TABLE 1. Chromosome numbers in *Lycopodium* sens. lat. based on 11.

Genus[1]	Base number 11	Common denominator	Numbers reported[2]	Anomalous numbers reported in genus[2]
Huperzia	6 × 11	66	132	
		67	67	
			134	
		68	68	
			136	
			204 (3×)	
Phlegmariurus (*Huperzia*)	6 × 11	66	132	ca. 128 *H. reflexa*
		68	136	
			ca. 275 (4 × 68?)	
Lycopodium	3 × 11	34	34	22 *L. clavatum*
			31	90–92 *L. jussiaei*
			68	
			102 (3 × 34)	
Diphasiastrum	2 × 11	23	23	48 *L. wightianum*
Lycopodiella	7 × 11	78	78	
			156	
Pseudolycopodiella (*Lycopodiella*)	6 × 11	35	35	
		68	68	
		70	70	
Palhinhaea (*Lycopodiella*)	5 × 11	52	104	136 *L. cernua*
			156 (3 × 52)	
		54	108	
		55	110	
			ca. 165 (3 × 55)	

[1] For a discussion of the classification used here see Wagner & Beitel (1992).
[2] For references to these numbers see Table 2. Chromosome numbers in *Lycopodium* sens. lat.

& Beitel, 1992). Aneuploid changes account for the common denominators shown here, and polyploidy results in further changes shown in the actual numbers reported.

The anomalous numbers listed in the last column of Table 1 can be interpreted in several ways. *Lycopodium clavatum* with $n = 22$ from Bolivia is most likely a taxon different from the worldwide species of that name that has $n = 34$. *Diphasiastrum wightianum* with $n = 48$ was counted by Ninan (1958), who wrote, "The bivalents at diakinesis exhibit very peculiar shapes and are of different sizes, presenting difficulties in interpretation." One is tempted to think that *D. wightianum* is a tetraploid based on $n = 23$, the only number in the genus, in which case *D. wightianum* would be the only tetraploid in *Diphasiastrum*.

Ecuadoran *Lycopodium jussiaei* was found by Øllgaard (1987) to have 90–92 pairs (Table 1). This number is difficult to relate to other numbers in the genus except perhaps *L. magellanicum* with $n = 31$. The two species, however, are placed in different groups by Øllgaard (1987).

The 128 pairs of chromosomes in *Huperzia reflexa* (Table 1) is an approximate count made by Walker (1966), who suggested that it is of the same order of magnitude as a count of $n = 132$, which is a number reported in other huperzias.

The photograph of a figure substantiating the count of 136 pairs in *Lycopodiella cernua* (Kuriachen, 1965) is difficult to interpret. When dealing with *Lycopodium* chromosomes in numbers of this size, a drawing in addition to a photograph is often needed to assist interpretation. With regard to the hypothesis that the base number for *Lycopodium* sens. lat. is 11, it is not unreasonable to assume that many aneuploid and polyploid changes could have accumulated during the long history of the genus. Such changes would be based on 11—for to assume a number other than 11, e.g., 7 or 17, would require an even greater number of alterations. Earlier studies have attempted to require the existence of exact multiples of a hypothesized base number as a criterion, e.g., 34 in *Lycopodium* sens. str., 68 and 136 in *Huperzia*, which are all exact multiples of 17 (e.g., Takamiya

TABLE 2. Chromosome numbers in *Lycopodium* sens. lat.

Species	Locality	Chromosome number	Photo or drawing	Reference*
Huperzia				
chinensis (Christ) Ching	Japan	$n = 68$	Photo	Takamiya & Kurita (1983)
herterana (Kumm) Sen & Sen[1]	India	$n = 132$	Drawing	Mehra & Verma (1957)
herterana[1]	India	$2n =$ ca. 405 (180 II + 45 I)	Photo, Drawing	Ninan (1958)
lucidula (Michx.) Trevisan	Canada	$2n = 264$		Löve & Löve (1958)
lucidula	U.S.A.	$n = 67$	Photo, Drawing	Beitel & F. Wagner (1982)
miyoshiana (Makino) Ching	U.S.A.	$n = 134$		Soltis & Soltis (1988a)
occidentalis (Clute) Beitel & Mickel	U.S.A.	$n =$ ca. 134		Soltis & Soltis (1988a)
selago (L.) C. Martius & Schrank	Canada	$2n = 264$		Löve & Löve (1958)
selago	Finland	$2n =$ ca. 90		Sorsa (1962)
		$n =$ ca. 45		Sorsa (1963b)
selago	Great Britain	ca. 113 II, 37 I	Photo, Drawing	Manton (1950)
selago	Iceland	$2n = 264$		Löve & Löve (1958)
selago	U.S.A.	$2n = 264$		Löve & Löve (1966)
selago	U.S.A.	$n = 134$	Photo, Drawing	F. Wagner (this paper)
selago var. *acuminatum* Sugimoto	Japan	$n = 136$		Tak & Kur in Mitui (1980)
serrata (Thunb. ex Murray) Trevisan	India	$n = 264$	Photo, Drawing	Ghatak (1965)
serrata	Japan	$n = 68$	Photo	Takamiya & Kurita (1983)
		$n = 136$	Photo	
serrata	Japan	$2n = 204$	Photo	Takamiya (1984)
vernicosa (Grev. & Hook.) Trevisan	India	$n = 136$	Photo, Drawing	Ninan (1958)
Huperzia (*Phlegmariurus*)				
cryptomerina (Maxim.) Dixit	Japan	$n = 136$	Photo, Drawing	Takamiya & Kurita (1983)
dichotoma (Jacq.) Trevisan	Puerto Rico	$n =$ ca. 132		Sorsa in Fabbri (1965)
fordii (Baker) Dixit	Japan	136	Photo, Drawing	Takamiya & Kurita (1983)
hamiltonii (Spreng.) Trevisan	India	$n = 136$	Photo, Drawing	Ninan (1958)
linifolia (L.) Trevisan	Puerto Rico	$n =$ ca. 130–140		Sorsa in Fabbri (1965)
macrostachys (Spring) Holub[2]	India	$n = 136$	Photo, Drawing	Ninan (1958)
phlegmaria (L.) Rothm.	India	$n = 136$	Photo, Drawing	Ninan (1958)
phlegmaria	Japan	$n =$ ca. 275	Photo, Drawing	Takamiya & Kurita (1983)
phyllantha (Hook. & Arn.) Holub	India	$n = 170$	Photo, Drawing	Ghatak (1965)
phyllantha[2]	India	$n = 136$	Photo, Drawing	Ninan (1958)
pulcherrima (Hook. & Grev.) Pichi-Serm[3]	India	$n = 136$	Photo, Drawing	Ninan (1958)

