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The phylogeny of land plants: a cladistic analysis based on male gametogenesis

David J. Garbary, Karen S. Renzaglia, and Jeffrey G. Duckett

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Abstract: A cladistic analysis was carried out to resolve phylogenetic pattern among bryophytes and other land plants. The analysis used 22 taxa of land plants and 90 characters relating to male gametogenesis. *Coleochaete* or *Chara*/*Nitella* were the outgroups in various analyses using HENNIG 86, PAUP, and MacClade, and the land plant phylogeny was unchanged regardless of outgroup utilized. The most parsimonious cladograms from HENNIG 86 (7 trees) have treelengths of 243 (C.I. = 0.58, R.I. = 0.82). Bryophytes are monophyletic as are hornworts, liverworts, and mosses, with hornworts identified as the sister group of a liverwort/moss assemblage. In vascular plants, lycophytes are polyphyletic and *Selaginella* is close to the bryophytes. *Lycopodium* is the sister group of the remaining vascular plants (minus *Selaginella*). Longer treelengths (over 250) are required to produce tree topologies in which either lycophytes are monophyletic or to reconstruct the paraphyletic bryophyte phylogeny of recent authors. This analysis challenges existing concepts of bryophyte phylogeny based on more classical data and interpretations, and provides new insight into land plant evolution.

The phylogeny of land plants is clearly one of the fundamental problems in evolution. Ultrastructural evidence accumulated over the last 20 years has demonstrated that the charophycean green algae form a monophyletic group with land plants (Pickett-Heaps 1975a, Mattox & Stewart 1984, Graham & Kaneko 1991). More recently, molecular information from sequence data has reinforced this interpretation (Hori & al. 1985, Devereux & al. 1990, Van De Peer & al. 1990, Chapman & Buchheim 1991, Waters & al. 1992). A number of cladistic analyses using mostly morphological and ultrastructural characters have been carried out on this complex in an attempt to understand patterns of relationships among the various taxonomic groups (Mishler & Churchill 1984, 1985; Bremer 1985; Bremer & al. 1987; Sluiman 1985; Theriot 1988; Graham & al. 1991). These studies were carried out using slightly different taxa and occasionally with different objectives. For example, Sluiman (1985) concentrated on the relationships among the green algae as a whole, whereas Mishler & Churchill (1984, 1985) were more concerned with evolution in the charophycean/bryophyte clade, and Graham & al. (1991) with relationships among the various charophycean algae and estab-

lishing the outgroup for embryophytes. On the whole, these studies utilized generalized features where homology among the different character states was not critically examined and has been questioned (e.g., ROBINSON 1985, KENRICK & CRANE 1991). Relationships among seed plants have been extensively studied with particular emphasis on the origin of angiosperms. The recent discovery of double fertilization and the details of endosperm development in *Ephedra* (FRIEDMAN 1990, 1992) and cladistics of spermatophytes (e.g., CRANE 1990, FRIEDMAN 1993, LOCONTE & STEVENSON 1990 and references therein) have gone a long way to resolve relationships among these organisms.

All these previous studies used a wide variety of taxa and an assortment of data types including morphological, ultrastructural, and biochemical information. Although these studies made considerable progress in understanding relationships among land plants, fundamental issues were unresolved including the extent of paraphyly among bryophytes, the homology of certain characters, the initial branching sequence at the base of the embryophyte clade, and relationships among the seedless vascular groups.

