*CYTOGENETIC STUDIES

IN THE *DRYOPTERIS SPINULOSA* COMPLEX – I

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Abstract

The inter-relationships of species within the *Dryopteris spinulosa* complex have been studied by investigating chromosome pairing during meiosis in wild and synthesised hybrids.

The three tetraploid species *D. cristata*, *D. spinulosa* and *D. dilatata* are shown to be allopolyploids requiring at least four ancestral diploids to account for their origin. Experimental hybridisations indicate that one of these ancestral genomes is now represented by two distinct diploid forms.

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(1) Introduction

A cytogenetic and taxonomic investigation of the *Dryopteris spinulosa* complex, as found in Europe and North America, is helping to elucidate the relationships between members of this circumboreal group of ferns. This paper is devoted entirely to the *D. spinulosa* complex in Europe. A report on the complex in N. America is being prepared, whilst further work, inter-relating species on both sides of the Atlantic, is in progress.

The *D. spinulosa* complex has long been a source of confusion for taxonomists. In modern Floras it is represented by three distinct species and the hybrids between them. The nomenclature varies somewhat but following Christensen (1905, 1913) they may be listed :

  (i) *Dryopteris cristata* (L.) A. Gray,
  (ii) *Dryopteris spinulosa* (O. F. Muell.) Watt,
  (iii) *Dryopteris dilatata* (Hoffm.) A. Gray,
  (iv) Hybrid, *D. × uliginosa* (Newm.) Druce (= *D. cristata × spinulosa*),
  (v) Hybrid, *D. dilatata × spinulosa*.

D. cristata was first named by Linnaeus in 1753 as *Polypodium cristatum*. *D. spinulosa* was not recognised as a separate species until described as *Polypodium spinulosum* by Müller in 1767 and *D. dilatata* was not considered distinct from *P. spinulosum* until 1795 when it was named *Polypodium dilatatum* by Hoffmann.

Since these dates of separate recognition the generic conception has changed and the plants have been placed under *Aspidium*, *Nephrodium*, *Lastrea*, *Thelypteris* and *Dryopteris*. It is more significant, however, that some authors have referred to the three species merely as subspecies or even varieties of a single species (Newman, 1844, 1865 ; Druery, 1912). The specific epithet *dilatata* has been replaced in the new *Flora of the British Isles* (Warburg, 1952) by *austriaca*, following the continental nomenclature, and *D. cristata*, *D. spinulosa* and *D. austriaca* (Jacq.) Woynar, 1915, are recognised as distinct species.

The use of the name *D. austriaca* raises problems of its own, however. Christensen (1913-16) states that " *D. austriaca = D. spinulosa* " and this was at a time when Christensen considered *D. spinulosa* and *D. dilatata* as separate species. The original name *Polypodium austriacum* was given to a fern collected by Jacquin (1764) but the type specimen is unavailable, even if one exists. Herbarium sheets from Jacquin's herbarium at the British Museum and labelled " *P. austriacum*", but not by Jacquin, are a mixed collection of genuine *D. spinulosa* and *D. dilatata*. Without returning to the type locality, a decision regarding the identity of *D. austriaca* cannot be reached and for this reason the writer prefers the retention of the name *D. dilatata*.

(2) MATERIALS

A number of collectors have helped to supply the material for the investigation, all of which has been checked for identification and cytological behaviour. Representative plants of all the specimens collected (except one) have been maintained in culture and herbarium specimens kept by the writer. A duplicate set of herbarium specimens is to be deposited with the British Museum (Natural History), London.

The material of *D. cristata* was collected and supplied originally from Surrey, England by its discoverer in that locality (Payne, 1939) and is now in cultivation at the Royal Botanic Gardens, Kew. From this specimen a number of plants have been raised and are now in cultivation at Liverpool. *D. spinulosa* has been sampled from England, France, Germany and Sweden. *D. dilatata* is known to include two cytological forms, one diploid and the other tetraploid (Manton, 1950). The tetraploid has been collected from France, Germany, Sweden, Ireland and many localities in England, and the diploid from Norway, Sweden, Switzerland, Scotland and also the island of Madeira (consideration of this plant is included here although it is from outside Europe).

Only one plant of the hybrid *D. × uliginosa* has been available owing to its relative rarity; this is maintained in cultivation at Leeds and was used also by Manton (1950) for cytological study. A number of specimens of the hybrid between *D. spinulosa* and *D. dilatata* have been collected from two Yorkshire (England) localities and a single specimen from Ireland, the latter having been found by Dr. R. L. Praeger.

(3) METHODS

Prothallial cultures were grown in 3 in. pots containing a sterilised compost comprising peat, loam and sand in the ratio of 3 : 2 : 1 respectively. The pots were protected from stray spores by clock glass covers and watered only from below, thus preventing access to free surface water and minimising the chances of fertilisation within parent cultures. The young prothalli could be maintained in a more juvenile state for a longer period if grown at temperatures of 70° to 80°F; in these conditions they were found to

(a) *D. cristata*

(b) Hybrid
D. × *uliginosa*
(= *D. cristata* × *spinulosa*)

