CYTOLOGICAL PROBLEMS IN *LYCOPODIUM* SENS. LAT.[1]

Florence S. Wagner[2]

ABSTRACT

Homosporous pteridophytes differ from seed plants most strikingly in their potential ability to produce completely homozygous offspring from a single haploid spore. The Lycopodiaceae share this characteristic with the ferns, but differ from them in the absence of apogamy and in the relatively high frequency of allohomoploid nothospeciation in certain genera. Determining chromosome numbers in this family is often difficult, and discrepancies are found in published accounts. Evidence in support of a base number of 11 is presented. Allohomoploid nothospeciation is described involving four species of *Diphasiastrum*. A table giving the published chromosome numbers in *Lycopodium* sens. lat. is included.

The lycopsids are spore-producing vascular plants that, along with *Psilotum* and *Equisetum*, are often referred to as "fern-allies." This is a designation badly in need of replacement. No apt substitute has been suggested. The category "homosporous pteridophytes" is inapplicable since that includes the ferns and excludes the heterosporous groups, *Selaginella*, *Isoetes*, and the heterosporous "water ferns."

However, for the purposes of this paper, which deals with cytology, homosporous pteridophytes is a useful classification supported by traits that contrast with the angiosperms, such as the relatively large size and high numbers of pteridophyte chromosomes, and the absence or paucity of multivalent formation in polyploid meioses. A guide to the nomenclature of the Lycopodiaceae is given in Øllgaard (1987, 1989).

The most important distinction between homosporous pteridophytes and seed plants (as well as heterosporous pteridophytes) has to do with fertilization. The gametes involved in "selfing" in seed plants arise from two different recombinant products of meiosis, and, therefore, selfing does not often produce homozygous offspring. All homosporous pteridophytes, on the other hand, have the capacity to produce completely homozygous offspring since a gametophyte and its gametes are produced from a single haploid spore. This is referred to as *intra*gametophytic selfing (Klekowski, 1970) as opposed to *inter*gametophytic mating, which results from fusion of gametes from the gametophytes of two spores.

The Lycopodiaceae as homosporous pteridophytes share these characteristics, but they differ from ferns in two notable respects: (1) apogamy, a specialized nonsexual life cycle that accounts for as much as 10% of fern species (Walker, 1979, 1985) but has never been reported in *Lycopodium* sens. lat.; and (2) allohomoploid nothospeciation, which is the production of fertile sporophytes with no change of ploidal level. Though rarely found in most pteridophytes, it is relatively common in Lycopodiaceae (Bruce, 1975; Hersey & Britton, 1981; F. Wagner, 1980). In contrast, allopolyploidy as a pathway to establishing nothospecies is well known in ferns (Wagner & Wagner, 1980), and in at least two genera of the Lycopodiaceae (Bruce, 1975; Wagner et al., 1985), but is rare in *Diphasiastrum* and *Lycopodium* sens. str.

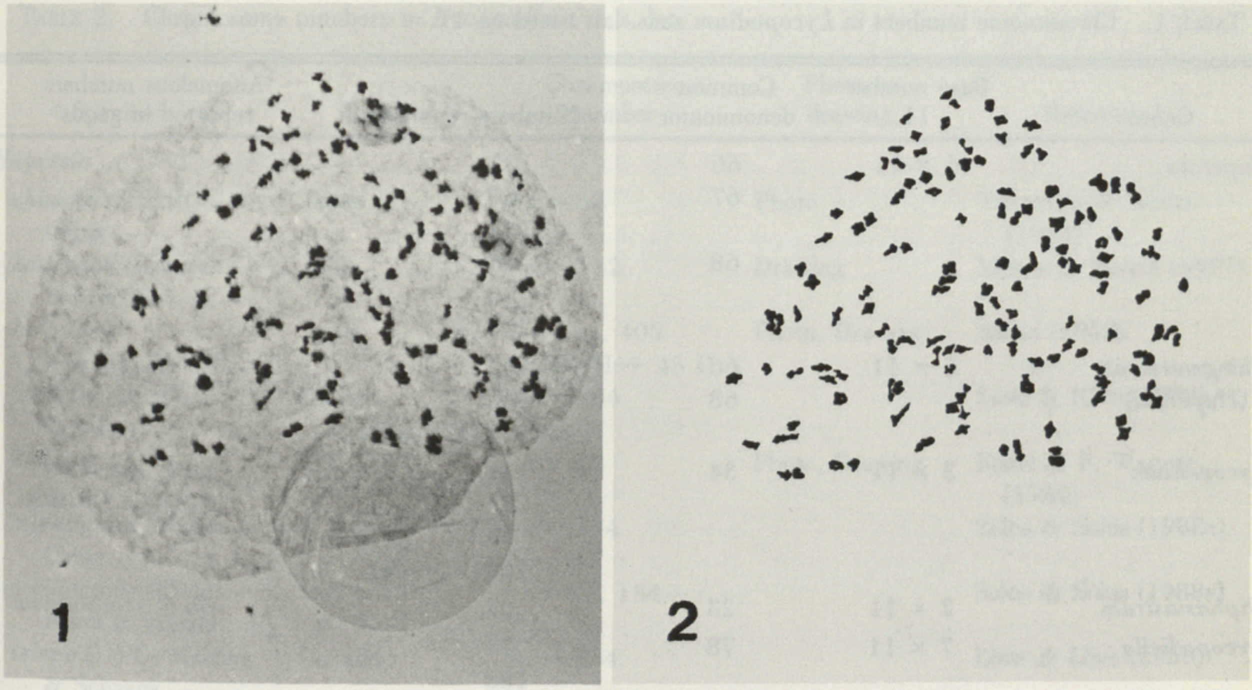
THE CHROMOSOMES OF *LYCOPODIUM* SENS. LAT.

