A population of *Botrychium lanuginosum* Wall. ex Hook. & Grev. from Papua New Guinea and its relevance to hybridisation in pteridophytes with subterranean gametophytes

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Abstract

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A population of *Botrychium lanuginosum* from a small abandoned garden area was cytologically examined, revealing octoploids, hypo-octoploids and hexaploid hybrids. The presence of the hybrids suggested that tetraploids may also be present. These three levels of ploidy were confirmed on spore characters and stomatal length on a representative sample of 53 plants. Nearly half the sample was hybrid and both octoploids and hybrids showed a wide range of morphology suggesting outcrossing and hybridisation between many different genotypes. As *Botrychium* has no accessory means of reproduction each hybrid represents a separate act of creation and the presence of so many hybrids demonstrates that the subterranean nature of the gametophyte need not be an effective barrier to crossing.

Introduction

A brief note on the cytology of *Botrychium lanuginosum* Wall. ex Hook. & Grev. was included in an account of the Malesian species of *Botrychium* (Jermy and Walker 1977). In that note only the barest facts were recorded and these need amplification as they have an important bearing on cross-fertilization and hybridisation in pteridophytes which have subterranean gametophytes.

Materials and methods

Whilst collecting ferns in 1964 at Moro, a village at 1680 m in the Finisterre Mountains in Madang District, eastern Papua New Guinea, Mr. A.C. Jermy of the British Museum (Natural

Dedicated to Prof. Reichstein on the occasion of his 90th birthday

History) and myself showed the local children some pictures of *Botrychium* in the hope that they knew where the plants grew. The children very rapidly collected for us large amounts of this fern from old garden sites which had been colonized by the grass *Imperata*. These specimens were sorted through for fixable material and a sample of about 50 individuals was also pressed to illustrate the range of morphological variation. Fixation of young sporangia was carried out in the field using a solution of 3 parts absolute alcohol : 1 part glacial acetic acid and these were later examined in the laboratory at Newcastle.

Mature spores were mounted in Euparal and their length measured, 50 spores being used for each sample. In addition small pieces of sterile pinnules were taken from the herbarium specimens, heated for a few minutes in water together with a few drops of detergent to rehydrate the tissues and these were used to measure stomatal lengths. Again, 50 measurements were taken of each sample.

Observations

The first two plants to be examined gave chromosome counts of $n = 176$ very clearly (fig. 1 A). This suggested an octoploid level on a base number of $x = 44$. However, another plant gave equally clear preparations (fig. 1 B) showing 180 bivalents at meiosis. This is in agreement with an octoploid level, founded on $x = 45$ which is the well-established base number for the genus as a whole. It was clear therefore that in this population there was a cytological mixture including some plants which were strict octoploids and others which were hypo-octoploid. These latter plants were perfectly normal in appearance. It may be concluded that the combination of a high basic chromosome number coupled with a high level of ploidy results in chromosomes and genes being present in multiplicate and that as a result there is no serious effect if a few chromosomes are omitted from the complement. This is comparable with the situation noted in *Ophioglossum costatum* R. Br. (Walker 1984 a) in which an aneuploid series ranging from $n = 116$ to 120 was present in a West African population. As these hypo-octoploids cannot be distinguished from the strict octoploids on any grounds other than cytological they are all treated as octoploids without distinction in the following account.

In addition to the octoploids four plants proved to be hexaploid with 270 chromosomes showing irregular meiosis in which bivalents and univalents were present (figs. 2 A, B). Typical analyses showed a small range from 85 bivalents plus 100 univalents to 87 bivalents plus 96 univalents i.e. approximating to $2x \, II + 2x \, I$. The morphological range of these hexaploids was also wide and paralleled that of the octoploids. The presence of the hexaploids showing both a hybrid meiosis and a similar variation in morphology to the octoploid suggests that they have been formed by individual acts of hybridisation between an octoploid and a tetraploid. Although no direct cytological proof has been obtained for the presence of the tetraploids their occurrence may be inferred in the light of more detailed morphological studies presented below. Indeed, elsewhere in the world only tetraploids have been recorded for this species, namely from the Himalayas (Verma and Loyal 1960), from S. India (Ninan 1956, Manikam 1984) and from Sri Lanka (Manton and Sledge 1954).