(c) *D. spinulosa*

(d) Hybrid
D. dilatata × *spinulosa*
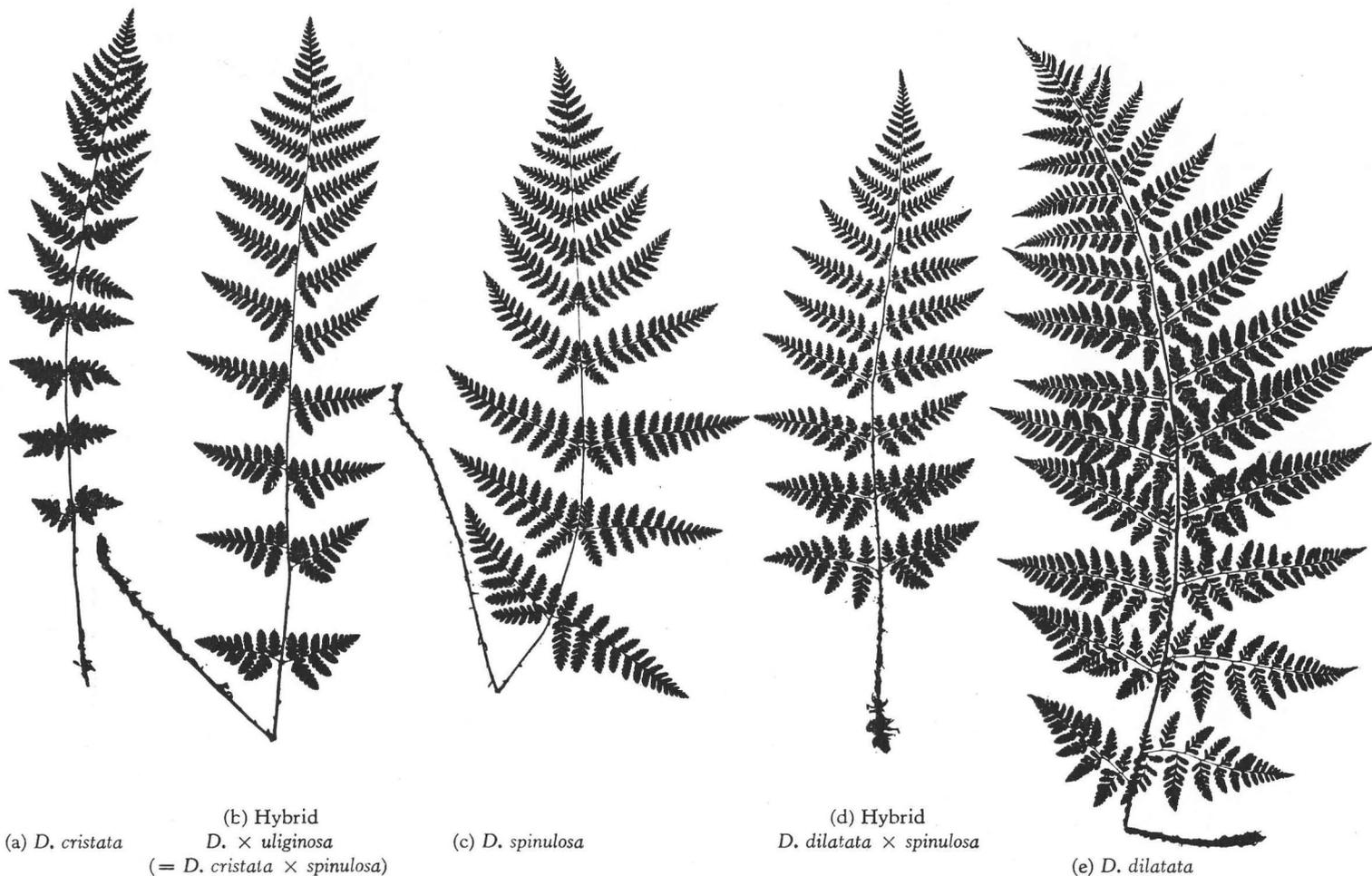
(e) *D. dilatata*

Fig. 1. Silhouettes of specimen fronds from the tetraploid species and hybrid. (¼ natural size).
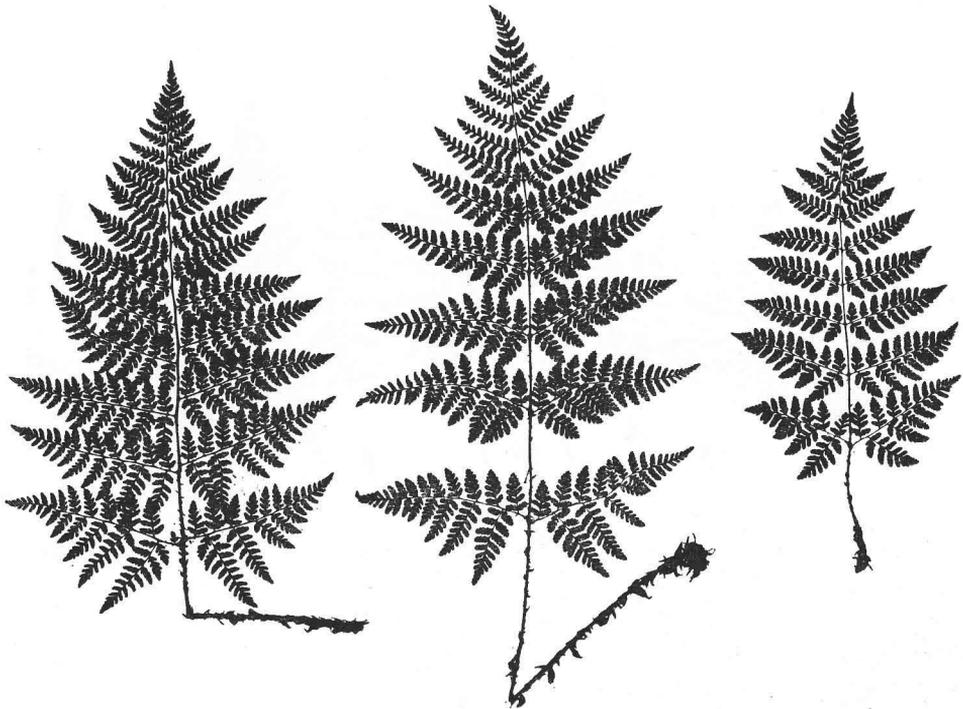
(a) " *D. dilatata* " (2n-Scotland).   (b) Hybrid (3n) " *D. dilatata* "   (c) Hybrid (3n) " *D. dilatata* "
                                         (2n-Scotland) × *D. dilatata* (4n).   (2n-Scotland) × *D. spinulosa.*

Fig. 2.   Silhouettes of specimen fronds, as labelled (quarter natural size).
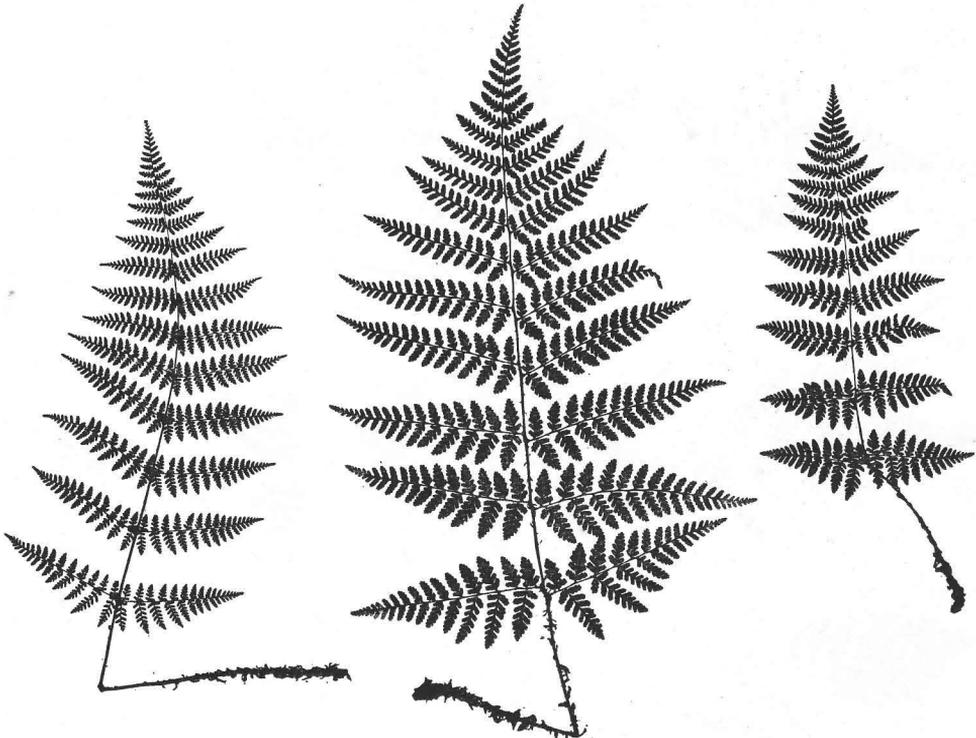
(a) " *D. dilatata* " (2n-Madeira).   (b) Hybrid (3n) " *D. dilatata* "   (c) Hybrid (3n) " *D. dilatata* "
                                        (2n-Madeira) × *D. dilatata* (4n).   (2n-Madeira) × *D. spinulosa.*