Determining the chromosome numbers of species in the traditional genus *Lycopodium* sens. lat. has not been popular with cytologists, and justifiably so because of the difficulties frequently encountered. *Lycopodium* spore mother cells, in general, have very thick walls. Indeed, at first glance, they look like young spores and may be full of cytoplasmic granules and oil droplets. Also, the chromosomes, unlike those of most ferns, are commonly of different sizes. Figure 1 illustrates a recent study I made of *Huperzia selago* with $n = 134$ (a tetraploid) and demonstrates some of the technical problems. *Huperzia selago* chromosomes were once categorized by Manton (1950) as the worst cytological object she had ever encountered. One figure she illustrated has lagging univalents, often

[1] The author gratefully acknowledges the help of D. Bay with photography, J. M. Beitel with material and information, D. B. Lellinger with literature, and W. H. Wagner with advice and assistance.

[2] Department of Biology and Herbarium, University of Michigan, Ann Arbor, Michigan 48109, U.S.A.

ANN. MISSOURI BOT. GARD. 79: 718–729. 1992.



FIGURES 1, 2. Meiotic chromosomes of *Huperzia selago*. — 1. Photomicrograph at diakinesis, with 134 pairs. — 2. Explanatory diagram. Material from Michigan, Mackinac Co., Carp River.

an indication of hybridity, and approximately 113 pairs, 37 univalents. Chromosome numbers of 132 and 136 have also been reported for this species (see Table 2). *Huperzia lucidula*, a related species, has 67 pairs of chromosomes at meiosis (Beitel & F. Wagner, 1982). The diploid number 68 and the tetraploid count 136 are numbers found frequently in *Huperzia* species in Japan (Takamiya & Kurita, 1983). The technical difficulties mentioned above, and the fact that *Lycopodium* chromosome pairs often show precocious disjunction, contrive to make differing counts possible. Do counts that differ in only one pair, for related species, truly reflect aneuploid changes, or mere prejudice in interpretation? Not all species of *Lycopodium* exhibit these pitfalls; chromosome figures in *Diphasiastrum*, almost all counts of which are $n = 23$, are usually cytological models. But, considering the more than 500 species in traditional broadly construed *Lycopodium*, we have very few dependable counts (see Table 2).

THE BASE CHROMOSOME NUMBER IN *LYCOPODIUM* SENS. LAT.

The average number of chromosomes in *Lycopodium* sens. lat. is around 80 pairs. This number in the angiosperms would indicate high polyploidy. Recent research, however (e.g., Haufler, 1987; see also Pichersky et al., 1990), suggests that pteridophytes, in spite of their high numbers, are genetically diploid, having essentially the same number of alleles as diploid angiosperms. One explanation for the genetic diploidy suggests a history of repeated allopolyploidization followed by gene silencing. Repeated polyploidization of originally low numbers is also supported by outside evidence (Wagner & Wagner, 1980). Not only do the heterosporous lycopsid pteridophytes, such as *Selaginella* and *Isoetes*, have low numbers (Klekowski & Baker, 1966; Löve et al., 1977; Wagner & Wagner, 1980), but heterosporous pteropsid ferns, such as *Marsilea* and *Azolla*, do also. In fact, all vascular plants probably have original base numbers of 7 to 13 (Wagner & Wagner, 1980).