It was evident even on the basis of the very limited sample of seven cytologically analysed plants that the gross morphology of both octoploids and hexaploids was not only very variable but that they apparently completely overlapped. An attempt was made to find if some means other than chromosome counts could be used to enable all the members in the population sample of herbarium specimens to be ascribed to a par-
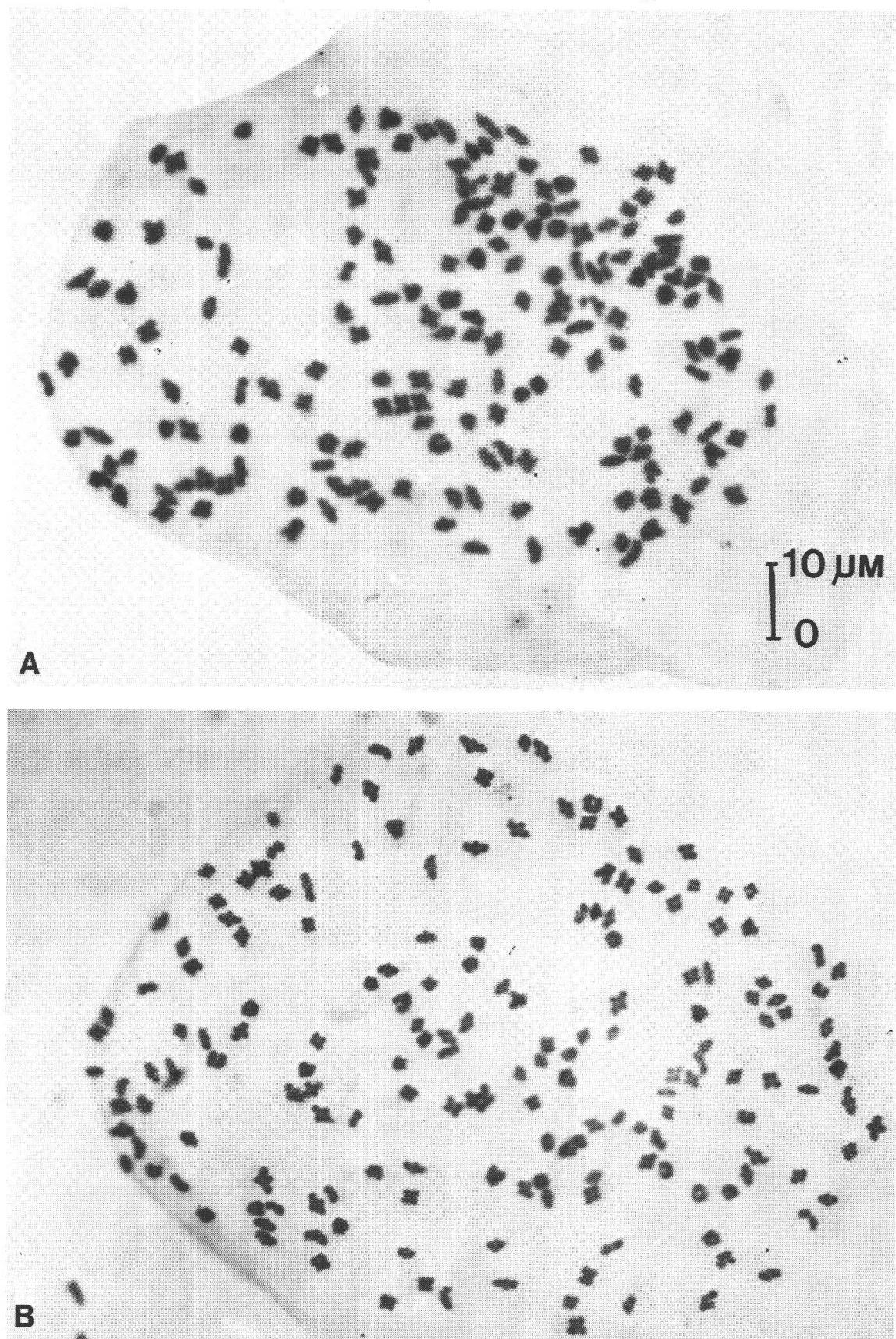
Fig. 1. Photographs of meiosis in *Botrychium lanuginosum*. A, hypo-octoploid 8329 with 176 bivalents; B, octoploid 8333 with 180 bivalents.
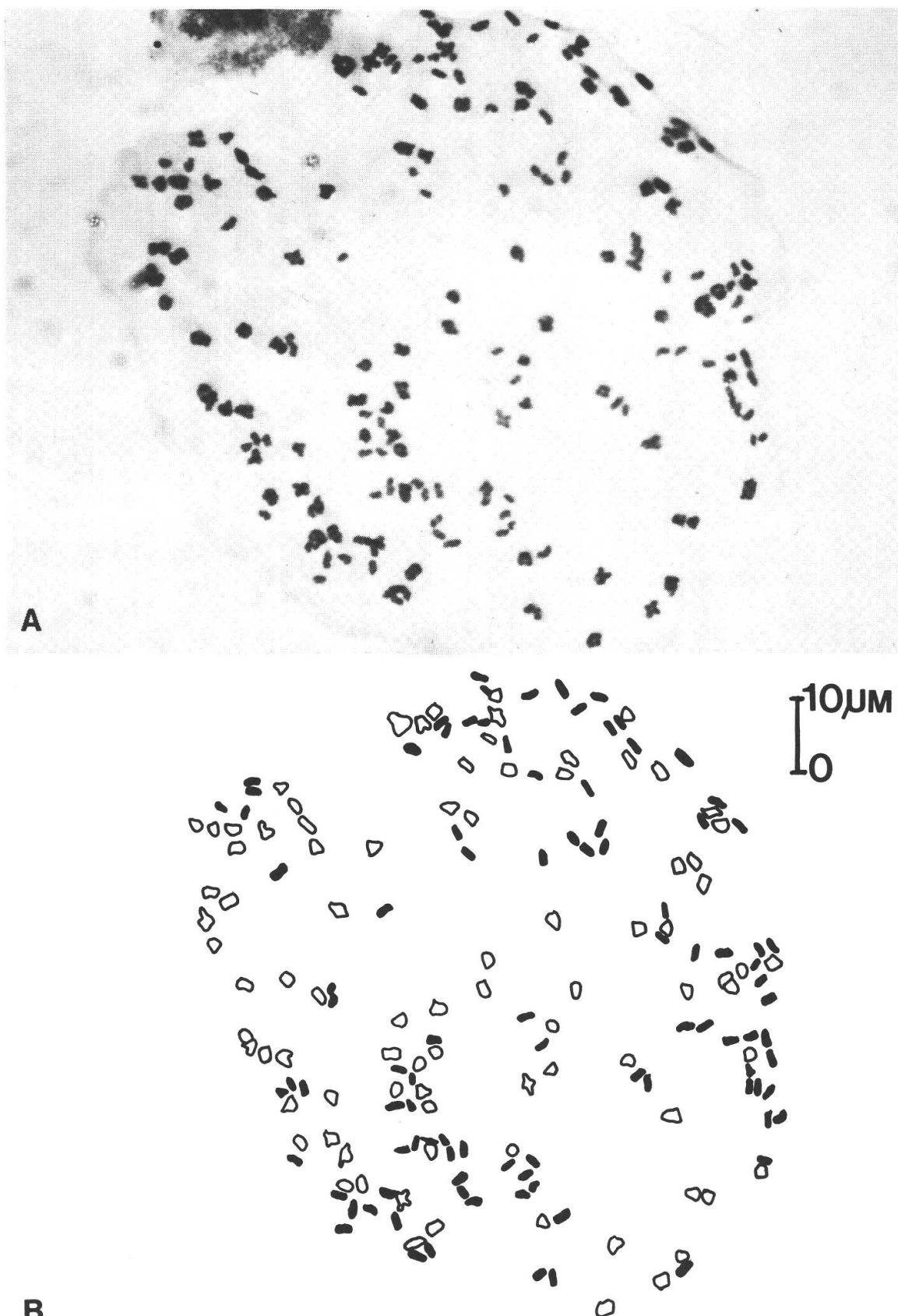
