Switzerland	x	Rumania	14	1	1	0	0	0
	Vermont, U.S.A.	x	Rumania	18	1	1	0	0	0
	Vermont, U.S.A.	x	B.C., Canada	55	11	5	3	0	3
	Switzerland	x	B.C., Canada	155	3	3	0	0	0
	B.C., Canada	x	Spain	13	5	1	4	0	0
	Japan	x	Switzerland	68	14	0	8	0	6
				756	58	31	18	0	9

Percentage success: 0.

8. P. braunii (4x) x P. setiferum (2x). Triploid hybrid.

♀				P	Sp.	Se.	D	H	Unc.
	Switzerland	x	England	54	4	2	1	0	1
R	England	x	Switzerland	86	1	1	0	0	0
	Norway	x	England	208	1	0	0	0	1
	Switzerland	x	Switzerland	68	1	1	0	0	0
	Norway	x	Switzerland	8	0	0	0	0	0
	Vermont, U.S.A.	x	England	43	5	0	4	0	1
	E. Canada	x	England	74	5	0	5	0	0
	B.C., Canada	x	Switzerland	15	5	4	1	0	0
	B.C., Canada	x	Wales	36	8	6	2	0	0
				592	30	14	13	0	3

Percentage success: 0.

9. P. aculeatum (4x) x P. lonchitis (2x). Triploid hybrid.

♀				P	Sp.	Se.	D	H	Unc.
	Spain	x	Switzerland	48	9	8	0	1	0
R	Switzerland	x	Spain	63	12	1	2	7	2

P. aculeatum x P. lonchitis - cont.

♀			P	Sp.	Se.	D	H	Unc.
Norway	x	Spain	11	1	0	0	0	1
Switzerland	x	Scotland	16	2	0	0	0	2
Norway	x	B.C., Canada	43	3	0	0	0	3
			181	27	9	2	8	8

Percentage success: 4.41%

10. P. aculeatum (4x) x P. setiferum (2x). Triploid hybrid.

♀			P	Sp.	Se.	D	H	Unc.
Spain	x	Madeira	54	23	0	0	23	0
Spain	x	England	16	6	0	0	6	0
R England	x	Spain	27	0	0	0	0	0
Spain	x	Switzerland	50	23	0	0	23	0
England	x	France	14	0	0	0	0	0
Wales	x	Switzerland	17	0	0	0	0	0
R Switzerland	x	Wales	15	0	0	0	0	0
			193	52	0	0	52	0

Percentage success: 2.70%

11. P. aculeatum (4x) x P. munitum (2x). Triploid hybrid.

♀			P	Sp.	Se.	D	H	Unc.
Norway	x	Oregon	17	10	1	1	0	8
Switzerland	x	Oregon	17	4	1	0	3	0
Spain	x	Oregon	114	40	5	4	6	25
			148	54	7	5	9	33

Percentage success: 6.10%

12. P. aculeatum (4x) x P. acrostichoides (2x). Triploid hybrid.

♀			P	Sp.	Se.	D	H	Unc.
Spain	x	Vermont	16	11	3	3	5	0
Spain	x	Ithaca	32	7	1	1	5	0
Switzerland	x	Ithaca	15	12	0	0	12	0
			63	30	4	4	22	0

Percentage success: 3.50%

13. P. braunii (4x) x P. acrostichoides (2x). Triploid hybrid.

♀				P	Sp.	Se.	D	H	Unc.
	E. Canada	x	Ithaca	12	0	0	0	0	0
	Norway	x	Ithaca	56	0	0	0	0	0
	Vermont, U.S.A.	x	Ithaca	17	4	2	2	0	0
	B.C., Canada	x	Ithaca	18	7	5	2	0	0
	Norway	x	Ithaca	10	0	0	0	0	0
	Switzerland	x	Ithaca	111	10	0	4	3	3
R	Ithaca	x	Switzerland	6	0	0	0	0	0
				230	21	7	8	3	3

Percentage success: 1.30%

14. P. braunii (4x) x P. munitum (2x). Triploid hybrid.

♀				P	Sp.	Se.	D	H	Unc.
	Switzerland	x	Oregon	151	13	5	2	0	6
R	Oregon	x	Switzerland	16	0	0	0	0	0
	Switzerland	x	California	17	1	1	0	0	0
	Vermont, U.S.A.	x	Oregon	47	15	1	5	3	6
	Vermont, U.S.A.	x	California	8	0	0	0	0	0
	B.C., Canada	x	Oregon	15	5	4	1	0	0
	Japan	x	Oregon	6	1	0	1	0	0
				260	35	11	9	3	12