In the present study we resolve relationships among the various groups of bryophytes and vascular plants by undertaking a phylogenetic analysis. We primarily use ultrastructural features associated with male gametogenesis, and emphasize features of the flagellar apparatus and its associated cytoskeleton and organelles. The use of flagellated stages (sperm in animals) has been used in a variety of algal, fungal, protozoan, and animal groups to infer phylogenetic relationships, and this approach in animal systems has been referred to as "spermiocladistics" (JAMIESON 1987). Spermiocladistics was considered an appropriate approach to making inferences relating to land plant phylogeny because sperms are homologous in all the organisms studied, and the necessary published (and unpublished) data with which to carry out the analysis are available. Cladograms produced using this data set may then be examined for congruence with those produced using other kinds of information. The importance of spermatogenesis in resolving bryophyte and vascular plant phylogenies was emphasized by a number of authors (e.g., DUCKETT & al. 1982, DUCKETT & CAROTHERS 1982, GRAHAM & al. 1991, RENZAGLIA & DUCKETT 1991), however, no formal phylogenetic analysis of these data has been previously undertaken.

Material and methods

Taxa. A list of the taxa included in the study is shown in Table 1 along with the primary sources for the data. *Coleochaete* and *Chara/Nitella* were initially selected as the outgroups after GRAHAM & al. (1991) and GRAHAM & KANEKO (1991) and others. Various bryophyte taxa were chosen based on availability of information, and an attempt to represent the greatest taxonomic diversity and to evaluate the widest range of systematic problems. Taxa included two genera of hornworts (*Phaeoceros, Notothylas*), seven genera or groups of genera of liverworts (*Marchantia, Sphaerocarpos, Pellia, Blasia, Jungermanniales, Treubia, Haplomitrium*), five genera of mosses (*Sphagnum, Andreaea, Polytrichum, Hypnum, Takakia*), six genera or groups of genera of spore-bearing vascular plants (*Lycopodium, Selaginella, Equisetum, Filicales, Osmunda, Marsilea*), and two genera of seed plants (*Zamia* and *Ginkgo*). The data are generally based on studies of one species in each genus, however, the characters for *Jungermanniales* and *Filicales* are composites from studies of several genera, i.e., *Bazzania, Cephalozia, Marsupella*, and *Chiloscyphus* for *Jungermanniales* and *Pteridium, Onoclea, Platyzoma*, and *Ceratopteris* for *Filicales*.

Table 1. List of taxa and order, class assignment. Sources for primary data on male gametogenesis indicated

Genus	Order, Class	References
Coleochaete	*Coleochaetales, Charophyceae*	GRAHAM & WEDEMAYER 1984, GRAHAM & REPAVICH 1989
Chara	*Charales, Charophyceae*	PICKETT-HEAPS 1968
Nitella	*Charales, Charophyceae*	TURNER 1968
Phaeoceros	*Anthocerotales, Anthoceropsida*	CAROTHERS & al. 1977, MOSER & al. 1977, RENZAGLIA & DUCKETT 1989
Notothylas	*Anthocerotales, Anthoceropsida*	RENZAGLIA & CAROTHERS 1986, RENZAGLIA & DUCKETT 1989
Treubia	*Treubiales, Hepatopsida*	CAROTHERS & RUSHING 1990
Haplomitrium	*Calobryales, Hepatopsida*	CAROTHERS & DUCKETT 1979, 1980; CAROTHERS & RUSHING 1988
"Jungermanniales" (Bazzania, Cephalozia, Chiloscyphus, Marsupella)	*Jungermanniales, Hepatopsida*	RUSHING & CAROTHERS 1986; RUSHING & al. 1984; MILLER & al. 1983; DUCKETT & al. 1982, 1984
Pellia	*Metzgeriales, Hepatopsida*	DUCKETT & al. 1984
Marchantia	*Marchantiales, Hepatopsida*	CAROTHERS & KREITNER 1968, KREITNER & CAROTHERS 1976
Blasia	*Metzgeriales, Hepatopsida*	RENZAGLIA & DUCKETT 1987 a, b
Sphaerocarpos	*Sphaerocarpales, Hepatopsida*	CAROTHERS & al. 1983
Sphagnum	*Sphagnales, Bryopsida*	DUCKETT & al. 1984, DUCKETT & CAROTHERS 1979, RENZAGLIA & DUCKETT 1991
Andreaea	*Andreaeales, Bryopsida*	CAROTHERS & DUCKETT 1977, DUCKETT (unpubl. data)
Polytrichum	*Polytrichales, Bryopsida*	PAOLILLO & al. 1968 a, b
Hypnum	*Bryales, Bryopsida*	DUCKETT & RENZAGLIA 1986, RENZAGLIA & DUCKETT 1991
Takakia	*Takakiales, Bryopsida*	RENZAGLIA (unpubl.)
Lycopodium	*Lycopodiales, Lycopodopsida*	CAROTHERS & al. 1975; ROBBINS & CAROTHERS 1975, 1978
Selaginella	*Selaginellales, Lycopodopsida*	ROBERT 1974
Equisetum	*Equisetales, Equisetopsida (Sphenopsida)*	DUCKETT 1973, DUCKETT & BELL 1977