The contrary hypothesis, that ancestral homosporous pteridophytes as well as contemporary species have had high base chromosome numbers from the beginning, is advanced as an alternative to the above by several biologists (see Duncan & Smith, 1978; Wagner & Wagner, 1980; Soltis & Soltis, 1988a).

If we can assume, however, that the high chromosome numbers of the homosporous pteridophytes (including the Lycopodiaceae) represent repeated polyploidization of ancestral low numbers, we can attempt to estimate what the base numbers are in the family.

Table 1 portrays a scheme with a base number of 11. *Lycopodium* sens. lat. is here divided into four genera (and three possible additional genera; the issue of how many genera are actually represented in the traditional *Lycopodium* sens. lat. is dealt with elsewhere in this symposium by Wagner

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 $n =$ ca. 45		Sorsa (1962) 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	$n = 68$ $n = 136$	Photo Photo	Takamiya & Kurita (1983)
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	2*n* = 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	2*n* = 68		Löve & Löve (1958)
annotinum	Finland	*n* = 34	Drawing	Sorsa (1958)
		2*n* = 68		Sorsa (1963b)
annotinum	Japan	*n* = 34	Photo	Takamiya & Kurita (1983)
annotinum	Sweden	2*n* = ca. 58		Ehrenberg (1945)
annotinum	Sweden	*n* = 34	Photo, Drawing	Manton (1950)
annotinum	U.S.A.	2*n* = ca. 50		Dunlop (1949)
annotinum var. *acrifolium* Fern.	Japan	*n* = 34	Photo	Takamiya & Kurita (1983)
annotinum subsp. *alpestre* Löve & Löve	Iceland	2*n* = 68		Löve & Löve (1958)
casuarinoides Spring	Japan	2*n* = 68	Photo	Takamiya & Tanaka (1983)
clavatum L.	Bolivia	*n* = 22		Rolleri (1982a)
clavatum	Canada	2*n* = 68		Löve & Löve (1958); Löve (1976)
clavatum	Ecuador	*n* = 34		Øllgaard (1987)
clavatum	Finland	*n* = 34	Drawing	Sorsa (1958)
		2*n* = 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	2*n* = 68	Photo	Tanaka & Takamiya (1981)
		2*n* = 102	Photo	Takamiya & Tanaka (1982)
		2*n* = 136	Photo	Takamiya (1989)
clavatum	Sweden	2*n* = 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.	2*n* = ca. 60		Dunlop (1949)
clavatum subsp. *megastachyon* (Fern. & Biss.) Löve & Löve	Canada	2*n* = 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	2*n* = 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	$n = 22-24$ $2n = 44$	Drawing	Sorsa (1963a, *b*)
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	$n = 104$	Photos	Ghatak (1965)
		$n = 156$	Drawings	
		$n = 208$		
cernua	India	$n = 104$	Photo	Kuriachen (1965)
		$n = 110$		
		$n = 136$		
		$n =$ ca. 160II, 20I		
cernua	Jamaica	$n =$ ca. 165	Photo, Drawing	Walker (1966)
	Trinidad	$n =$ ca. 165		
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*,