TABLE 2. Continued.

Species	Locality	Chromosome number	Photo or drawing	Reference*
pulcherrima[3]	India	$2n = 330–340$		Mehra & Verma (1957)
reflexa (Lam.) Trevisan	Jamaica	$n =$ ca. 128		Walker (1966)
saururus (Lam.) Trevisan	Bolivia	$n = 132$	Drawing	Rolleri (1982b)
sieboldii (Miq.) Holub	Japan	$n = 136$	Photo, Drawing	Takamiya & Kurita (1983)
squarrosa (G. Forster) Trevisan	India	$n = 136$	Photo	Ninan (1958)
		$n = 138$	Drawing	
Lycopodium				
annotinum L.	Canada	$2n = 68$		Löve & Löve (1958)
annotinum	Finland	$n = 34$	Drawing	Sorsa (1958)
		$2n = 68$		Sorsa (1963b)
annotinum	Japan	$n = 34$	Photo	Takamiya & Kurita (1983)
annotinum	Sweden	$2n =$ ca. 58		Ehrenberg (1945)
annotinum	Sweden	$n = 34$	Photo, Drawing	Manton (1950)
annotinum	U.S.A.	$2n =$ ca. 50		Dunlop (1949)
annotinum var. *acrifolium* Fern.	Japan	$n = 34$	Photo	Takamiya & Kurita (1983)
annotinum subsp. *alpestre* Löve & Löve	Iceland	$2n = 68$		Löve & Löve (1958)
casuarinoides Spring	Japan	$2n = 68$	Photo	Takamiya & Tanaka (1983)
clavatum L.	Bolivia	$n = 22$		Rolleri (1982a)
clavatum	Canada	$2n = 68$		Löve & Löve (1958); Löve (1976)
clavatum	Ecuador	$n = 34$		Øllgaard (1987)
clavatum	Finland	$n = 34$	Drawing	Sorsa (1958)
		$2n = 68$		Sorsa (1963b)
clavatum	India	$n = 34$	Drawing	Mehra & Verma (1957)
clavatum sens. lat.	India	$n = 68$	Drawing	Ghatak (1965)
clavatum	Great Britain	$n = 34$	Photo, Drawing	Manton (1950)
clavatum	Jamaica	$n = 34$	Photo	Walker (1966)
clavatum	Japan	$2n = 68$	Photo	Tanaka & Takamiya (1981)
		$2n = 102$	Photo	Takamiya & Tanaka (1982)
		$2n = 136$	Photo	Takamiya (1989)
clavatum	Sweden	$2n =$ ca. 66		Ehrenberg (1945)
clavatum	Taiwan	$n = 34$	Photo	Tsai & Shieh (1983)
clavatum	U.S.S.R.	$n = 14$	Drawing	Baranov (1925)
clavatum × *vestitum*	Ecuador	$n = 34$		Øllgaard (1987)
clavatum var.?.	U.S.A.	$2n =$ ca. 60		Dunlop (1949)
clavatum subsp. *megastachyon* (Fern. & Biss.) Löve & Löve	Canada	$2n = 68$		Löve & Löve (1958)
clavatum var. *nipponicum* Nakai	Japan	$n = 34$	Photo	Takamiya & Kurita (1983)
contiguum Klotzsch	Ecuador	$n = 34$		Øllgaard (1987)
dendroideum Michx.	Canada	$2n = 68$		Löve (1976)

TABLE 2. Continued.