Percentage success: 1.15%

III. TETRAPLOID : TETRAPLOID CROSSES.

15. P. braunii (4x) x P. aculeatum (4x). Tetraploid hybrid.

♀				P	Sp.	Se.	D	H	Unc.
	Spain	x	Switzerland	77	2	2	0	0	0
R	Switzerland	x	Spain	36	3	0	0	0	3
	Switzerland	x	Switzerland	23	5	4	0	1	0
	Vermont, U.S.A.	x	Spain	10	2	0	2	0	0
				146	12	6	2	1	3

Percentage success: 0.68%

SUPPLEMENT I.

A. CHROMOSOME COUNTS OF SOME JAPANESE POLYSTICHUM SPECIES. (not illustrated).

As stated on page 146, a collection of living Japanese Polystichum species belonging to the P. braunii group (Section Metapolystichum (Tagawa, 1940)) were obtained for the purpose of this investigation by Professor T. Reichstein. These were sent to Kew by Professor H. Itô of Tokyo. Unfortunately the plants did not respond well to cultural conditions in this country and many died before an accurate chromosome count could be made. They produced few roots and during the first two years many showed irregular meioses. Some results have nevertheless been obtained and the plants listed below have all been examined cytologically. In one case (starred) ploidy has been inferred from a hybrid incorporating the same material.

Name and locality.	n	2n	Ploidy	Reproduction
P. neo-lobatum Nakai. Tanzawayama, Kanagawa Pref., Honshû. Itô 109	'123'	-	Triploid	Apogamous
P. tsus-simense (Hooker) J. Smith Yugawara, Kanagawa Pref., Honshû. Itô 127	'123'	-	Triploid	Apogamous
P. makinoi (Tagawa) Tagawa Kurobaruyama, Kumamoto Pref., Kyûshû. Itô 107	82	-	Tetraploid	Sexual
P. tagawanum Kurata Kurobaruyama, Kumamoto Pref., Kyûshû. Itô 125	82	-	Tetraploid	Sexual
P. polyblepharum (Roem. ex Kunze) Presl. Kurobaruyama, Kumamoto Pref., Kyûshû. Itô 113	82	-	Tetraploid	Sexual
P. ovato-paleaceum (Kodama) Kurata Ongata, Tokyo Pref., Honshû. Itô 120	-	164	Tetraploid	Sexual

Name and locality.	n	2n	Ploidy	Reproduction
P. fibrilloso-paleaceum * (Kodama) Tagawa. Odawara, Kanagawa Pref., Honshû. Itô 116	-	-	Diploid	Sexual
P. x namaegatae Kurata Mizugaki-mura, Kumamoto Pref., Kyûshû. Ito 207	Meiosis irregular	-	Hybrid	Sterile
P. x kiyozumianum Kurata Kiyozumiyama, Chiba Pref., Honshû. Itô 106	Meiosis irregular	-	Hybrid	Sterile
P. x ongataense Kurata Ongata, Tokyo Pref., Honshû. Ito 111	Meiosis irregular	-	Hybrid	Sterile

Counts of P. braunii and P. ohmurae are listed in Table VII, page 172.

Nomenclature follows Kurata (1964).

B. OTHER SPECIES COUNTED DURING THE COURSE OF THE INVESTIGATION.

Chromosome counts have been obtained from the following African species which were collected and sent to Kew by A. F. Braithwaite in 1961.

P. fuscopaleaceum Alston Cathkin Peak, Drakensberg Mts., Natal. E	82	-	Tetraploid	Sexual
P. fuscopaleaceum Alston Aberdare Mtns., W. of Nyeri, Kenya. 443	82	-	Tetraploid	Sexual
P. fuscopaleaceum Alston Mt. Kilimanjaro, S.E.side. N. Tanganyika. 403	82	-	Tetraploid	Sexual
P. lucidum (Burm.) Becherer * Vumba Mtns., nr Umtali, S. Rhodesia. VUM 38	-	-	Tetraploid	Sexual
P. luctuosum (Kze.) Moore Pilgrims Rest, E. Transvaal. 229	-	-	-	Apogamous

* Ploidy inferred from the meiotic behaviour of the hybrid AS/437

(see page 249).

SUPPLEMENT II. SOME FURTHER HYBRIDS WITHIN THE GENUS POLYSTICHUM.