Table 1 (continued)

Genus	Order, Class	References
Marsilea	*Marsileales, Filicopsida*	MYLES & BELL 1975, MYLES & HEPLER 1977, HEPLER 1976
"Filicales" (Pteridium, Platyzoma, Ceratopteris, Onoclea)	*Filicales, Filicopsida*	MANTON 1959, BELL 1974, DUCKETT 1975, BELL & DUCKETT 1976, DUCKETT & CAROTHERS 1982, DOONAN & al. 1986, KOTENKO 1990
Osmunda	*Osmundales, Filicopsida*	MILLER & al. 1985
Zamia	*Cycadales, Cycadopsida*	MIZUKAMI & GALL 1966; NORSTOG 1986, 1990
Ginkgo	*Ginkgoales, Ginkgopsida*	LI & al. 1989

Characters for land plant phylogeny. In our analysis, 90 characters were defined to describe all organisms being considered. These features were described in a general way by DUCKETT & CAROTHERS (1982), DUCKETT & al. (1982), RENZAGLIA & DUCKETT (1988, 1991). Characters refer to morphogenesis of antheridia and development and morphology/microanatomy of sperm. The characters and character states are outlined in Appendix 1, and problems of homology and coding are discussed. Some characters are self-explanatory and require no discussion (see standard plant morphology texts for elaboration, e.g., EAMES 1936, SMITH 1955, PARIHAR 1965, FOSTER & GIFFORD 1974, BIERHORST 1971, BOLD & al. 1987). The complete data matrix is shown in Table 2.

Analysis. Cladistic analysis (see WILEY 1980 for introduction to methodology) was carried out using HENNIG 86 version 1.5 (FARRIS 1988), MacClade version 2.1 (MADDISON & MADDISON 1987), and PAUP version 3.0s (SWOFFORD 1991). Because of the number of taxa and the time required for each run of PAUP, only partial data sets could be analyzed with this software. Various runs were carried out using either *Coleochaete* or *Chara/Nitella* as outgroups to determine principal dichotomies in an overall cladogram. Using MacClade, remaining taxa were included to produce complete cladograms for all OTUs, and for mapping character changes. Final cladograms were produced in HENNIG 86 using the "ie" command which generates all of the most parsimonious trees resulting from the data set. HENNIG 86 is extremely effective for cladistic analysis and it has been suggested that it "become the tool of choice for practising systematists" (PLATNICK 1989). Additional cladograms were also constructed in HENNIG 86 and MacClade to model various phylogenetic hypotheses including some previously suggested by MISHLER & CHURCHILL (1985), SLUIMAN (1985), and BREMER & al. (1987). Although bootstrapping has become widely used in the evaluation of molecular data, this procedure has been questioned on theoretical grounds (THERIOT 1992) and was not employed here.