Species	Locality	Chromosome number	Photo or drawing	Reference*
jussiaei Desv. in Poiret	Ecuador	$n = 90–92$	Photo, Drawing	Øllgaard (1987)
jussiaei		$n = 34–36$		Wilce (1972)
lagopus (Læst. ex Hartm.) I. Zinzerl. ex Kuzen.-Proch[4]		$2n = 68$		Löve & Löve (1958)
magellanicum (Beauv.) Sw.	Ecuador	$n = 31$		Øllgaard (1987)
magellanicum	Argentina	$n = 31$	Photo	Øllgaard (1987)
obscurum L.	Canada	$2n = 68$		Löve & Löve (1958)
obscurum	Japan	$n = 34$	Photo	Takamiya & Kurita (1983)
obscurum	U.S.A.	$n = 34$	Photo, Drawing	Wagner & Wagner (1966)
obscurum	U.S.A.	$2n =$ ca. 50		Dunlop (1949)
vestitum Poiret	Ecuador	$n = 34$		Øllgaard (1987)
Diphasiastrum				
alpinum (L.) Holub	Canada	$2n = 48$		Löve & Löve (1958)
alpinum	Finland	$n = 22–24$	Drawing	Sorsa (1963a, b)
		$2n = 44$		
alpinum	Great Britain	$n = 24–25$	Photo, Drawing	Manton (1950)
alpinum	Scandinavia & Canada	$2n = 46$		Löve & Löve (1961)
complanatum (L.) Holub	Canada	$2n = 46$		Hersey & Britton (1981)
complanatum	Canada & Scandinavia	$2n =$ ca 48 $2n = 46$		Löve & Löve (1958, 1961)
complanatum	Finland	$n = 22–24$	Drawing	Sorsa (1963a)
complanatum	Finland	$n =$ ca. 24		Kukkonen (1967)
complanatum	Japan	$n = 23$		Tak & Kur in Mitui (1980)
complanatum	Labrador	$n = 23$	Drawing	Wilce (1965)
complanatum × *tristachyum*?	Canada	$2n = 46$	Photo	Hersey & Britton (1981)
complanatum var. *elongatum*	U.S.A.	$n = 40$	Drawing	Dunlop (1949)
digitatum (A. Braun) Holub	Canada	$2n = 46$		Hersey & Britton (1981)
digitatum[5]	Canada	$2n =$ ca. 48		Löve & Löve (1958)
digitatum	Canada	$2n = 46$		Löve (1976)
digitatum	U.S.A.	$2n = 46$	Drawing	Wilce (1965)
fawcettii (Lloyd & Underwood) Holub	Jamaica	$n = 23$	Photo	Walker (1966)
×*habereri* (House) Holub	Canada	$2n = 46$	Photo	Hersey & Britton (1981)
×*habereri*	U.S.A.	$n = 23$		F. Wagner (1980)
×*issleri* (Rouy) Holub	Germany	$2n = 46$	Drawing	Damboldt (1962)
×*sabinifolium* (Willd.) Holub	Canada	$2n = 46$		Löve (1976)
×*sabinifolium*	Canada	$n = 23$		F. Wagner (1980)
sitchense (Rupr.) Holub	Canada	$2n = 46$		Löve (1976)
sitchense	Labrador	$n = 23$		Wilce (1965)
sitchense	U.S.A.	$2n = 46$		Löve & Löve (1966)

TABLE 2. Continued.

Species	Locality	Chromosome number	Photo or drawing	Reference*
sitchense subsp. *nikoënse* L. & L.	Japan	$2n = 46$		Löve (1976)
sitchense var. *nikoense* Takeda	Japan	$n = 23$	Photo	Takamiya & Kurita (1983)
thyoides (Willd.) Holub	Ecuador	$n = 23$		Øllgaard (1987)
tristachyum (Pursh) Holub	Canada	$2n = 46$	Photo	Hersey & Britton (1981)
tristachyum	Canada	$2n =$ ca. 48		Löve & Löve (1958)
tristachyum	Canada	$2n = 46$		Löve (1976)
tristachyum	U.S.A.	$n = 23$	Drawing	Wilce (1965)
veitchii (Christ) Holub	Taiwan	$n = 68$	Photo	Tsai & Shieh (1983)
wightianum (Grev. & Hook.) Holub	India	$n = 48$	Photo, Drawing	Ninan (1958)
× *zeilleri* (Rouy) Holub	Germany	$2n = 46$	Drawing	Damboldt (1962)
× *zeilleri*	U.S.A.	$n = 23$		F. Wagner (1980)
Lycopodiella				
alopecuroides (L.) Cran.	U.S.A.	$n = 78$	Photo, Drawing	Bruce (1975)
alopecuroides × *appressa*	U.S.A.	$n = 78$	Photo, Drawing	Bruce (1975)
alopecuroides × *prostrata*	U.S.A.	$n = 78$	Photo, Drawing	Bruce (1975)
appressa (Chapman) Cranfill	U.S.A.	$n = 78$	Photo, Drawing	Bruce (1975)
appressa × *prostrata*	U.S.A.	$n = 78$	Photo, Drawing	Bruce (1975)
inundata (L.) Holub	Canada	$2n = 156$		Löve & Löve (1958)
inundata	Canada	$2n = 156$		Löve (1976)
inundata	Finland	$n = 78$	Drawing	Sorsa (1961)
inundata	Great Britain	$n = 78$	Photo, Drawing	Manton (1950)
inundata	U.S.A.	$n = 78$	Photo, Drawing	Bruce (1975)
margueritae Bruce, Wagner & Beitel[7]	U.S.A.	$n = 156$	Photo, Drawing	Bruce (1975)
prostrata (Harper) Cranf.	U.S.A.	$n = 78$	Photo, Drawing	Bruce (1975)
subappressa Bruce, Wagner & Beitel[6]	U.S.A.	$n = 156$	Photo, Drawing	Bruce (1975)
Pseudolycopodiella (*Lycopodiella*)				
caroliniana (L.) Holub	Japan	$n = 68$	Photo	Takamiya & Kurita (1983)
caroliniana	Japan	$n = 68$	Photo	Takamiya & Kurita (1983)
caroliniana	U.S.A.	$n = 35$ $n = 70$ $2n = 115$[8]	Photo, Drawing	Bruce (1976)
meridionalis (L. Underw. & F. Lloyd) Holub	Jamaica	$n =$ ca. 69		Walker (1966)
Palhinhaea (*Lycopodiella*)				
cernua (L.) Carv. Vasc. & Franco	Japan	$n = 108$	Photo	Takamiya & Kurita (1983)
cernua	India	$n = 104$	Photo, Drawing	Ninan (1958)

TABLE 2. Continued.