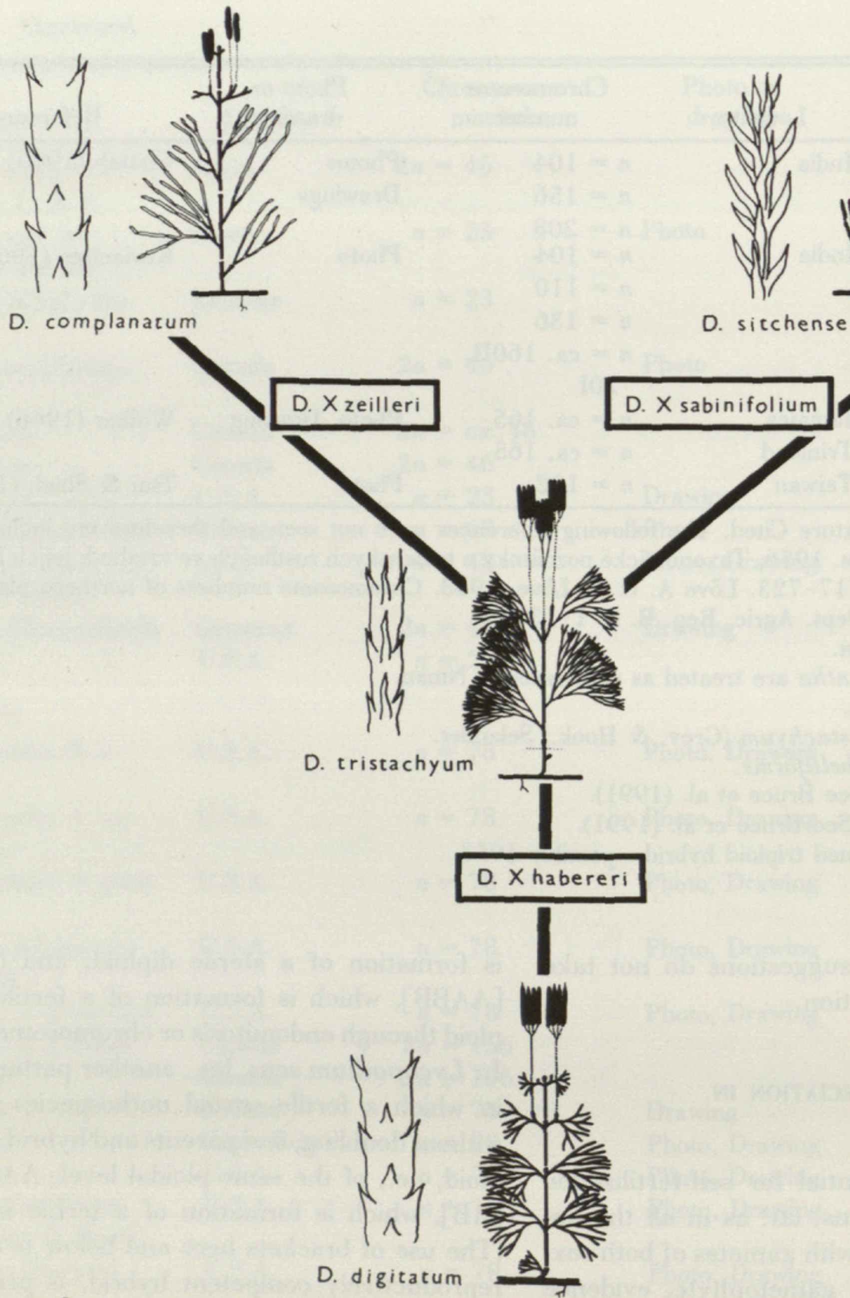


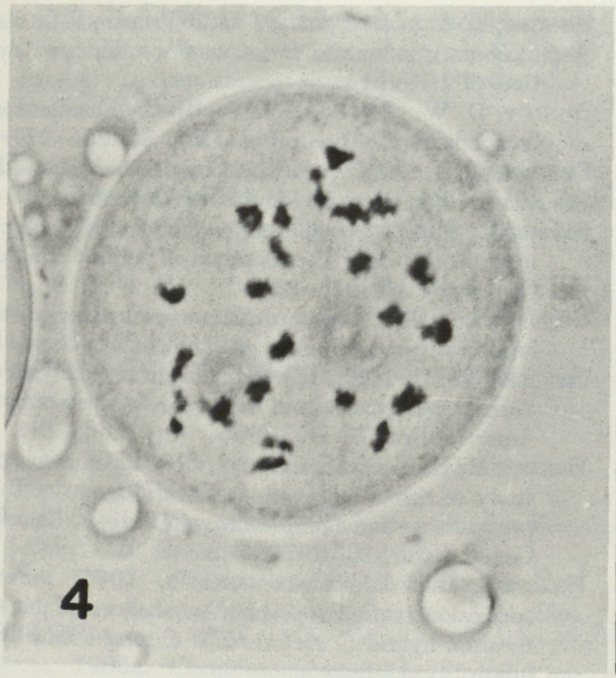
FIGURE 3. *Diphasiastrum.* Diagram showing hybridization of *D. tristachyum* with *D. complanatum* to form *D.* ×*zeilleri*; with *D. sitchense* to form *D.* ×*sabinifolium*; and with *D. digitatum* to form *D.* ×*habereri*. All taxa have $n = 23$ pairs of chromosomes. Branchlet drawings show relative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