Results

Outgroups and cladogram topology. In different "ie" analyses using HENNIG either *Coleochaete* or *Chara/Nitella* were designated as outgroups. In the former case a polytomy was formed with *Coleochaete, Chara/Nitella*, and land plants joining at a basal node (e.g., Figs. 1, 2). When *Chara/Nitella* was the outgroup, *Coleochaete* was the sister group to land plants (not shown). Choice of outgroup had no effect

Table 2. Character number and character state coding of 90 characters for 25 taxa used in the analysis

	1–10	11–20	21–30	31–40	41–50	51–60	61–70	71–80	81–90
Coleochaete	0?00000000	??0?000?00	0000000000	?00000??00	00?01?0000	000000000??	0?0???0000	00?01??000	000??0??1?
Chara	10?1010012	0?00030?00	000000?010	?000?0??00	00?00?0000	0000001100	0000000?10	0011??0001	20?100??21
Nitella	10?1010013	0?00030?00	000000?0?0	?00000??00	00?00?0000	0000001100	0000000?10	0001??0001	20?100??21
Phaeoceros	1022110013	0000110111	1100002020	?1?0100000	0111000000	3001001100	1000110111	1110001110	1111001021
Notothylas	1022110013	0000110111	1100002020	?1?0100000	0111000000	3001001100	1000110111	1110001110	1111001021
Marchantia	1022010003	0000110121	2001112021	1130111000	0010010000	3001001100	0100101210	0110000110	1102011020
Sphaerocarpos	1022010013	0000110121	2001112021	1130111000	0010011000	3001001100	0100101210	01?0000110	110?0?1020
Pellia	1012010003	0000110121	2001112021	1130100000	0110110000	3001001100	0110101210	0110000110	1102011020
Blasia	10?2010003	0000110121	2001112021	1130111000	0010010000	3001001100	0100101210	0110000110	1102011020
Jungermanniales	1012010013	0000110121	2001112021	1130100000	0010010000	3001001100	0110101210	0110000110	1102011020
Haplomitrium	1012010013	0000110101	?001112021	0130100001	10101?0010	3001001100	010015??10	0110000110	?11?0?1020
Treubia	1012010?3	0000110121	200111?021	013010000?	10101?00?0	3?01001??0	01001????0	??1000?1?0	?10?0?1020
Sphagnum	1112010003	1111110121	21?0111?0?	1111000120	0011010100	3001001100	0101220310	1110000110	0110200120
Andreaea	1112013003	1111110121	2001112021	1110100100	0011010000	3001001100	0101220310	1110000110	0100200120
Polytrichum	1112013013	1111110121	2001112021	1110100110	0011010000	3001001100	0101220310	1110000110	0100200120
Hypnum	1112013013	1111110121	2001112021	1110100110	0011010000	3001001100	0101220310	1110000110	0100200120
Takakia	1112013013	1111110121	200111?021	11?01001??	00?10?00?0	3?01?0110?	01??????10	?1?000?110	010?2??020
Lycopodium	10?3000002	?101110140	000?000130	?1?0100001	000?3?00?0	0101001071	0?00??0000	01100?0110	??0000?01?
Selaginella	10?3?0?012	??1?110?22	1000002070	?1?0100000	00010?0000	0101001101	0000?40?10	01100?0110	?00000?020
Equisetum	10?3004012	2101111030	0001000100	?121100201	0000400011	0001211111	0100330310	0000110001	20?0100110
Filicales	10?3001001	2101121032	1012000100	?121100201	0000200011	2?01211111	0100330310	0000110001	20?0100110
Marsilea	10?3?0?001	2101121030	0001000110	?121100200	00?0000000	2001211100	0100160120	0000010001	20?010?010
Osmunda	10?3001001	2101121032	1012000140	?121100201	0000200011	1001211111	0100330310	0000110001	20?0100110
Ginkgo	2??????1?4	?0?11?1030	0001000100	?12?100200	00005?0011	0011122072	0?2???0000	00012?0001	20?010??00
Zamia	2??????1?4	?0?11?1030	0001000100	?12?100200	00005?0011	0011122072	0?2???0000	00012?0001	20?010??00