Species	Locality	Chromosome number	Photo or drawing	Reference*
cernua	India	$n = 104$ $n = 156$ $n = 208$	Photos Drawings	Ghatak (1965)
cernua	India	$n = 104$ $n = 110$ $n = 136$ $n =$ ca. 160II, 20I	Photo	Kuriachen (1965)
cernua	Jamaica Trinidad	$n =$ ca. 165 $n =$ ca. 165	Photo, Drawing	Walker (1966)
cernua	Taiwan	$n = 102$	Photo	Tsai & Shieh (1983)

* For references, see Literature Cited. The following references were not seen and therefore not included in this table: Hadac, E. & V. Haskova. 1956. Taxonomické poznámky o tatranskych rostlinách ve vztahu k jejich Bratislava/ cytologii. Biológia Brat. 11: 717–723. Löve A. & D. Löve. 1948. Chromosome numbers of northern plant species. Icel. Univ. Inst. Appl. Sci., Dept. Agric. Rep. B. 3: 1–131.

[1] As *Lycopodium lucidulum.*

[2] *macrostachys* and *phyllantha* are treated as synonyms by Ninan.

[3] As *Lycopodium setaceum.*

[4] As *clavatum* subsp. *monostachyum* (Grev. & Hook.) Selander.

[5] As *complanatum* var. *flabelliforme.*

[6] As "northern appressa" See Bruce et al. (1991).

[7] As "appressed inundata" See Bruce et al. (1991).

[8] Somatic count of a presumed triploid hybrid—possibly 105?

& Kurita, 1983). Such suggestions do not take aneuploidy into consideration.

ALLOHOMOPLOID NOTHOSPECIATION IN *LYCOPODIUM* SENS. LAT.

Even though the potential for self-fertilization exists, in *Lycopodium* sens. lat. as in all the homosporous pteridophytes with gametes of both sexes produced in the same gametophyte, evidence for high frequencies of intergametophytic matings has been found. Soltis & Soltis (1988b) studied a total of 22 widely scattered populations of *L. clavatum*, *L. annotinum*, and *Huperzia miyoshiana*, and, using electrophoretic analyses of polymorphic loci, calculated low estimates of intragametophytic self-fertilization. They concluded, therefore, that these species predominantly cross-fertilize. Because *Lycopodium* sens. lat., with the exception of *Lycopodiella* sens. lat., has entirely underground gametophytes, it had been presumed in the past that sperms would have difficulty swimming underground through the soil, with the result that selfing would be the rule and hybridization would be difficult. On the contrary, intergametophytic mating and interspecific hybridization have turned out to be common in the Lycopodiaceae (Wagner et al., 1985).

Typically in plants, nothospeciation (hybridization) involves two steps: (1) AA × BB → AB, which is formation of a sterile diploid, and (2) AB → [AABB], which is formation of a fertile allopolyploid through endomitosis or chromosome doubling. In *Lycopodium* sens. lat., another pattern is found in which a fertile sexual nothospecies is formed without doubling, and parents and hybrid are homoploid, i.e., of the same ploidal level, AA × BB → [AB], which is formation of a fertile homoploid. (The use of brackets here and below to indicate a reproductively competent hybrid, is proposed by Werth & Wagner (1990).)

In Figure 3 three species of *Diphasiastrum*—*digitatum*, *complanatum*, and *sitchense*—are shown with *D. tristachyum*, a species that hybridizes with all three. The hybrids resulting from these crosses, *D.* [×] *habereri*, [×] *zeilleri*, and [×] *sabinifolium* (all of which have been found in the wild), are fertile to the extent that their genomes show complete pairing of chromosomes, and their spores are apparently normal (Figs. 4, 5). The number of chromosome pairs in the hybrids ($n = 23$) is the same as that for all the parents involved (F. Wagner, 1980; Hersey & Britton, 1981).

Unfortunately, germination of *Lycopodium* spores can only be carried out with difficulty (see Whittier, 1977, 1981; Whittier & Webster, 1986). Tests of the germinability of these morphologically normal spores have yet to be made. Some indication of their fertility, however, is attested to by the fact that we find isolated populations of *D.* ×*habereri*,