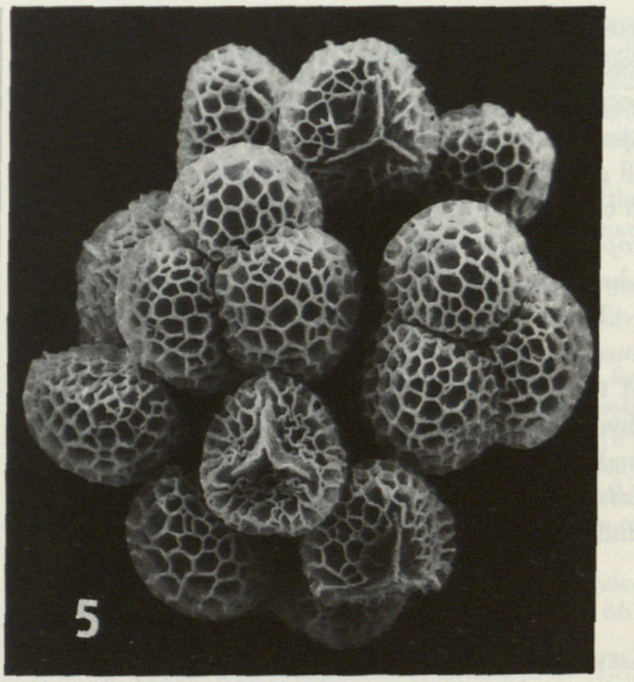
for example, presumably initiated by a single fertile spore with no parental species or only one parent in the area (Wagner & Wagner, unpublished).

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 heterosporous 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.

Literature Cited

Baranov, P. 1925. Entwicklungsgeschichte des Sporangiums und der Sporen von *Lycopodium clavatum* L. Ber. Deutsch. Bot. Ges. 43: 352–360.

Beitel, J. M. 1979a. The clubmosses *Lycopodium sitchense* and *L. sabinifolium* in the upper Great Lakes region. Michigan Bot. 18: 3–13.

———. 1979b. Clubmosses (*Lycopodium*) in North America. Fiddlehead Forum (Bull. Amer. Fern Soc.). 6: 1–8.

———. 1986. The *Huperzia selago* (Lycopodiaceae) complex in the Pacific Northwest. Amer. J. Bot. Suppl. 73: 733–734.

———. 1988. The *Huperzia selago* (Lycopodiaceae) complex in the Hawaiian Islands. Amer. J. Bot. Suppl. 76: 137.

——— & J. T. Mickel. 1992. Taxonomic changes in firmosses, *Huperzia*. Amer. Fern J. 82 (in press).

——— & F. S. Wagner. 1982. The chromosomes of *Lycopodium lucidulum*. Amer. Fern J. 72: 33–35.

———, M. K. Hansen & K. S. Walter. 1982. The effect of sun and shade on *Lycopodium* ×*habereri* (*digitatum* × *tristachyum*). Bot. Soc. Amer. Misc. Publ. No. 162: 74–75.

Bruce, J. G. 1975. Systematics and morphology of subgenus *Lepidotis* of the genus *Lycopodium* (Lycopodiaceae). Doctoral Thesis, University of Michigan, Ann Arbor.

———. 1976. Comparative studies in the biology of *Lycopodium carolinianum*. Amer. Fern J. 66: 125–137.

———, W. H. Wagner, Jr. & J. M. Beitel. 1991. Two new species of bog clubmosses, *Lycopodiella* (Lycopodiaceae), from southwestern Michigan. Michigan Bot. 30: 3–10

Conant, D. S. & G. Cooper-Driver. 1980. Autogamous allohomoploidy in *Alsophila* and *Nephelea* (Cyatheaceae): a new hypothesis for speciation in homoploid homosporous ferns. Amer. J. Bot. 67: 1269–1288.

Damboldt, J. 1962. *Lycopodium issleri* in Bayern. Ber. Bayer. Bot. Ges. 35: 20–22.

———. 1963. Zur Kenntnis der Flachen Bärlappe in Bayern. Ber. Bayer. Bot. Ges. 36: 25–27.

Duncan, T. & A. R. Smith. 1978. Primary basic chromosome numbers in ferns: facts or fantasies. Syst. Bot. 3: 105–114.

Dunlop, D. W. 1949. Notes on the cytology of some lycopods. Bull. Torrey Bot. Club. 76: 266–277.