Fig. 2. Meiosis in hexaploid 8336 *Botrychium lanuginosum*. A, photograph; B, explanatory diagram showing 87 bivalents (outlined) plus 96 univalents (solid black).
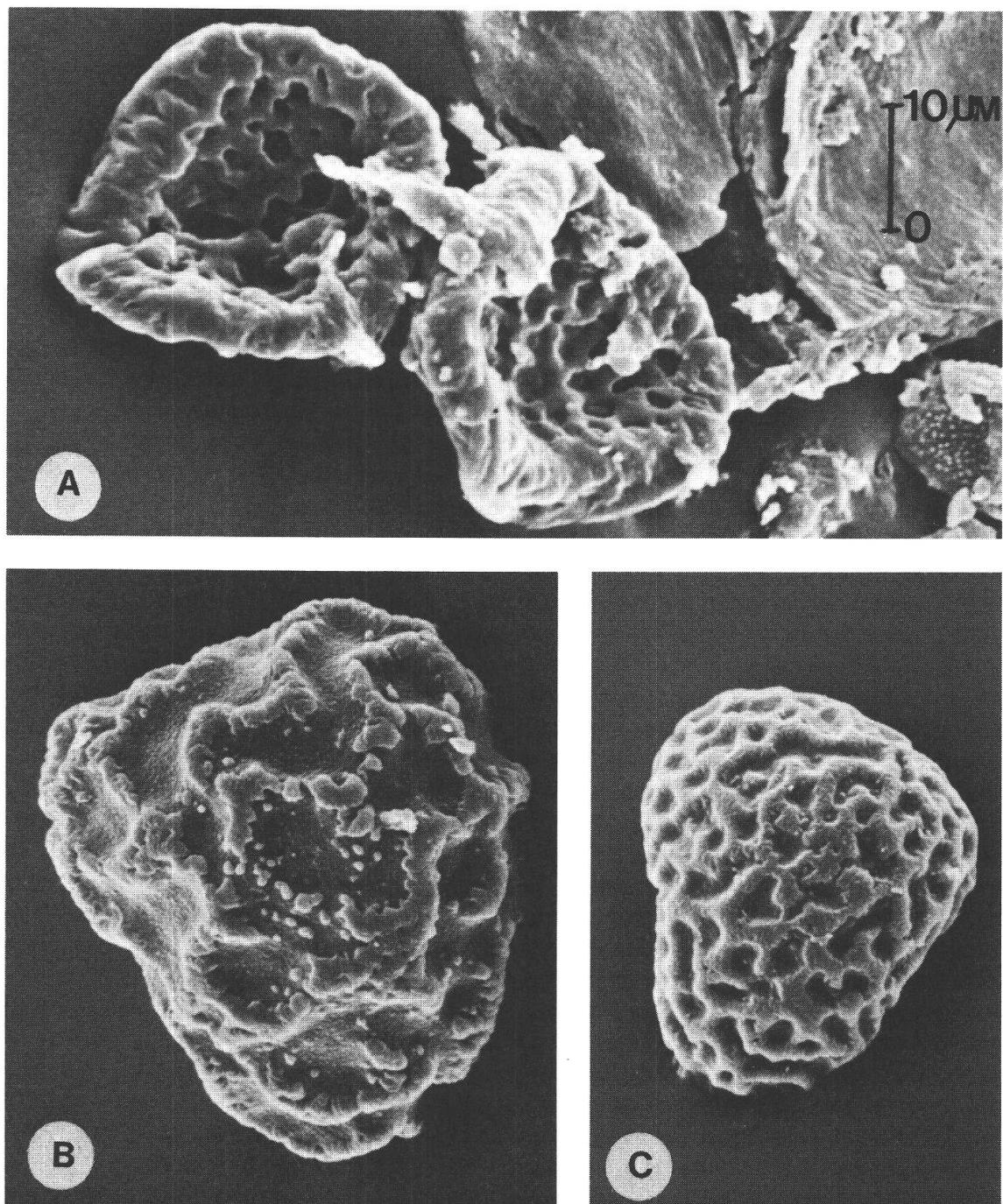
Fig. 3. SEM micrographs of spores of *Botrychium lanuginosum*. A, abortive ones of J3891, hexa-
ploid hybrid; B, 8352 octoploid; C, 8353 putative tetraploid.