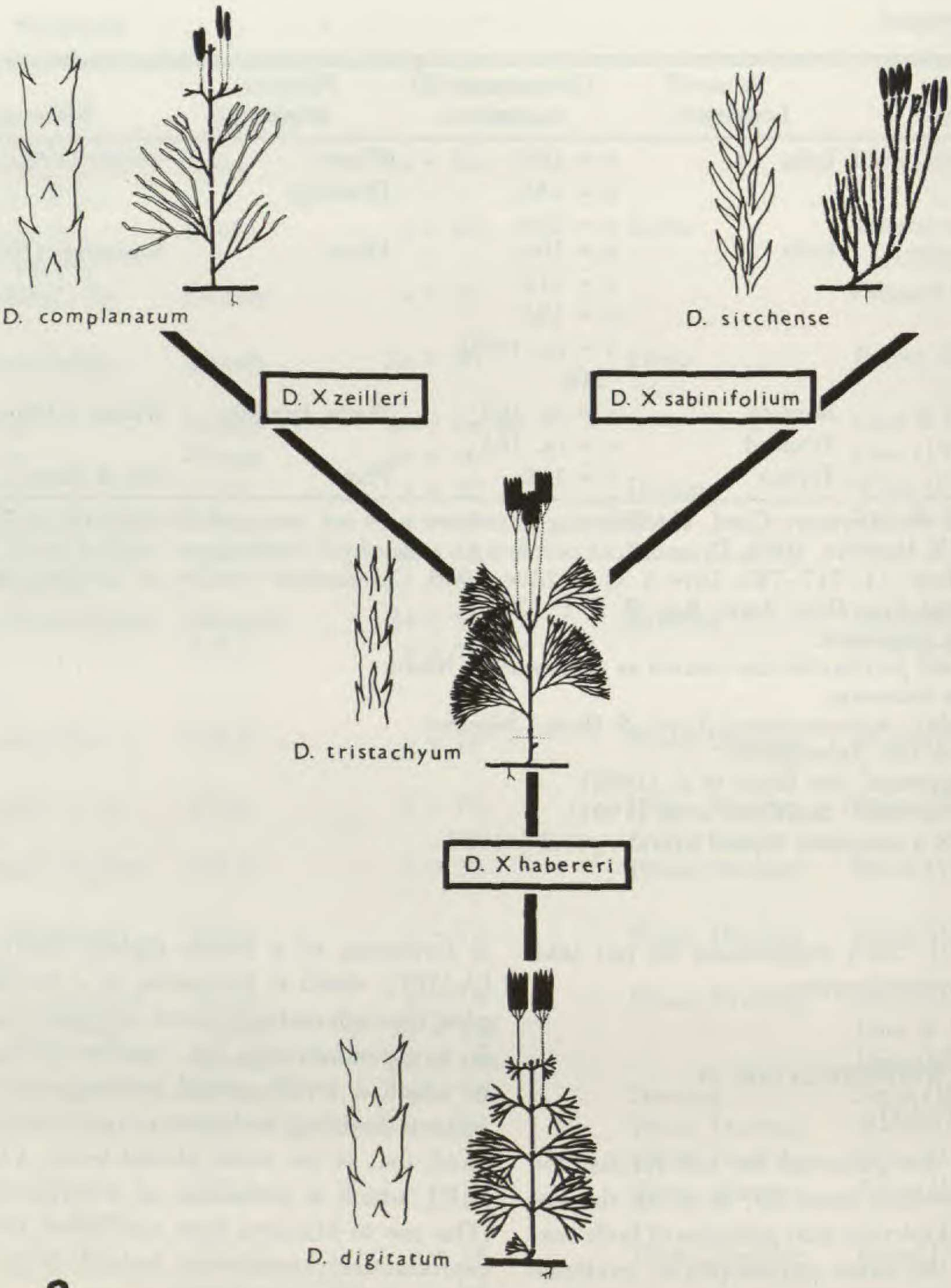


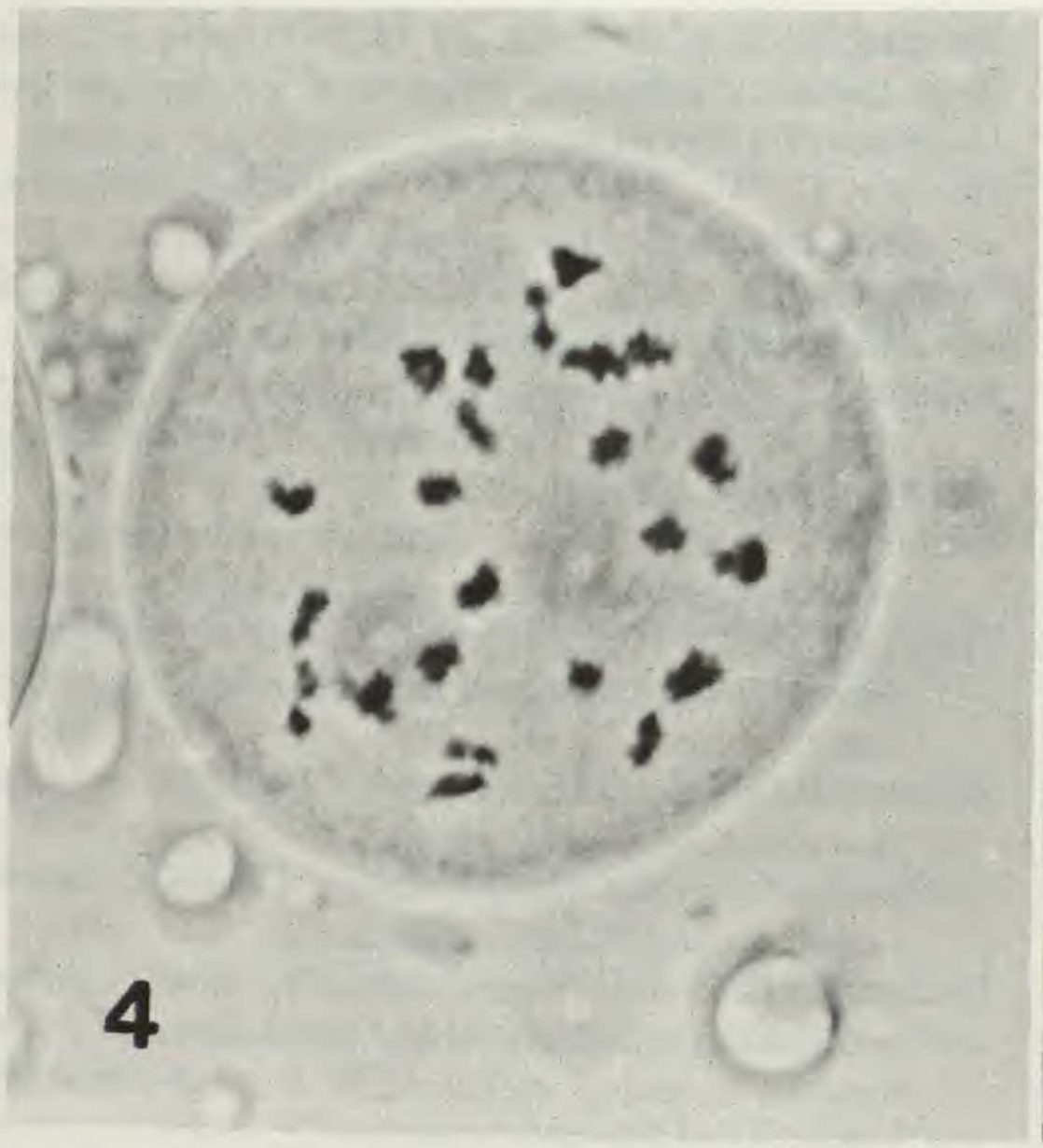
FIGURE 3. *Diphasiastrum*. Diagram showing hybridization of *D. tristachyum* with *D. complanatum* to form *D.* ×*zeilleri*; with *D. sitchense* to form *D.* ×*sabinifolium*; and with *D. digitatum* to form *D.* ×*habereri*. All taxa have $n = 23$ pairs of chromosomes. Branchlet drawings show relative sizes of leaves. Habit drawings are from Wilce (1965).