Fig. 3.   Silhouettes of specimen fronds, as labelled (quarter natural size).

produce large numbers of antheridia. Prothalli required as females were pricked out into pots at an early stage so that each prothallus was separated from its neighbour by about ¾ in.; this almost precludes fertilisation within the cultures before hybridisation.

Somatic chromosome counts have been made mainly by embedding and sectioning root tips and using iron haematoxylin stain, though some aceto-carmine root-tip squashes have also been made after hydroxyquinoline pretreatment (Tjio and Levan, 1950; Meyer, 1952). Hybrids became fertile approximately 12-18 months after fertilisation and detailed analyses of meiotic chromosome pairing have been made, using the aceto-carmine squash technique (Manton, 1950). Photographic evidence is supported by explanatory diagrams where necessary. In most cases the analyses have been made from several plants of the same hybrid combination.

(4) Survey of the previous work

The taxonomic history has been outlined very briefly above. The three recognised species of the complex, D. cristata, D. spinulosa and D. dilatata, have a circumpolar distribution in the Northern Hemisphere, particularly within the Temperate Zone, and can be separated by their morphology and type of habitat (Warburg, 1952; Hyde & Wade, 1948). D. cristata is distinguished more readily from the other two species than these two are from one another. Consequently the exact distribution of D. spinulosa and D. dilatata is incompletely known as the two species have not always been treated as distinct.

The hybrids D. × uliginosa and D. dilatata × spinulosa can be found in habitats which will support both parents; D. × uliginosa is therefore restricted to very wet situations whereas D. dilatata × spinulosa abounds in marshy woods which are gradually drying out. Both hybrids exhibit morphological characters intermediate between those of their parents and produce abortive spores. The differences in frond shape and degree of pinnation in the species and species hybrids are illustrated by Fig. 1.

D. cristata, D. spinulosa and D. dilatata occur as tetraploids (Manton, 1950), each with regular chromosome pairing at meiosis and a gametic count of n = 82 (see Plate 15, fig. (a)). Manton also gave cytological evidence of hybridity for D. × uliginosa and the suspected hybrid between D. spinulosa and D. dilatata; both are tetraploid and show a number of paired and unpaired chromosomes during meiosis.

In 1948 a diploid form of D. dilatata was found on the continent and collected from Trondheim in Norway, Storlien in Sweden and Arolla in Switzerland (Manton, 1950). All show typical D. dilatata features but possess a more finely cut pinnation. This character was mentioned by Moore (1863) in his description of D. dilatata var. alpina. In 1949 a specimen agreeing with the characters of var. alpina was collected from the upper regions of Ben Lawers in Perthshire, Scotland (this was the location of the type specimen in Moore's herbarium) by Mr. A. H. G. Alston of the British Museum. This specimen is diploid and Ben Lawers is, as yet, the only confirmed locality in the British Isles where it can be found.

Another form of D. dilatata was collected from the island of Madeira in 1948 and is also diploid (Manton, unpublished). This is a very elegant form with an even more finely cut lamina and is quite distinct from the European diploids as illustrated by Figs. 2 (a) and 3 (a). All these diploids exhibit 41 bivalents during meiosis as seen in Plate 16, figs. (a) and (b).

(5) Experimental

(A) *Hybrids of wild origin*

The extent to which hybridisation occurs in the wild is relatively unknown in

Europe. Apart from *D. × uliginosa* and *D. dilatata × spinulosa*, only one other hybrid plant has been located which represents a cross between the diploid and tetraploid forms of *D. dilatata*. This plant was collected in 1951 by Mr. Alston near the Huberspitze in Bavaria. At the time of collection it was not recognised as a hybrid. It agrees morphologically with *D. dilatata* but differs cytologically in being triploid with $2n = 123$. A systematic search for more hybrids is yet to be made; probably the most reliable guide to hybridity in the field will be the production of abortive spores.

Cytological investigation of the wild hybrids is outlined below :

(1) *D. × uliginosa* (Newm.) Druce – Many squash preparations were sufficiently good to indicate that the number of univalents outnumbered that of the bivalents by approximately 2 : 1 but only four were available for complete meiotic analysis. Three of these contain 39 paired and 86 unpaired chromosomes and the remaining cell has 38 and 88 respectively. One plate showing the former arrangement is illustrated by Fig. 4 corresponding to the cell photographed in Plate 15 fig. (b).

Fig. 4. Explanatory diagram of Plate 15 fig. (b) showing meiosis in the tetraploid hybrid *D. × uliginosa*.
n = 39 pairs + 86 singles (× 1500).

(2) *D. dilatata × spinulosa* – Three plants, each from a different locality, have been used in the study of meiosis in this hybrid. Ten cells have been analysed in which the numbers of bivalents vary from 32 to 35 and those of univalents from 100 to 94 correspondingly. An example of one plate showing the more constant number of 33 pairs and 98 singles can be seen on Plate 15, fig. (c), and explained by Fig. 5.