ticular level of ploidy. One possible source of information was in the size and appear-
ance of the mature spores. Here there is an immediately obvious difference as might be
expected between a hexaploid with an irregular meiosis resulting in crumpled abortive
spores (fig. 3 A) and an octoploid with a regular meiosis giving rise to large well-filled
spores (fig. 3 B) with a mean length of 43 µm. During this survey a third type of spore
was found (fig. 3 C) being well-filled, of a different morphology and having a mean
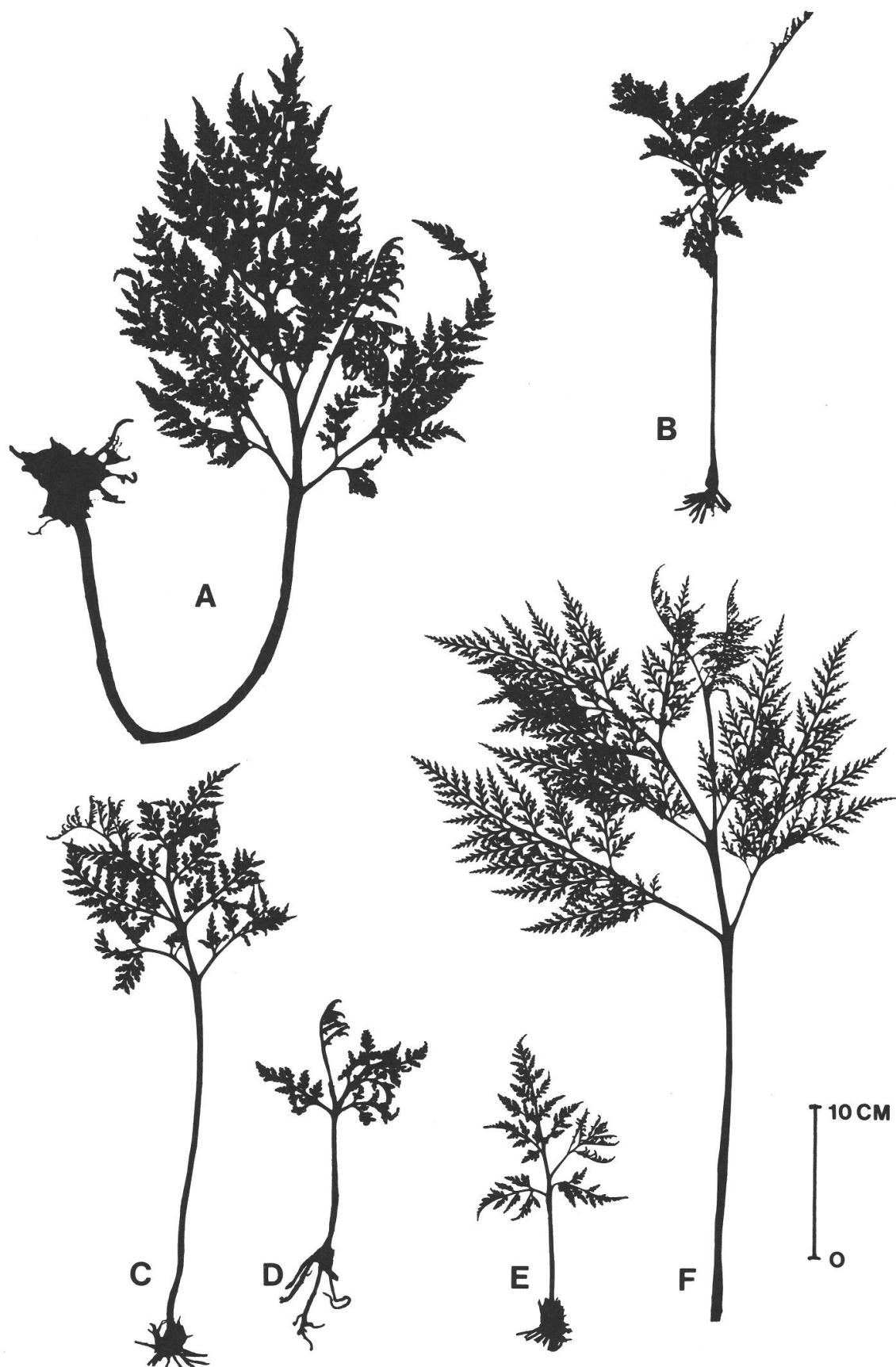
Fig. 4. Silhouettes of octoploid *Botrychium lanuginosum*. A, 8324; B, 8364; C, 8357; D, 8329; E, 8333; F, 8353.
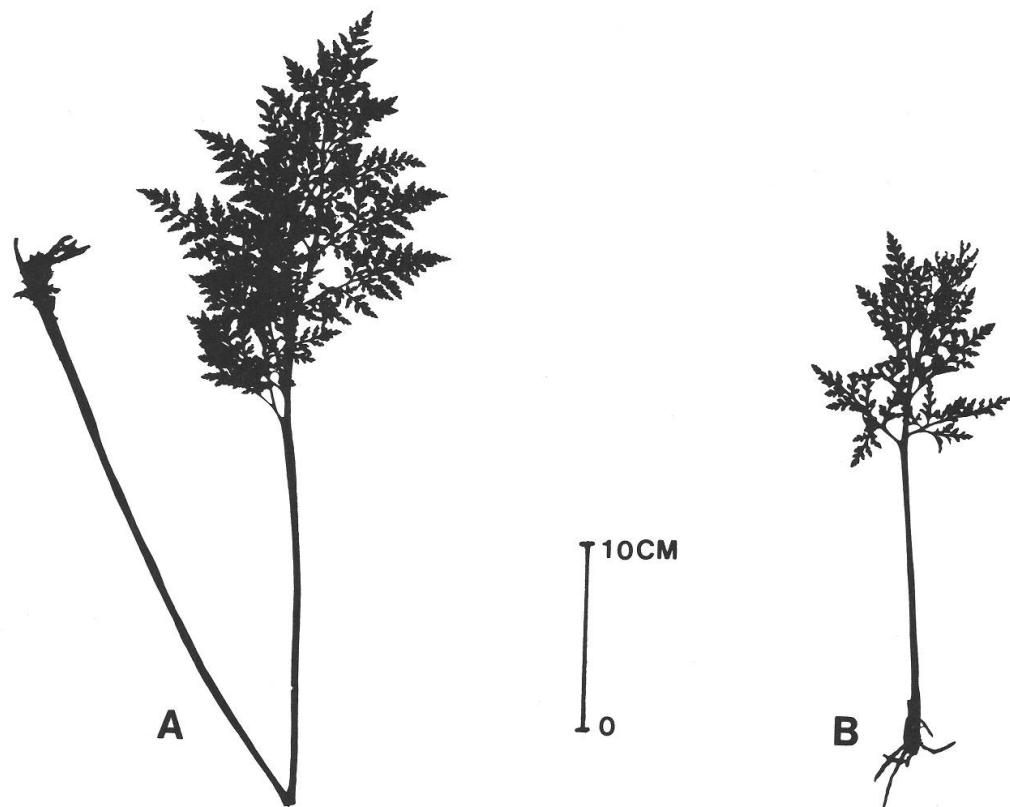
Fig. 5. Silhouettes of putative tetraploid *Botrychium lanuginosum.* A, 8341; B, 8343.