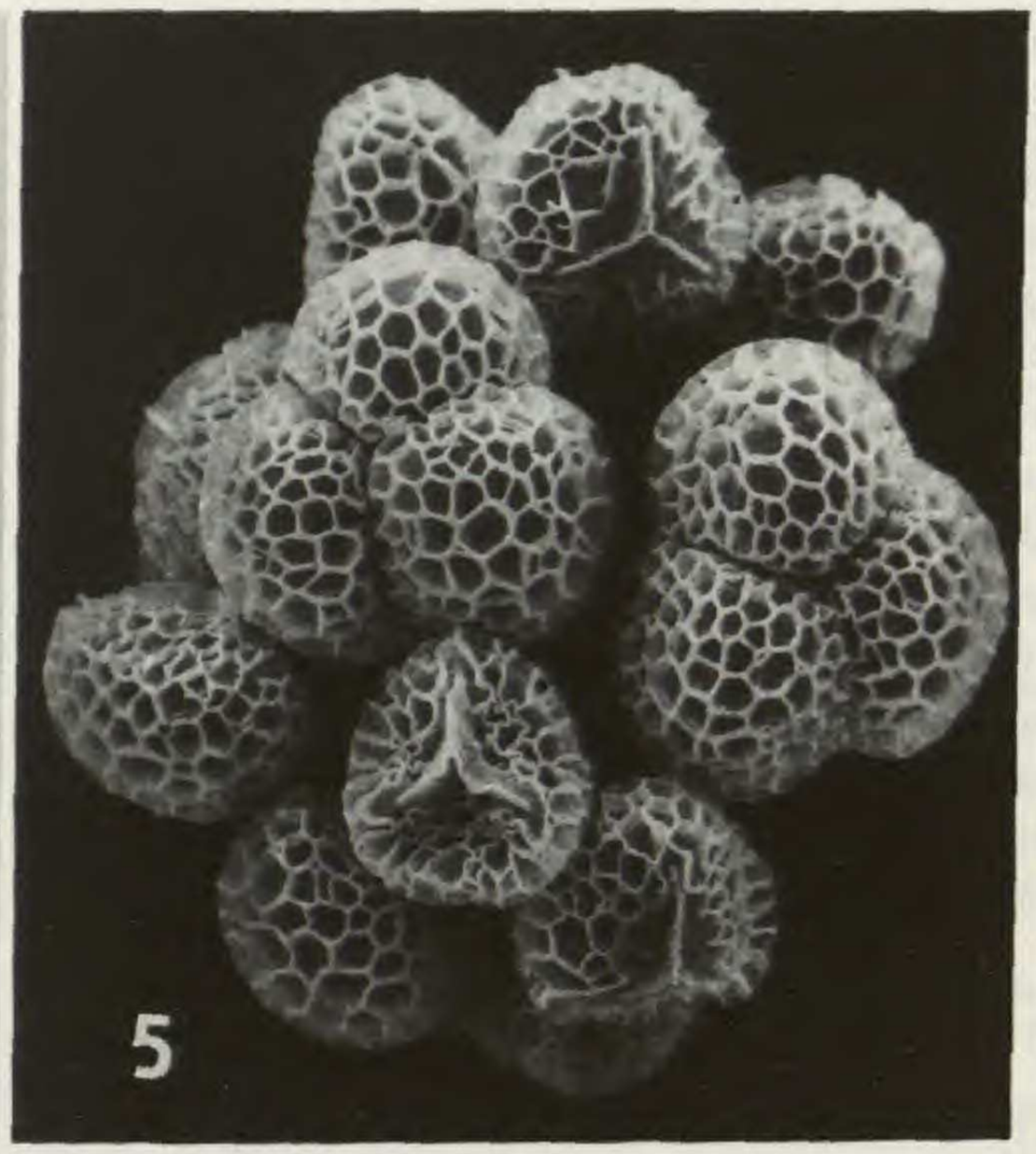
for example, presumably initiated by a single fertile spore with no parental species or only one parent in the area (Wagner & Wagner, unpublished).

Homoploid nothospecies in *Lycopodium* sens. lat. have not been confined to *Diphasiastrum*, although most reported examples are in that genus. Bruce (1975) found hybrids in *Lycopodiella* between *L. alopecuroides* and *L. appressa*, and between *L. alopecuroides* and *L. prostrata*, with pairing of genomes, the same chromosome number as the parents, and morphologically normal spores. Øllgaard (1987) has reported a homoploid nothospecies, *L. clavatum* × *vestitum*, in the genus *Lycopodium* sens. str.

Fertile homoploid nothospeciation in pteridophytes was first reported in a classic study by Trevor Walker (1958) in the fern genus *Pteris*. Two species, *P. multiaurita* and *P. quadriaurita* in Ceylon, formed a hybrid swarm of intermediates occupying an ecotone between the parents. The hybrids were fertile but without chromosome doubling; all had the same chromosome number as the parental species.

Homoploid hybrids of *Ceratopteris* have also





FIGURES 4, 5. 4. Photomicrograph of chromosomes of *Diphasiastrum* ×*sabinifolium* at diakinesis with 23 pairs of chromosomes.—5. Scanning electron photomicrograph of spores of *D.* ×*habereri*.

been produced in culture by Hickok & Klekowski (1974), and homoploid hybrids in the Cyatheaceae, first reported by Conant & Cooper-Driver (1980), are found in Puerto Rico. The cyatheoid hybrids backcross and form hybrid swarms, but recombinant second generation hybrids may become stabilized and maintain their genetic integrity by means of autogamy, i.e., intragametophytic selfing.