(3) Hybrid " *D. dilatata* " – This was not examined cytologically until the Spring of 1953 by which time a number of similar hybrids had been synthesised. Seven cells in 1st division meiosis were analysable; six show 38 bivalents and 47 univalents, as seen in Plate 15, fig. (d), and explained by Fig. 6 and the other has 39 bivalents and 45 univalents.
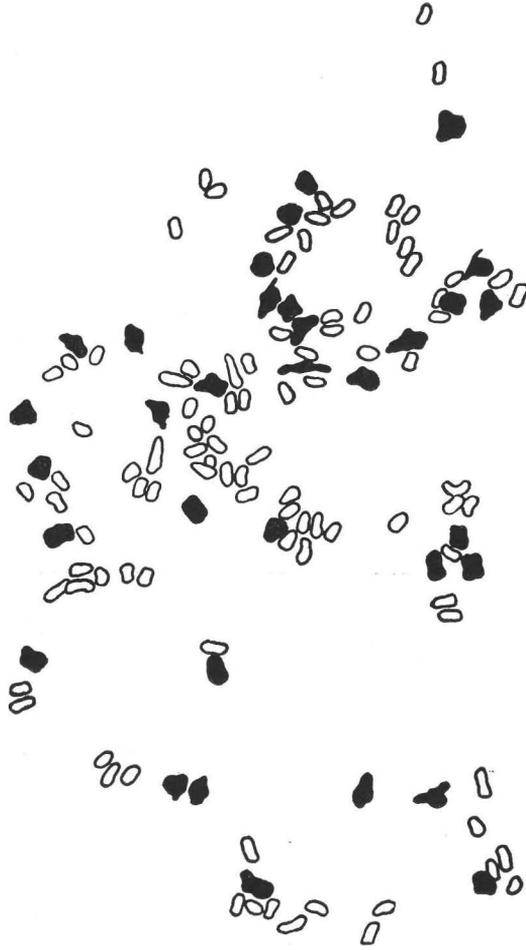
Fig. 5. Explanatory diagram of Plate 15, fig. (c) showing meiosis in the tetraploid
hybrid *D. dilatata* × *D. spinulosa*.   n = 33 pairs + 98 singles (× 1500).

(B) *Synthesised hybrids*

The programme of hybridisation is conveniently summarised by Table 1, in which is indicated the number of attempts made at any one hybrid combination and the relative amounts of success achieved.   Further information is given below.

(1)   *D. cristata* × *D. spinulosa* – This attempt to synthesise *D.* × *uliginosa* has so far been unsuccessful but more trials are being made.

(2)   *D. dilatata* × *D. spinulosa* (4n) – Using *D. spinulosa* from France as the female and a British *D. dilatata* as male, it is probable that this wild hybrid has been synthesised; cytological proof, however, is wanting.   Two plants out of four potential hybrids were normal *D. spinulosa*.   The other two plants, however, exhibit glands on the rhachis and rhacheolae and possess an almost erect rhizome; these are now 3 years old but have never developed beyond a juvenile stage with fronds of 2 in. to 3 in. high.   A reason for this lack of development is not evident. They have not produced fertile fronds and an investigation of the somatic chromosome number would not give confirmation of hybridity since both parents are tetraploid and the hybrid would be also.

(3)   *D. cristata* × *D. dilatata* (4n) – The few attempts to cross these species have

TABLE 1

Hybridisation		No. of prothalli used	Fertilised Prothalli		Confirmed Selfs		Confirmed Hybrids		Unconfirmed	
♀	♂		No.	%	No.	%	No.	%	No.	%
D. cristata	D. spinulosa	12	—	—	—	—	—	—	—	—
D. spinulosa	D. dilatata (4n)	12	4	33	2	50	—	—	2	50
D. cristata	D. dilatata (4n)	12	—	—	—	—	—	—	—	—
" D. dilatata " (2n-Scotland)	D. dilatata (4n)	36	30	83	2	7	28	93	—	—
D. dilatata (4n)	" D. dilatata" (2n-Scotland)	8	3	38	—	—	3	100	—	—
" D. dilatata " (2n-Norway)	D. dilatata (4n)	16	2	13	—	—	2	100	—	—
D. dilatata (4n)	" D. dilatata " (2n-Norway)	24	2	8	1	50	1	50	—	—
"D. dilatata" (2n-Switzerland)	D. dilatata (4n)	24	21	88	1	5	19	90	1	5
" D. dilatata " (2n-Madeira)	D. dilatata (4n)	34	15	44	2	13	13	87	—	—
D. dilatata (4n)	" D. dilatata " (2n-Madeira)	18	2	11	1	50	1	50	—	—
" D. dilatata " (2n-Scotland)	D. spinulosa	12	7	58	6	86	1	14	—	—
D. spinulosa	" D. dilatata " (2n-Scotland)	12	9	75	6	67	3	33	—	—
" D. dilatata " (2n-Madeira)	D. spinulosa	24	6	25	—	—	6	100	—	—
D. spinulosa	" D. dilatata " (2n-Madeira)	18	4	22	3	75	1	25	—	—
D. cristata	" D. dilatata " (2n-Scotland)	36	—	—	—	—	—	—	—	—
D. cristata	" D. dilatata " (2n-Madeira)	36	2	6	2	100	—	—	—	—

Fig. 6. Explanatory diagram of Plate 15, fig. (d) showing meiosis in the triploid hybrid " *D. dilatata* " (Bavaria). n = 38 pairs + 47 singles (× 1500).

been unsuccessful. The two species are ecologically separated in the wild and, so far, a wild hybrid is unknown.

(4) *D. dilatata* (4n) × " *D. dilatata* " (2n-Scotland) – A frond from a 3 years old plant of this combination is illustrated by Fig. 2 (b). In this particular plant the tetraploid had been used as the female parent. The morphology of the hybrid tends to be inter-mediate between that of its parents but likeness with the diploid is most evident. Tetra-ploids of different origin have been used with similar success and the reciprocal cross has also been made. Six chromosome analyses of meiosis in one cross and eight of the reciprocal show, in the majority of cases, 39 bivalents and 45 univalents. This can be seen in the photographed cell, Plate 16, fig. (c) and explained by Fig. 7. In two cases the number of bivalents formed was 38 and in another was 40.

(5) *D. dilatata* (4n) × " *D. dilatata* " (2n-Norway) – Spores collected from a plant in Trondheim, Norway were used as the source of the diploid in this hybrid. The original diploid plant was not maintained in culture but was examined cytologically by Manton (1950) and a herbarium specimen kept. Reciprocal crosses have been made. Altogether nine cells have been analysed at meiosis, seven of which show 39 paired and 45 unpaired chromosomes. The other two show 38 bivalents and 47 univalents.

(6) *D. dilatata* (4n) × " *D. dilatata* " (2n-Switzerland) – Using the diploid as female, 19 hybrids have, so far, been confirmed. The reciprocal cross has not been attempted. Only three cells have been completely analysed for chromosome pairing and, as in the previous hybrids involving European diploid and tetraploid forms of *D. dilatata*, 39 pairs and 45 singles can be recognised.

Fig. 7.   Explanatory diagram of Plate 16, fig. (c) showing meiosis in the triploid hybrid *D. dilatata* (4n) ×
" *D. dilatata* " (2n-Scotland).   n = 39 pairs + 45 singles (× 1500).