Ehrenberg, L. 1945. Kromosomtalen hos några kärlväxter. Bot. Not. 1945: 430–437.

Fabbri, F. 1965. Secondo supplemento alle Tavole cromosomische delle Pteridophyta di Alberto Chiarugi. Caryologia 18: 675–731.

Ghatak, J. 1965. Some evidences of cytological evolution in *Lycopodium* L. s.l. The Nucleus 8: 45–58.

Haufler, C. H. 1987. Electrophoresis is modifying our concepts of evolution in homosporous pteridophytes. Amer. J. Bot. 74: 953–966.

Hersey, R. E. & D. M. Britton. 1981. A cytological study of three species and a hybrid taxon of *Lycopodium* (section *Complanata*) in Ontario. Canad. J. Genet. Cytol. 23: 497–504.

Hickok, L. G. & E. J. Klekowski, Jr. 1974. Inchoate speciation in *Ceratopteris*: An analysis of the synthesized hybrid *C. richardii* × *C. pteridoides*. Evolution 28: 439–446.

Klekowski, E. J., Jr. 1970. Populational and genetic studies of a homosporous fern—*Osmunda regalis*. Amer. J. Bot. 57: 1122–1138.

——— & H. G. Baker. 1966. Evolutionary significance of polyploidy in the Pteridophyta. Science 153: 305–307.

Kukkonen, I. 1967. Studies on the variability of *Diphasium* (*Lycopodium*) *complanatum*. Ann. Bot. Fenn. 4: 441–470.

Kuriachen, P. I. 1965. Cytology of *Lycopodium cernuum*. Caryologia 18: 633–636.

Löve, A. 1976. IOPB chromosome number reports. LIII. Taxon 25: 483–500.

——— & D. Löve. 1958. Cytotaxonomy and classification of lycopods. The Nucleus 1: 1–10.

——— & ———. 1961. Some nomenclatural changes in the European flora. I. Species and supraspecific categories. Bot. Not. 114: 33–47.

——— & ———. 1966. Cytotaxonomy of the alpine vascular plants of Mount Washington. Univ. Colorado Stud., Ser. Biol. 24: 1–74.

———, ——— & R. E. G. Pichi Sermolli. 1977. Cytotaxonomical atlas of the Pteridophyta. J. Cramer, Vaduz.

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

Mehra, P. N. & S. C. Verma. 1957. Cytology of *Lycopodium*. Current Science 26: 55–56.

Mitui, K. 1980. Chromosome numbers of Japanese Pteridophytes (2). Bull. Nippon Dental Univ. Gen. Education 9: 217–229.

Ninan, C. A. 1958. Studies on the cytology and phylogeny of the pteridophytes. II. Observations on the genus *Lycopodium*. Proc. Natl. Inst. Sci. India 24: 54–66.

Øllgaard, B. 1987. A revised classification of the Lycopodiaceae s. lat. Opera Bot. 92: 153–178.

———. 1989. Index of the Lycopodiaceae. Biol. Skr. 34: 1–135.

Pichersky, E., D. Soltis & P. Soltis. 1990. Defective chlorophyll a/b-binding protein genes in the genome of a homosporous fern. Proc. Natl. Acad. Sci. 87: 195–199.

ROLLERI, C. H. 1982a. El número cromosómico de *Lycopodium clavatum* L. ssp. *clavatum* (Lycopodiaceae) y sus implicancias sistemáticas. Revista Mus. La Plata (N.S.) Bot. 13: 115–118.

———. 1982b. El número cromosómico de *Lycopodium saururus* Lam (Secc. Crassistachys Herter, Lycopodiaceae, Pteridophyta) y sus implicancias sistemáticas. Revista Mus. La Plata (N.S.) Bot. 13: 119–122.

SOLTIS, D. E. & P. S. SOLTIS. 1988a. Are lycopods with high chromosome numbers ancient polyploids? Amer. J. Bot. 5: 238–247.

——— & ———. 1988b. Estimated rates of intragametophytic selfing in lycopods. Amer. J. Bot. 75: 248–256.

SORSA, V. 1958. Chromosome studies on Finnish Pteridophyta. I. Hereditas 44: 541–546.

———. 1961. Chromosome studies on Finnish Pteridophyta. II. Hereditas 47: 480–488.