Further material was incorporated into the basic hybridisation programme as it became available. Hybrids so far examined involve the following additional species:

Name.	Locality.	Source of material.	Ploidy.
P. andersoni	Smithers, British Columbia, Canada.	S. Holland. Plant in cultivation at Victoria, B.C.	Tetraploid.
P. proliferum.	Australia.	Stock plant at Kew. 304/53.	Tetraploid.
P. lucidum	Vumba Mtns., Southern Rhodesia.	Wild collection A.F.B. 38.	Tetraploid.
P. makinoi	Kurobaruyama, Kumamoto Pref., Kyūshū, Japan.	Stock plant in Basel. Itô 107.	Tetraploid.
P. fibrillosopaleaceum	Odawara, Kanagawa Pref. Honshū, Japan.	Stock plant in Basel. Itô 116.	Diploid.

A large number of putative hybrids involving these taxa with other parental stocks have also been obtained, but as yet are still too young to yield cytological data.

The results obtained are not illustrated, but analyses of meiosis for each type of hybrid so far examined are given below:

1. AS/480. P. andersoni (4x) x P. setiferum (2x).

Ninety-three prothalli were inseminated, and of the nine sporelings obtained only one proved to be hybrid. This plant was triploid, and preliminary analyses showed c. 20 paired chromosomes in each of the cells examined.

Wagner (in Fabbri, 1963) reports a perfectly regular meiosis in this species, and states that the hybrid P. munitum x P. andersoni shows 41 bivalents and 41 univalents (cf. P. lonchitis x P. aculeatum). The result quoted above is thus an interesting parallel to the results obtained from the triploid hybrids P. acrostichoides x P. aculeatum and P. munitum x P. aculeatum.

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2. AS/36. P. aculeatum (4x) x P. proliferum (4x).

35 prothalli were inseminated and five hybrid plants were obtained. The ones examined were uniformly tetraploid and at meiosis 16 - 24 paired chromosomes were seen. There was some indication of multivalent formation in a few of the cells examined. However, the high number of univalent chromosomes (116 - 132) indicates that the Australian plant is not closely related to the British tetraploid, a fact of interest in view of previous taxonomic treatment (e.g. Christensen (1906), who considered P. proliferum to be a variety of P. aculeatum).

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3. AS/437. P. aculeatum (4x) x P. lucidum (4x) (= VUM 38).

20 prothalli were inseminated and thirteen hybrid plants were obtained. A selection of these hybrids were examined cytologically and gave the following results:

Bivalents per cell:	17	19	19	23	25
Univalents per cell:	130	126	126	118	114

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4. AS/443. P. aculeatum (4x) x P. fibrillosopaleaceum (2x)

30 prothalli were inseminated and two hybrid plants have been obtained. Preliminary analyses revealed a high number of paired chromosomes (fifty or more) and approximate counts showed these plants to be tetraploid and not triploid, as expected. It is therefore possible that this is yet another example of the production of an unreduced gamete by the diploid parent.

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5. AS/459. P. braunii (4x) x P. fibrillosopaleaceum (2x).

43 prothalli were inseminated and six triploid hybrids have been obtained. In the cells examined chromosome pairing of the order of 20 bivalents per cell was observed, the highest number recorded being 26 pairs. These bivalents are in all probability the result of homoeologous pairing and cannot therefore be taken as any indication of close relationship between P. braunii and the Japanese diploid.

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6. AS/428. P. munitum (2x) x P. fibrillosopaleaceum (2x).

10 prothalli were inseminated and one hybrid plant was obtained. Analyses of cells at meiosis showed 19 - 23 paired chromosomes. This is therefore a further example of the widespread pairing which is present at the diploid level in Polystichum.

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7. AS/503. P. makinoi (4x) x P. braunii (4x).

17 prothalli were inseminated and two morphologically hybrid plants were obtained. The one plant which has so far produced sporangia

is tetraploid. Only one cell has been fully analysed and this showed 44 paired chromosomes. Other cells seen indicated the presence of a high number of pairs and also the possibility of multivalent formation. Although this result could imply a common relationship between an ancestral diploid and both the European and Japenese tetraploids, similar pairing could be due to autosyndesis within the Japanese P. makinoi. It is apparent that any pairing found, particularly at the tetraploid level, could be due to any of several factors, and consequently great care is necessary in interpreting any bivalent formation as indicative of a close relationship between the species involved.

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