(7)   *D. dilatata* (4n) × " *D. dilatata* " (2n-Madeira) – A frond from a 3 year old
hybrid plant in which the female parent was diploid is illustrated by Fig. 3 (b).   Com-
parison of this with Fig. 2 (b) shows clearly the morphological differences found in
triploid " *D. dilatata* " when the European or Madeiran diploid forms are used.   Eleven
analyses of meiotic chromosome pairing in one cross and four analyses from the reciprocal
cross have been made.   In all cells except one the number of bivalents ranges from 34 to
37 together with the corresponding 55 to 49 univalents; nine cells show 36 pairs and 51
singles. In one cell, however, as illustrated by Plate 16, fig. (d) and explanatory Fig. 8
one quadrivalent and one trivalent were found.   One or two cells in anaphase have also
suggested multivalent formation but the percentage is small.   Small segmental inter-
changes between chromosomes otherwise non-homologous are probably the cause of
multivalents in this case.

(8)   " *D. dilatata* " (2n-Scotland) × *D. spinulosa* – Fig. 2 (c) shows the outline of a
frond from a hybrid plant when 18 months old.   Intermediate characters between the
parents such as pinnation, non-prostrate rhizome and two-coloured scales are evident
in living specimens.   Reciprocal crosses have been made and, in all, eight cells analysed
during meiosis; 38 paired and 47 single chromosomes can be seen as in Plate 16, fig. (e)
and explained by Fig. 9.   Variations of ± one bivalent have been found in three of the
plates examined.

(9)   " *D. dilatata* " (2n-Madeira) × *D. spinulosa* – Again reciprocal crosses have
been made and Fig. 3 (c) shows a frond from a hybrid, when 18 months old.   Cytological
investigation of meiosis has shown 35 bivalents and 53 univalents in 7 analysed cells and
34 bivalents with 55 univalents in two other cells.   An example of the former arrangement
can be seen on Plate 16, fig. (f) and explanatory Fig. 10.

(10)   *D. cristata* × " *D. dilatata* " (2n) – No hybrids have been formed when
attempting to cross *D. cristata* with either of the diploid forms of *D. dilatata* from Scotland
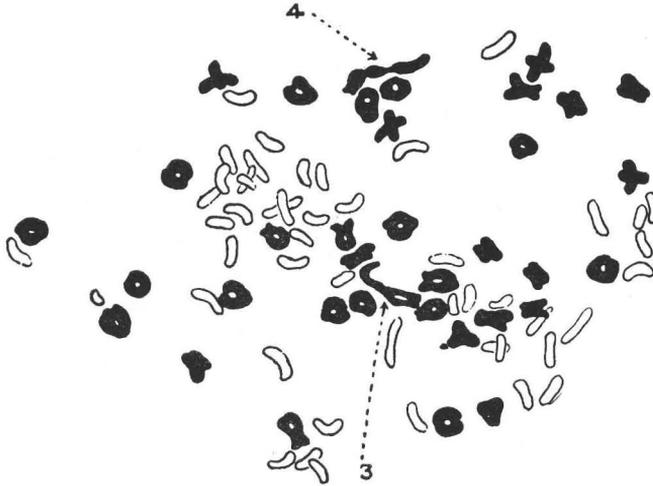
Fig. 8. Explanatory diagram of Plate 16, fig. (d) showing meiosis in the triploid hybrid *D. dilatata* (4n) × " *D. dilatata* " (2n-Madeira). n = 1 quadrivalent + 1 trivalent + 34 pairs + 48 singles (× 1500).
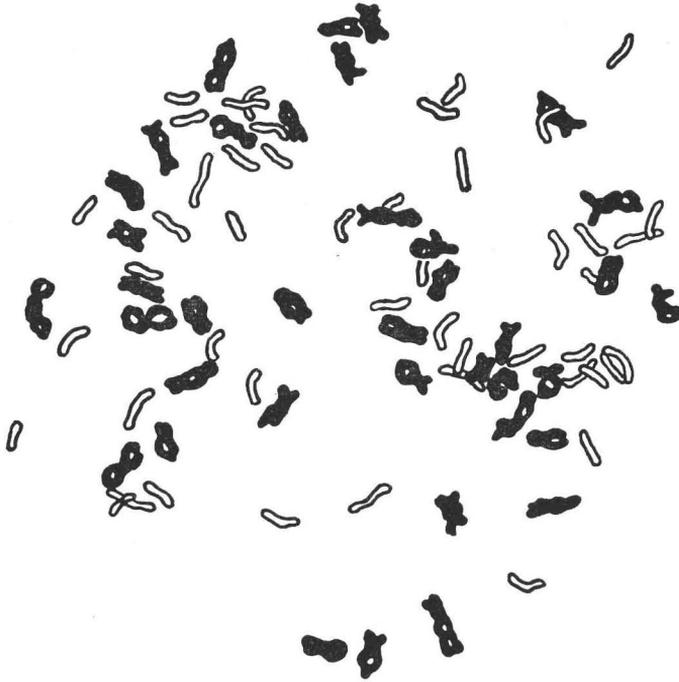
Fig. 9. Explanatory diagram of Plate 16, fig. (e) showing meiosis in the triploid hybrid *D. spinulosa* × " *D. dilatata* " (2n-Scotland). n = 38 pairs + 47 singles (× 1500).

or Madeira. Two young plants which did develop were found to be normal *D. cristata* which had been used as the female parent and probably selfed at the time of attempted hybridisation.

Attempts to synthesise any hybrid utilising *D. cristata* as one parent have not been successful. The prothallial cultures of this species have never produced sex organs in quantity; the numbers of swimming spermatozoids have been relatively small and mature archegonia few at any one time. This may be attributed to the present cultural conditions

Fig. 10. Explanatory diagram of Plate 16, fig. (f) showing meiosis in the triploid hybrid *D. spinulosa* ×
" *D. dilatata* " (2n-Madeira). n = 35 pairs + 53 singles (× 1500).

which, though satisfactory for other species, may have to be varied for a species like
D. cristata which normally inhabits acid bogs and swampy places. The need for preventing
access to free water within cultures may be disadvantageous for the normal growth and
sex organ development in prothalli of *D. cristata*.

(6) DISCUSSION

Hybridisation within the *D. spinulosa* complex and the corresponding cytological
evidence given above is represented by Fig. 11. Reciprocal hybrids have been studied
in all the synthesised lines but no cytological differences found. Chromosome counts
of the species and wild or synthesised hybrids so far investigated are summarised in
Table 2.

In the genus *Dryopteris* the basic haploid chromosome number is $x = 41$ (Manton,
1950). The presence of 39 bivalents during meiosis in $D. \times uliginosa$ and of 33 in the
hybrid *D. dilatata* × *spinulosa* is therefore highly significant since these numbers are
not far removed from 41. ¯Either autoploidy exists in one or more of the tetraploid
species *D. cristata*, *D. spinulosa* and *D. dilatata*, in which case the bivalents could be
formed from the gametic chromosome set of the autoploid parent, or there is evidence
of alloploidy with common ancestral diploids, one between *D. cristata* and *D. spinulosa*
and another between *D. spinulosa* and *D. dilatata*.