———. 1962. Chromosomenzahlen finnischer Kormophyten I. Ann. Acad. Sci. Fenn. Ser. A, 4. Biol. 58: 1–14.

———. 1963a. Chromosome studies on Finnish Pteridophyta. III. Hereditas 49: 337–344.

———. 1963b. Chromosomenzahlen finnischer Kormophyten II. Ann. Acad. Sci. Fenn. Ser. A, 4. Biol. 68: 1–14.

TAKAMIYA, M. 1984. A triploid cytotype of *Lycopodium serratum*, pteridophyte. Chrom. Info. Serv. No. 37: 25–26.

———. 1989. Cytological and ecological studies on the speciation of *Lycopodium clavatum* L. in the Japanese Archipelago. J. Sci. Hiroshima Univ., Ser. B, Div. 2 (Bot.) 22: 353–430.

——— & S. KURITA. 1983. Cytotaxonomic studies on Japanese species of the genus *Lycopodium* sensu lato. Acta Phytotax. Geobot. 34: 66–79.

——— & R. TANAKA. 1982. Polyploid cytotypes and their habitat preferences in *Lycopodium clavatum*. Bot. Mag. Tokyo 95: 419–434.

——— & ———. 1983. Chromosomes of *Lycopodium casuarinoides*, a fern ally. Chrom. Info. Serv. No. 35: 27–28.

TANAKA, R. & M. TAKAMIYA. 1981. Polyploidy in *Lycopodium clavatum* of Japan. Chrom. Info. Serv. No. 31: 5–6.

TSAI, J-L. & W-C. SHIEH. 1983. A cytotaxonomic survey of the pteridophytes in Taiwan. J. Sci. and Engineering 20: 137–159.

WAGNER, F. S. 1980. Chromosome behavior in three interspecific hybrids of *Diphasiastrum* (Lycopodiaceae). Misc. Publ. Bot. Soc. Amer. 158:121–122.

WAGNER, W. H., JR. & J. M. BEITEL. 1992. Generic classification of modern North American Lycopodiaceae. Ann. Missouri Bot. Gard. 79: 676–686.

——— & F. S. WAGNER. 1966. Pteridophytes of the Mountain Lake area, Giles Co., Virginia: biosystematic studies, 1964–65. Castanea 31: 121–140.

——— & ———. 1980. Polyploidy in pteridophytes. Pp. 199–214 *in* W. H. Lewis (editor), Polyploidy: Biological Relevance. Plenum, New York.

———, ——— & J. M. BEITEL. 1985. Evidence for interspecific hybridization in pteridophytes with subterranean mycoparasitic gametophytes. Pp. 273–281 *in* A. F. Dyer & C. N. Page (editors), Biology of Pteridophytes. Proc. Roy. Soc. Edinburgh 86B.

WALKER, T. G. 1958. Hybridization in some species of *Pteris* L. Evolution 12: 82–92.

———. 1966. A cytotaxonomic survey of the pteridophytes of Jamaica. Trans. Roy. Soc. Edinburgh 66: 169–237.

———. 1979. The cytogenetics of ferns. Pp. 87–132 *in* A. F. Dyer (editor), The Experimental Biology of Ferns. Academic Press, New York.

———. 1985. Some aspects of agamospory in ferns—the Braithwaite system. Pp. 59–65 *in* A. F. Dyer & C. N. Page (editors), Biology of Pteridophytes. Proc. Roy. Soc. Edinburgh 86B.

WERTH, C. R. & W. H. WAGNER, JR. 1990. Proposal to designate reproductively competent species of hybrid origin by a multiplication sign placed in brackets (reword Article H.3, *Note 1*). Taxon 39: 699–702.

WHITTIER, D. P. 1977. Gametophytes of *Lycopodium obscurum* as grown in axenic culture. Canad. J. Bot. 55: 563–567.

———. 1981. Gametophytes of *Lycopodium digitatum* (formerly *L. complanatum* var. *flabelliforme*) as grown in axenic culture. Bot. Gaz. (Crawfordsville) 142: 519–524.

——— & T. R. WEBSTER. 1986. Gametophytes of *Lycopodium lucidulum* from axenic culture. Amer. Fern J. 76: 48–55.

WILCE, J. H. 1965. Section *Complanata* of the genus *Lycopodium*. Nova Hedwigia 19: 1–233.

———. 1972. Lycopod spores, I. General spore patterns and the generic segregates of *Lycopodium*. Amer. Fern J. 62: 65–79.