In the North American *Lycopodium* sens. lat., the morphological variation seen in the *Diphasiastrum* hybrids seems clearly to be environmentally produced, i.e., sun and shade forms (Beitel, 1979a, b; Beitel et al., 1982). However, although we have searched for years, we have not found backcrosses in these hybrids. This seems surprising since *Diphasiastrum* species have been found to be primarily outcrossers (see above and Soltis & Soltis, 1988b). Hybridization produces the original hybrid and if such hybrids retain this capacity, then continued outcrossing should ultimately lead to backcrossing, introgression, and hybrid swarms. Apparently this is not happening in *Diphasiastrum*, and it may be that rarity is a factor; there may not be enough individuals of associated parental species to cross with. Related perhaps, is the fact that species of *Lycopodium* sens. lat. are great clone formers and rely heavily on vegetative reproduction. It may be that there is in reality very little sexual reproduction.

Unlike *Diphasiastrum* and *Lycopodiella*, hybridization in *Huperzia* follows a course more familiar in the ferns resulting in either sterile allodiploids or fertile allopolyploids (Beitel, 1986, 1988). No allohomoploid hybrids have been reported in the genus.

DISCUSSION

A number of generalizations can now be made regarding the cytology of Lycopodiaceae. The basic chromosome numbers are high, the lowest being $x = 23$. In this respect the clubmosses are like other homosporous pteridophytes and unlike the heterosporous Selaginellaceae and Isoetaceae, which have x numbers like seed plants. Also, like other homosporous pteridophytes, Lycopodiaceae bear both sex organs on the same gametophyte and potentially can undergo intragametophytic mating. The Lycopodiaceae differ from homosporous ferns in the apparent absence of apogamy and in a greater tendency for allohomoploidy, as illustrated primarily by *Diphasiastrum*.

To explain the curious "step-wise" increases now known in *Lycopodium* chromosome base numbers, i.e., 23, 31–34, 52–55, 66–70, and 78, I can offer only a hypothesis that we are dealing here with a polyploid series, involving some aneuploid changes as a minor element, i.e., 2 × 11, 3 × 11, 5 × 11, 6 × 11, and 7 × 11. The graded nature of the base numbers tends to negate the possibility that the original clubmosses had high chromosome numbers. Also, the fact that the het-

erosporous lycopsids have low numbers as do the seed plants supports the idea that paleopolyploidy accounts for the genome sizes known today in the Lycopodiaceae. Neopolyploidy probably occurs in all genera of Lycopodiaceae, but seems to be rare in certain groups, notably *Diphasiastrum* and *Lycopodium* sens. str., in comparison to *Huperzia*, where neopolyploidy is common.

The chromosomes of these plants are, for various reasons, often difficult to study, especially those of the polyploid fir mosses, *Huperzia*. The great diversity of numbers already known in the Lycopodiaceae indicates that further work will be informative, but care must be taken to find precise and thoroughly documented numbers.

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THE YOUNG GAMETOPHYTE OF *PHYLLOGLOSSUM* (LYCOPODIACEAE)[1]

Dean P. Whittier[2] *and*
John E. Braggins[3]

ABSTRACT

Any similarities between *Phylloglossum* and specific subgenera of *Lycopodium* sens. lat. have been difficult to determine, because the reduced sporophyte of *Phylloglossum* has few characters for comparison. Gametophytes can provide useful characters, but it is difficult to find *Phylloglossum* gametophytes in nature. These gametophytes have been grown in axenic culture for study. Spores of *Phylloglossum* germinate in the dark on a nutrient medium containing minerals and glucose. No germination occurs in the light. In early development, a globular gametophyte forms that later becomes cylindrical. The cylindrical gametophytes are negatively gravitropic and grow vertically away from the surface of the nutrient medium. As long as the gametophytes are cultured in the dark they remain cylindrical and nonphotosynthetic. Moving the gametophytes into light initiates chlorophyll development and brings about a new growth habit that is oriented more or less horizontally rather than vertically. Growth in the light is elongated but deltoid in cross section. Sexually mature, photosynthetic gametophytes have not yet been grown. Information from axenic culture helps to explain the habit of these gametophytes described from nature. It would appear that the spores have to be covered with soil before they germinate. The young gametophyte, which is mycorrhizal, becomes cylindrical and grows to the surface of the soil. Once exposed to light the mature habit develops. Germination in the dark, mycorrhizal young gametophytes, and other characters suggest that *Phylloglossum* is not as similar to the subgenus of *Lycopodium* (*Lepidotis*) having photosynthetic gametophytes as once thought.

The extant Lycopodiaceae are often regarded as being composed of two genera, *Phylloglossum* (one species) and *Lycopodium* sens. lat. (more than 200 species). *Lycopodium* is complex, comprising discrete groups of species recognized as subgenera (Wilce, 1972), genera (Øllgaard, 1987) or even higher categories (see Wagner & Beitel, 1992).

Phylloglossum is a small, homosporous member of the Lycopodiaceae of shrubland areas from New Zealand and Australia. Its shortened, erect, underground stem bears a pseudowhorl of up to 10 quill-like microphylls at the soil surface. Even reproductively mature plants rarely exceed 5 cm in height. A few roots are produced from the side of the stem. The plant bears reniform sporangia on the adaxial surface of sporophylls in a small, stalked strobilus.