length of 35 µm. This suggested the presence of a third cytotype – possibly the postulated tetraploid.

Because the population sample had been collected not only to show some of the morphological range present but primarily with a view to obtaining as many cytological preparations as possible, the fertile pinnae were young and only a few had mature spores present, hence this character could only be used in a very few cases. Therefore another differentiating character was sought which could be used on all the specimens regardless of their state of reproductive maturity. Stomatal length was such a possible character and was initially measured on the cytologically confirmed specimens of octoploids and hexaploids. Each sample consisted of 50 measurements and the mean lengths of both cytotypes proved to be distinct and showed no overlap, ranging from 81.4 to 82.9 µm in the octoploids and from 75.8 to 76.9 µm in the hexaploids. As this seemed to be a satisfactory means of distinguishing cytotypes, stomatal lengths of all members of the population were measured, and with the exception of five individuals they separated clearly into two categories with their means corresponding to those found in the confirmed octoploids and hexaploids respectively. The five exceptions all had smaller stomata with means ranging from 69.8 to 70.9 µm. Three of these plants had good spores and they were much smaller than those of the octoploids with means ranging from 34.5 to 35.6 µm and having the morphology of the spores of the presumed tetraploid. These five exceptional plants are therefore assumed to be tetraploid.

The means of all the stomatal lengths, the spore lengths where available and the confirmed cytology of the individual plants are given in Table 1. It will be noted that on

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Table 1. Summary of the cytology, mean spore lengths and mean stomatal lengths of the sample
of 53 specimens

Collectors' Number [1]	Cytology	$\bar{M}$ spore length (in μm)	$\bar{M}$ stomatal length (in μm)
8333	n = 180 (8x)	–	82.9
8324	n = 176 (hypo-8x)	–	82,1
8329	n = 176 (hypo-8x)	–	81.4
J3885	–	–	81.0
J3887	–	–	83.3
J3889	–	–	81.0
J3898	–	–	82.5
J3901	–	–	83.3
J3904	–	–	79.9
8339	–	–	81.4
8344	–	43,0	81.8
8345	–	–	80.6
8350	–	–	81.0
8351	–	–	79.9
8352	–	43.5	82.5
8355	–	–	82.5
8356	–	–	81.0
8357	–	43.1	80.3
8359	–	–	80.6
8360	–	–	80.3
8363	–	–	81.4
8364	–	–	82.9
8366	–	–	79.9
8332	279 chr., irreg. (6x)	–	76.9
8334	270 chr., irreg. (6x)	–	76.9
8335	270 chr., irreg. (6x)	–	75.8
8336	270 chr., irreg. (6x)	–	76.1
J3886	–	–	77.6
J3888	–	–	76.5
J3890	–	–	75.4
J3891	–	–	75.8
J3893	–	–	75.8
J3896	–	–	76.5
J3897	–	–	76.1
J3900	–	–	76.9
J3902	–	–	76.1
8338	–	–	75.8
8340	–	–	76.9
8342	–	–	76.9
8346	–	–	75.4
8347	–	–	76.1
8348	–	–	75.1
8349	–	–	76.1
8354	–	–	75.0
8358	–	–	76.1
8361	–	–	76.1
8362	–	–	75.0
8365	–	–	76.5