Not one of the three parent tetraploids exhibit multivalent formation during meiosis,
which would indicate duplicated sets of chromosomes common to autoploids, but this
ability may have been lost and yet the tendency for homologous pairing to occur in the
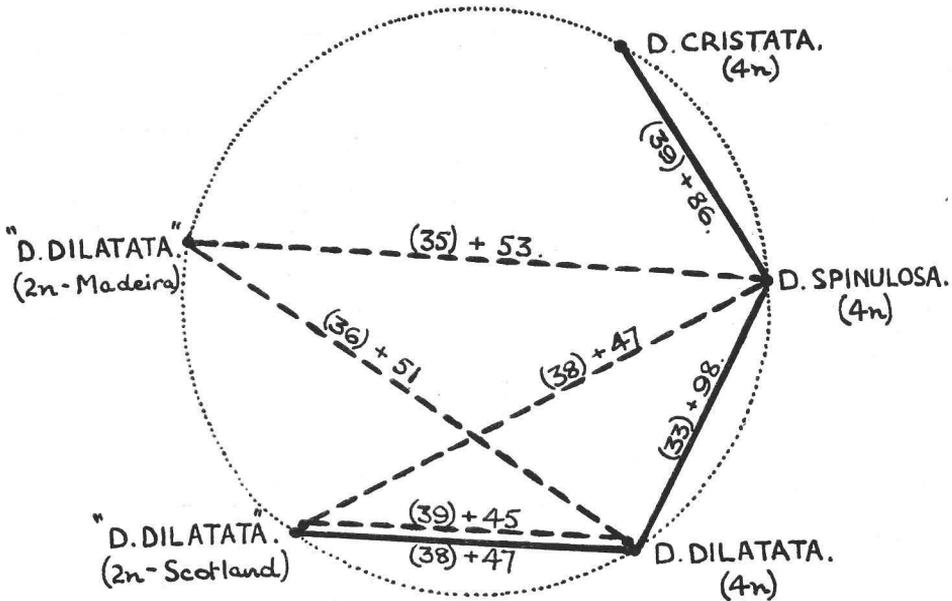
Fig. 11. Hybridisation within the complex. Continuous lines show wild hybrids and broken lines those synthesised. Numbers of bivalents formed during meiosis are shown in brackets; other numbers represent the univalents formed.

gametic chromosome set present in a species-hybrid still remain. The available evidence, however, suggests that *D. cristata*, *D. spinulosa* and *D. dilatata* are in fact allotetraploids.

In Eastern North America and Canada *D. cristata* is involved in a number of hybrids which have been cytologically examined (Manton & Walker, 1953; Walker, unpublished). Two of these are triploid and show complete failure of pairing at meiosis but another is tetraploid with pairs and univalents; bivalents are not formed therefore where there is a lack of chromosome homology between the parent species and this excludes auto-ploidy in *D. cristata*.

Evidence against autoploidy in *D. dilatata* has been provided by an investigation of induced apogamy in this species (Manton & Walker, 1954). An apogamous sporophyte with a somatic complement of 82 (normal sexually reproduced sporophytes have 2n=164) shows complete failure of pairing between these chromosomes during meiotic division.

For *D. spinulosa* the evidence is less complete. This species does not, so far as is known, form hybrids, which show complete failure of meiotic chromosome pairing, nor has the formation of an apogamous plant yet been induced. Reference to Fig. 11 however, will indicate the variation in the number of bivalents (33 to 39) formed in hybrids involving *D. spinulosa*. If this species were an autotetraploid, the number of bivalents, arising from homologous pairs within the gametic chromosome set, should remain more constant in the different hybrids.

The *D. spinulosa* complex includes, on available evidence, three allotetraploid species inter-related by common diploid ancestors. If the genomes of the ancestral diploids are represented by letters, the genetic constitution of the three allotetraploids can be illustrated as below :

D. cristata	..	A + B
D. spinulosa	..	B + C
D. dilatata	..	C + D (or B + D, or C + A)

TABLE 2

Species or Hybrid	Source	2n (Roots)	n (bivalents in brackets)	Ploidy
D. cristata (L.) A. Gray	England	c. 160	(82)	Tetraploid
D. spinulosa (Müll.) Watt	England	–	(82)	Tetraploid
	France	c. 160	(82)	Tetraploid
	Germany	–	(82)	Tetraploid
	Sweden	–	(82)	Tetraploid
D. dilatata (Hoffm.) A. Gray	England	c. 160	(82)	Tetraploid
	France	–	(82)	Tetraploid
	Germany	–	(82)	Tetraploid
	Ireland	–	(82)	Tetraploid
	Shetlands	–	(82)	Tetraploid
	Sweden	–	(82)	Tetraploid
" *D. dilatata* "	Madeira	82	(41)	Diploid
	Norway	–	(41)*	Diploid
	Scotland	82	(41)	Diploid
	Sweden	82	(41)	Diploid
	Switzerland	–	(41)	Diploid
" *D. dilatata* "	Bavaria	–	(38) + 47	Triploid
D. × *uliginosa* (Newm.) Druce	Continent	c. 160	(39) + 86	Tetraploid
D. dilatata × *spinulosa*	England	–	(33) + 98	Tetraploid
	Ireland	c. 160	(34) + 96	Tetraploid
D. dilatata (induced apogamy)	England	82	82	Diploid
D. dilatata (4n) × " *D. dilatata* " (2n-Britain)	Synthetic	c. 120	(39) + 45	Triploid
D. dilatata (4n) × " *D. dilatata* " (2n-Norway)	Synthetic	c. 120	(39) + 45	Triploid
D. dilatata (4n) × " *D. dilatata* " (2n-Switzerland)	Synthetic	c. 120	(39) + 45	Triploid
D. dilatata (4n) × " *D. dilatata* " (2n-Madeira)	Synthetic	c. 120	(36) + 51	Triploid
" *D. dilatata* " (2n-Britain) × *D. spinulosa*	Synthetic	c. 120	(38) + 47	Triploid
" *D. dilatata* " (2n-Madeira)× *D. spinulosa*	Synthetic	c. 120	(35) + 53	Triploid

* (Manton, 1950)

The ancestral genome B is common to *D. cristata* and *D. spinulosa*, whilst C is common to *D. spinulosa* and *D. dilatata*.   That genome B cannot be common to all three species is evident from hybrids known in N. America and those synthesised between European and American species of the complex (Walker, unpublished); this excludes the alternative B + D combination for *D. dilatata* as above.   The combination C + A can also be excluded since genome A must carry the factors responsible for simple pinnation in *D. cristata* and the suggested genome D will be responsible for the erect rhizome habit and scale colour in *D. dilatata*.