Phylloglossum is a tuberous perennial. Each growing season (winter) the stem branches and sends a new tuber down in the soil. The tuber apex, which is the shoot apex for the next year's plant, is not external or terminal but rather is internal (marsupial) and oriented toward the soil surface and away from the tip of the downwardly growing tuber. The tuber is fleshy and contains storage materials. At the beginning of the next growing season, the tuber apex grows upward, producing the new stem with leaves, roots, and cone.

The relationship of *Phylloglossum* to *Lycopodium* has been discussed by a number of workers (Bower, 1886; Thomas, 1901; Holloway, 1935; Hackney, 1950; Breckon & Falk, 1974). However, the extremely reduced structure of the *Phylloglossum* sporophyte makes comparisons with *Lycopodium* difficult.

For the purposes of this paper we follow Wilce (1972) in recognizing the following subgenera of *Lycopodium*:

> *Urostachys*, with stems isodichotomous; sporophylls usually little differentiated from leaves, persistent, and not subpeltate; walls of sporangial epidermal cells sinuate and lignified; spores foveolate-fossulate; mature gametophytes mycorrhizal, cylindrical, branched or unbranched with radial or bilateral symmetry.
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> *Lepidotis*, with stems anisodichotomous, main stems with indefinite growth; lateral, usually determinate, branchlet systems; sporophylls modified, ephemeral, and subpeltate; walls of spo-

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[2] Department of Biology, Vanderbilt University, Nashville, Tennessee 37235, U.S.A.
[3] Department of Botany, University of Auckland, Private Bag, Auckland, New Zealand.

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rangial epidermal cells straight and nonlignified; spores rugulate; mature gametophytes photosynthetic, tuberous with dorsal lobes.

Lycopodium, as for *Lepidotis* but walls of the sporangial epidermal cells sinuate and lignified throughout; spores baculate or saccate; mature gametophytes mycorrhizal, carrot- or disc-shaped.

The morphology of *Lycopodium* gametophytes is variable and has been considered of taxonomic value (Bruchmann, 1898; Rothmaler, 1944; Boivin, 1950; Bruce, 1976b). Comparisons between the gametophytes of *Lycopodium* and *Phylloglossum* could be of value in determining relationships between the two genera. Although little is known about *Phylloglossum* gametophytes, they do not appear to have a reduced morphology. Of the three studies carried out in the 1900s (Thomas, 1901; Sampson, 1916b; Holloway, 1935), only Thomas based his report on observations of more than one gametophyte. The gametophytes are described as being photosynthetic, but Thomas (1901) noted that their basal portions can be white.

No twentieth-century worker has been successful in germinating the spores of *Phylloglossum*. However, Crié (1883) reported that the spores germinated to form gametophytes similar to those of the Ophioglossaceae. Crié's very brief report has generally been ignored in the recent literature on *Phylloglossum* and will be considered later in this report.

The present study employed axenic culture techniques to germinate the spores and grow the gametophytes of *Phylloglossum* in order to gain more information on their structure and physiology that might be useful in determining affinities *Phylloglossum* may have with subgenera of *Lycopodium*. These techniques are useful in germinating the spores and growing gametophytes of various pteridophytes that have proven difficult to find in nature or grow in culture (Whittier, 1972, 1981).

Materials and Methods

Strobili of *Phylloglossum drummondii* Kunze collected at Lake Ohia and Ahipara, New Zealand, provided spores for this study. Vouchers of sporophytes are deposited at the Vanderbilt University Herbarium (VDB). Strobili were removed from the plants and dried to release the spores that fit the description for *Phylloglossum* spores (Knox, 1950; Harris, 1955; Breckon & Falk, 1974). They were trilete and had an average diameter of 40.5 μm (measured fresh in water). The proximal face of the spore was essentially smooth (Fig. 1); however, the hemispherical distal face of the spore had dense regular foveolate ornamentation.

Often spores obtained from pteridophytes that bear their sporangia close to the soil give high rates of bacterial and fungal contamination when inoculated in axenic culture. To reduce the possibility of contamination, the spores were wetted with a 2% Tween 80 solution, rinsed in several changes of water, and then soaked in the final water rinse overnight. The following day the spores were surface sterilized with 20% Clorox for 2 minutes. The sterilized spores were collected by filtration and washed on the filter paper with several changes of sterile distilled water. They were finally suspended in sterile distilled water and pipetted into culture tubes.

The spores were inoculated onto 15 ml of nutrient medium in 20 × 125 mm culture tubes with screw caps, which were tightened after inoculation. A liter of nutrient medium contained 100 mg $Mg_2SO_4 \cdot 7H_2O$, 100 mg NH_4NO_3, 40 mg $CaCl_2$, and 100 mg K_2HPO_4. The medium was completed with 0.25 ml of a minor element solution (Whittier & Steeves, 1960), 8.5 ml of a FeEDTA solution (Sheat et al., 1959), and 0.1% glucose. It was adjusted to pH 5.0 and solidified with 0.8% agar. The cultures were maintained at 24 ± 1°C in light for 12 of every 24 hours or in darkness. The irradiance level was 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ from Grolux fluorescent lamps.

Results

Twenty percent of the spores germinated after three months in dark culture. Since 3- and 4-celled gametophytes were found at this time, the earliest germination was initiated prior to three months. No germination occurred in illuminated cultures after one year. Because spores cultured in the light for one year germinate if moved into the dark for three months, it may be assumed that light inhibited germination.

Germination occurs as the cell expands and ruptures the triradiate ridge of the spore coat (Fig. 2). Shortly after the cell bulges out of the spore coat, the first cell division takes place (Fig. 3). This division, which produces the 2-celled gametophyte, is oblique to the polar axis of the spore. However, it forms more or less proximal (toward the triradiate ridge) and distal (away from the triradiate ridge) cells. A portion of the distal cell remains inside the spore coat while the proximal cell becomes free of it.

The second cell division occurs in the proximal cell to produce a 3-celled gametophyte (large ar-