Table 1. (continued)

Collectors' Number[1]	Cytology	M̄ spore length (in µm)	M̄ stomatal length (in µm)
J3889	–	35.6	69.0
8337	–	–	69.0
8341	–	34.5	70.5
8343	–	–	69.8
8353	–	34.8	70.9

[1] Specimens with a J preceding the Collector's number were collected by A. C. Jermy, other specimens by the Author.

these characters the population falls into three discrete classes corresponding to the octoploid level, the presumed tetraploid, and the hexaploid hybrid between them. It will be further noted that out of this more or less randomly selected sample the octoploids and hybrids are more or less equally common, whilst the presumed tetraploid represents less than 10% of the total.

Morphologically the octoploid plants are extremely variable in height, lamina area, and degree of dissection of the pinnae, as may be seen in fig. 4. The range in variation shown by the presumed tetraploids was restricted (fig. 5) although this may be a reflection of the small numbers of this group rather than being a genuine feature. Many of the hexaploids showed extreme vigour and their range of variation paralleled those of the octoploids (fig. 6), a point which will be returned to in the following discussion. In addition to the gross morphological features which can be seen in the silhouettes, a further variable character was shown in the hairiness of the fronds, ranging from sparsely to quite densely hairy.

Discussion

Botrychium shares with a number of other pteridophytes the possession of a subterranean gametophyte. This habit occurs in a number of genera belonging to several different families, i.e. *Psilotum* and *Tmesipteris* (Psilotaceae); *Huperzia* and *Lycopodium* sensu Øllgaard 1987 (Lycopodiaceae); *Ophioglossum, Botrychium* and *Helminthostachys* (Ophioglossaceae); *Stromatopteris* (Gleicheniaceae); and part of *Schizaea* (Schizaeaceae).