The three tetraploid species within the complex can have been formed therefore only as a result of hybridisation amongst at least four ancestral diploids, and as the present-day distribution of *D. cristata*, *D. spinulosa* and *D. dilatata* is wide so is the field of search for ancient diploids.

Hybrids which have been synthesised between the diploids from Europe and Madeira and tetraploid *D. dilatata* or *D. spinulosa* are all triploid and show an approximation to equal numbers of bivalents and univalents during meiosis (Table 2). This suggests that the ancestral diploid common to *D. dilatata* and *D. spinulosa* is now represented by diploid forms of "*D. dilatata*" in Europe and Madeira. The diploids from Britain, Norway, Sweden and Switzerland are similar in their morphology and cytological behaviour; hybrids between any one of them and tetraploid *D. dilatata* show the same number of bivalents when analysed during meiosis. The diploid from Madeira, however, is distinct in its morphology, being more finely cut than the European diploids and also producing spores smaller in size, which average 42 μ in length as against an average of 55 μ. It differs cytologically when involved in triploid hybrids; the number of bivalents formed in hybrids with the Madeiran form is constantly lower than in triploids involving the European form; in those with the European form there are 38 to 39 pairs and with the Madeira form 35 to 36 pairs with a possibility of multivalent formation to a very small degree.

These differences between the diploid forms are significant but nevertheless insufficient to suggest that the diploids represent other than the same ancestral genome from which they evolved separately; their separation may well have taken place even before the origin of the tetraploid species. That the two diploid forms represent the same ancestral genome can be confirmed by hybridisation between them. This is now in progress. If the two forms are closely related, as seems to be the case from experimental hybrids, chromosome pairing should be high in the hybrid with a possibility of a few univalents or one or two multivalents.

Separation of the Madeiran form from the normal tetraploid *D. dilatata* is simple on the type of pinnation alone, although spore size differences apply also as in the separation of the Madeiran and European diploids. It is more difficult to distinguish the European diploid from the tetraploid, particularly in the field. The problem is enhanced by the wide range of form exhibited by the tetraploid itself which may vary in mature size and shape even in the same locality. The most reliable macroscopic characters in the diploid are the marked unevenly deltoid basal pinnae and the greater tendency to tripinnation. However, microscopic characters are more useful and recently Crane (1955) has shown that the spores of the tetraploid and diploid *D. dilatata* differ in their ornamentation, though not in their size. Other characters are being studied.

The diploids from Europe and Madeira differ from tetraploid *D. dilatata* both cytologically and in their morphology. Triploid hybrids, wild and synthesised, are sterile with abortive spores. On this evidence the diploids represent a distinct species and should be separated from *D. dilatata*. Further, the European and Madeiran forms of the diploid can be separated from one another by their morphology and slight chromosomal differences exhibited in the synthesised hybrids; they are worthy of subspecific recognition. The final details of nomenclature and taxonomic descriptions of the diploids will be considered in a later paper when the inter-relationships of species in Europe and N. America have been made known.

The *D. spinulosa* complex in Europe now consists of four recognisable species, three tetraploid and one diploid, with hybrids between them, both tetraploid and triploid. To what extent, if any, the hybrids are able to back-cross is unknown, though the appearance of mixed populations of *D. dilatata* and *D. spinulosa* suggests there may be slight fertility

in the hybrids. Spore sowings have not confirmed this and the apparent intergrading is probably not a result of introgression but arises from the polymorphism present in *D. dilatata*. A detailed cytological survey of mixed populations could give further information. An alternative explanation of the appearance of mixed populations may simply be the effect of matrocliny; Chandler (1948) working on the morphology of *Dryopteris* hybrids in N. America does suggest that this may occur in the wild. In the experimental hybridisation between species of the *D. spinulosa* complex reciprocal crosses have been made without any evidence of matrocliny; in almost every synthesised triploid there has been evidence of the diploid involved, irrespective of whether the diploid was the male or female parent. This could be explained by gene dosage, each triploid possessing twice the genome of the diploid present, and would not discount matrocliny as a possibility in tetraploid hybrids.

(7) Summary

Cytological observations have been made on the species and species-hybrids, both wild and synthesised, in the *Dryopteris spinulosa* complex as represented in Europe (see Table 2 and Fig. 11).

There are three tetraploid species in the complex, namely *D. cristata*, *D. spinulosa* and *D. dilatata*, all of which are allopolyploids. At least four ancestral diploid species would be required to account for the origin of these triploids. One of the ancestral genomes is common to *D. cristata* and *D. spinulosa*, and another is common to *D. spinulosa* and *D. dilatata*.

Chromosome pairing in synthesised hybrids suggests that the common ancestor of *D. spinulosa* and *D. dilatata* is now represented by two distinct diploid forms of " *D. dilatata* "; one is found in Britain and on the continent of Europe and the other on the island of Madeira. It is suggested that these forms should be recognised as subspecies of a single diploid species which is additional to the polyploid complex. The nomenclature and taxonomic description must be left for discussion in a later paper. These diploids differ slightly in their cytological behaviour when hybridised with the tetraploid species *D. spinulosa* or *D. dilatata*, indicating that there is differentiation within the diploid genome resulting in a loss of chromosome homology.

Reciprocal crosses between species in different grades of ploidy have been made without difficulty or evidence of matrocliny.

(8) Acknowledgments

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PLATE 15
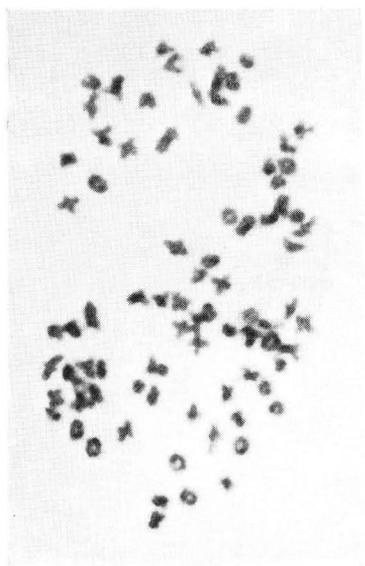
(a)
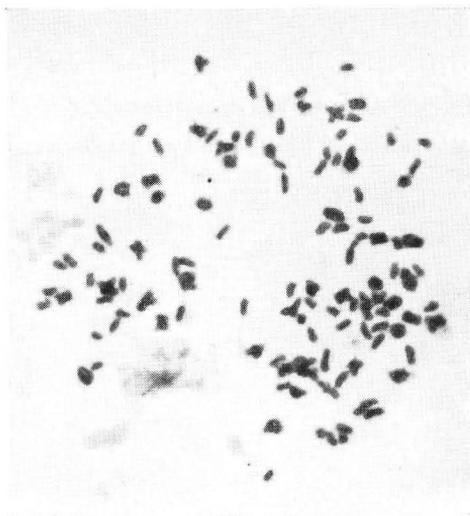
(b)
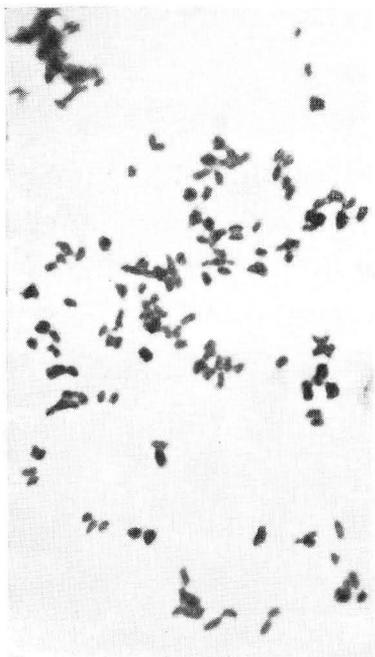
(c)
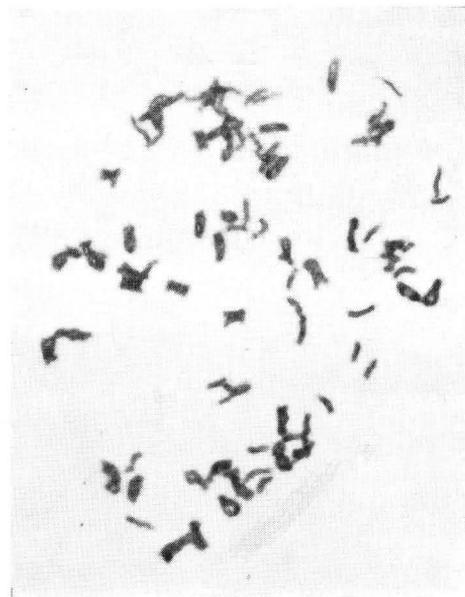
(d)

Shows meiosis in the following :

(a) *D. cristata*. 1st diakinesis. $n = 82$ pairs ($\times$ 1,000).

(b) *D. $\times$ uliginosa*. 1st metaphase. n $= 39$ pairs $+ 86$ singles ($\times$ 1,000). See explanatory Fig. 4.

(c) Hybrid *D. dilatata $\times$ spinulosa*. 1st metaphase. n $= 33$ pairs $+ 98$ singles. ($\times$ 1,000). See explanatory Fig. 5.

(d) Hybrid " *D. dilatata* " (Bavaria). 1st diakinesis, early. n $= 38$ pairs $+ 47$ singles ($\times$ 1,000). See explanatory Fig. 6.

PLATE 16.
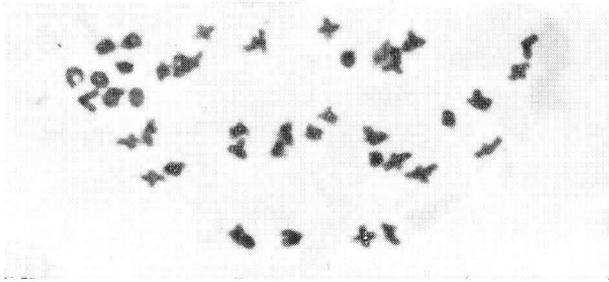
(a)
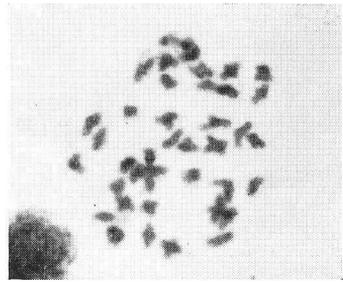
(b)

(c)
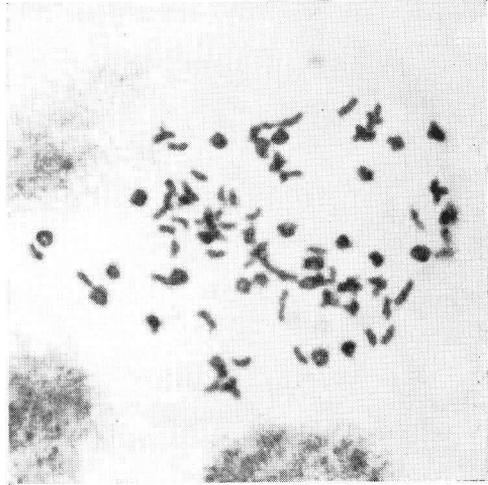
(d)

(e)
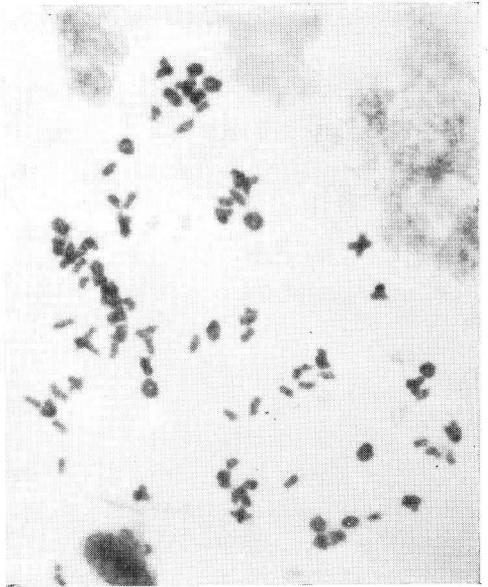
(f)

Shows meiosis in the following :

(a) " *D. dilatata* " (Scotland). 1st diakinesis. n = 41 pairs (× 1,000).
(b) " *D. dilatata* " (Madeira). 1st diakinesis. n = 41 pairs (× 1,000).
(c) Hybrid *D. dilatata* (4n) × " *D. dilatata* " (2n, Scotland). 1st diakinesis, early. n = 39 pairs + 45 singles (× 1,000). See explanatory Fig. 7.
(d) Hybrid *D. dilatata* (4n) × " *D. dilatata* " (2n, Madeira). 1st diakinesis. n = 1 quadrivalent + 1 trivalent + 34 pairs + 48 singles (× 1,000). See explanatory Fig. 8.
(e) Hybrid " *D. dilatata* " (2n, Scotland) × *D. spinulosa*. 1st diakinesis, early. n = 38 pairs + 47 singles (× 1,000). See explanatory Fig. 9.
(f) Hybrid " *D. dilatata* " (2n, Madeira) × *D. spinulosa*. 1st diakinesis. n = 35 pairs + 53 singles (× 1,000). See explanatory Fig. 10.

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