In the past doubts have been expressed (Lloyd 1974) about the ability of underground gametophytes to outcross and hybridise to any great extent due to the supposed difficulties inherent in this environment. Such difficulties were assumed to include wide dispersal of the gametophytes and a general physical impedence of movement of the spermatozoids through the soil. However, in a recent review (Wagner, Wagner and Beitel 1985) these alleged restrictions have been shown not to operate to a great extent and these authors list some 35 interspecific hybrids, mainly in Lycopodiaceae and *Botrychium*. In addition to the interspecific hybrid quoted of *Psilotum nudum × complanatum* in Hawaii it has been shown (Walker 1984 b) that in Papua New
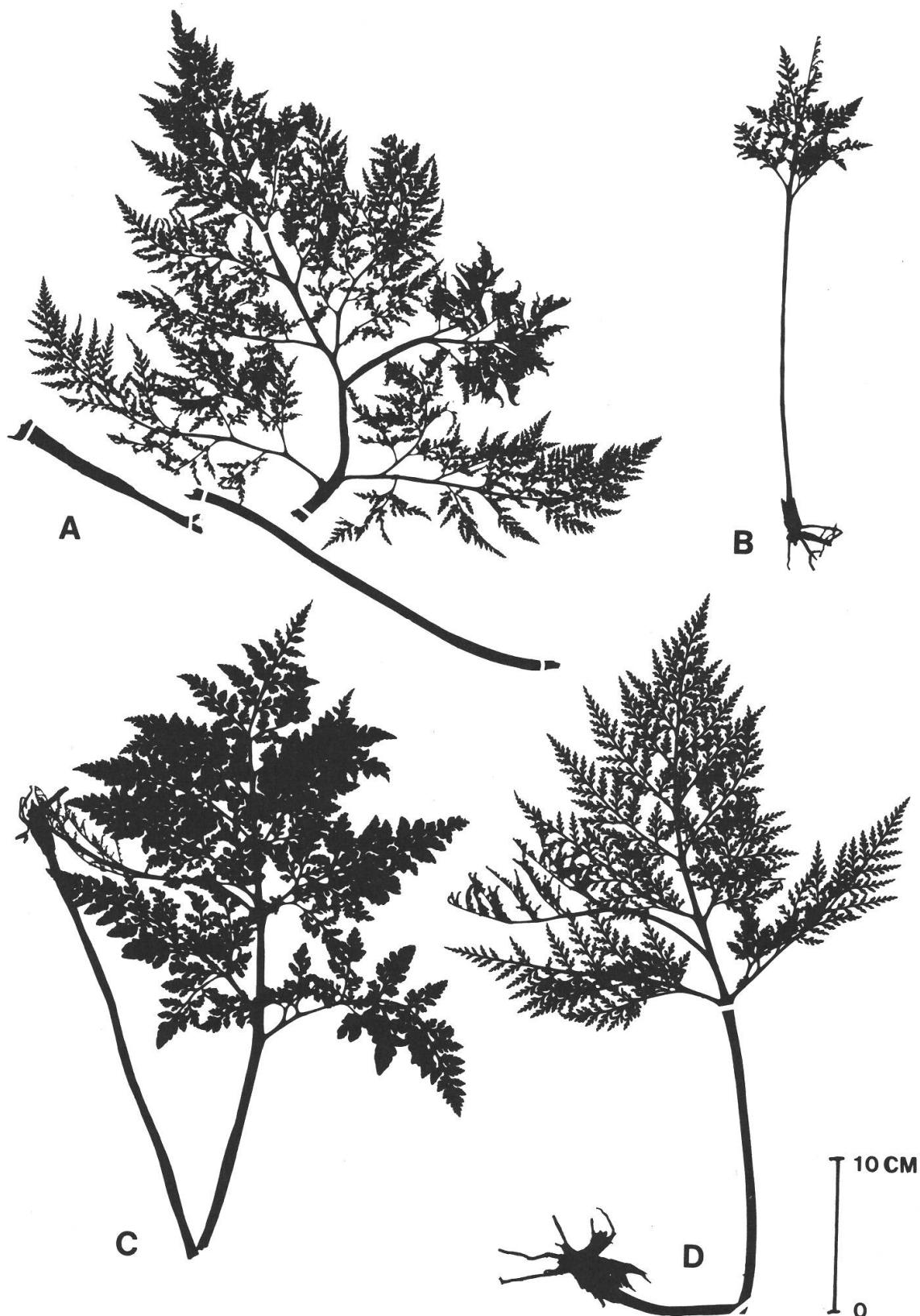
Fig. 6. Silhouettes of hexaploid hybrid *Botrychium lanuginosum*. A, 8335; B, 8361; C, 8347; D, J3900.

Guinea wild triploids of *P. nudum* occur as a result of hybridization between diploid and tetraploid races.

The same interspecific combination may arise independently at different times and in different places; e.g. *Lycopodium alpinum × tristachyum* has 11 separate colonies in Denmark (Øllgaard 1985) and from Japan Takamiya and Tanaka (1982) have reported finding 45 triploid plants of *Lycopodium clavatum* (formed by hybridisation between diploid and tetraploid cytotypes) from 18 different localities. These examples are sufficient to show that hybridisation in plants with subterranean gametophytes is not necessarily a unique event but may occur several times independently. However, in these cases the size of the hybrid population is no guide to the number of acts of hybridisation that have occurred since in the case of *Lycopodium* there are very effective means of vegetative reproduction by creeping, branched rhizomes, bulbils, etc. whilst in *Psilotum* copious gemmae are produced. Hence a single act of hybridisation may result in many plants being formed.

In the case of *Botrychium* by contrast the situation is very clear as there are no means of vegetative reproduction and therefore each hybrid plant represents a separate act of creation. In a dozen different interspecific combinations recorded for this genus by Wagner et al. nearly all are known either as rather rare specimens or as more or less individual plants from a fairly large number of localities. The one exception reported is *B. × watertonense* (= *B. hesperium × paradoxum*) where over 50 individuals were recorded from an area covering a third of a square mile in Canada.

In the case of *B. lanuginosum* reported here the evidence is that hybridisation has taken place on a massive scale in that about half of our population sample gathered from a small area was hybrid. Because of the great morphological variation shown by at least the octoploid cytotype which is also reflected in the similar range of variation in the hybrid, the further deduction may be made that these hybrids have been produced as a result of crossing between many different genotypes and that outcrossing is general in this species. Finally, these hybrids must be of very recent origin and not the accumulation over many years as they were produced in temporarily abandoned garden land which is subject to intensive cultivation on a cyclical basis. This example supports the view that under certain circumstances at least the subterranean character of the gametophyte and its physical environment are not barriers to outcrossing and hybridisation.

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