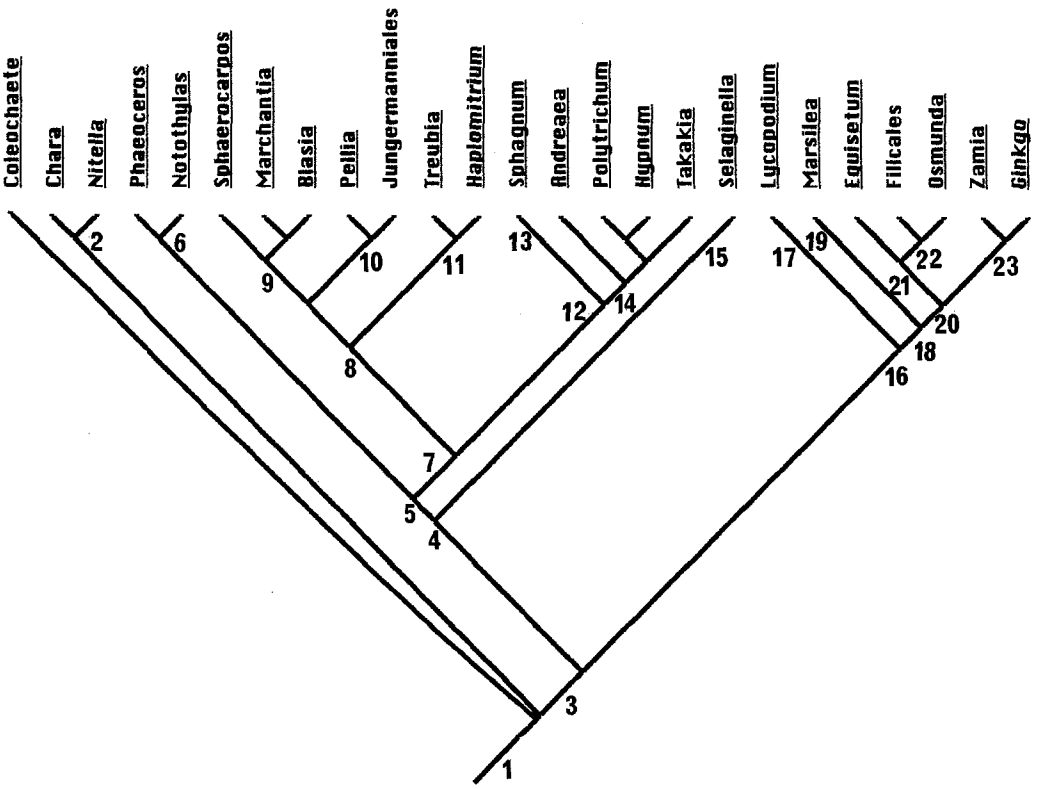

Fig. 1. The phylogeny of land plants. One of seven most parsimonious trees produced by "ie"analysis in HENNIG 86. Treelength = 243 (MacClade treelength = 198). Note positions of *Selaginella* and *Lycopodium*. Apomorphic characters associated with each node indicated in Table 3

on relationships within the land plant clade which was always monophyletic. All subsequent discussion refers to cladograms produced with *Coleochaete* as the outgroup.

Land plants. The "ie" analysis in HENNIG produced seven equally parsimonious trees (treelength 243, CI 58, RI 82) in which there was one primary configuration of a monophyletic land plant clade (Figs. 1, 2) (apomorphies of major clades are indicated in Table 3). In these cladograms lycopods are polyphyletic with *Selaginella* associated with the bryophyte clade, and *Lycopodium* associated with the remaining land plants (Fig. 1). In none of the most parsimonious trees did *Lycopodium* and *Selaginella* form a monophyletic group. All of the variation in the equally parsimonious cladograms occurred within the mosses, and this variation is demonstrated in the strict consensus tree (Fig. 2). Thus according to the data from male gametogenesis, land plants are those green plants in which: (1) there is no centriolar replication in spermatogenous cells (15)[1], (2) BBs are not at right angles throughout MLS development (19), (3) the developing MLS is beneath the BBs (32), (4) there

[1] Figures in parentheses are the character numbers (see Appendix 1).

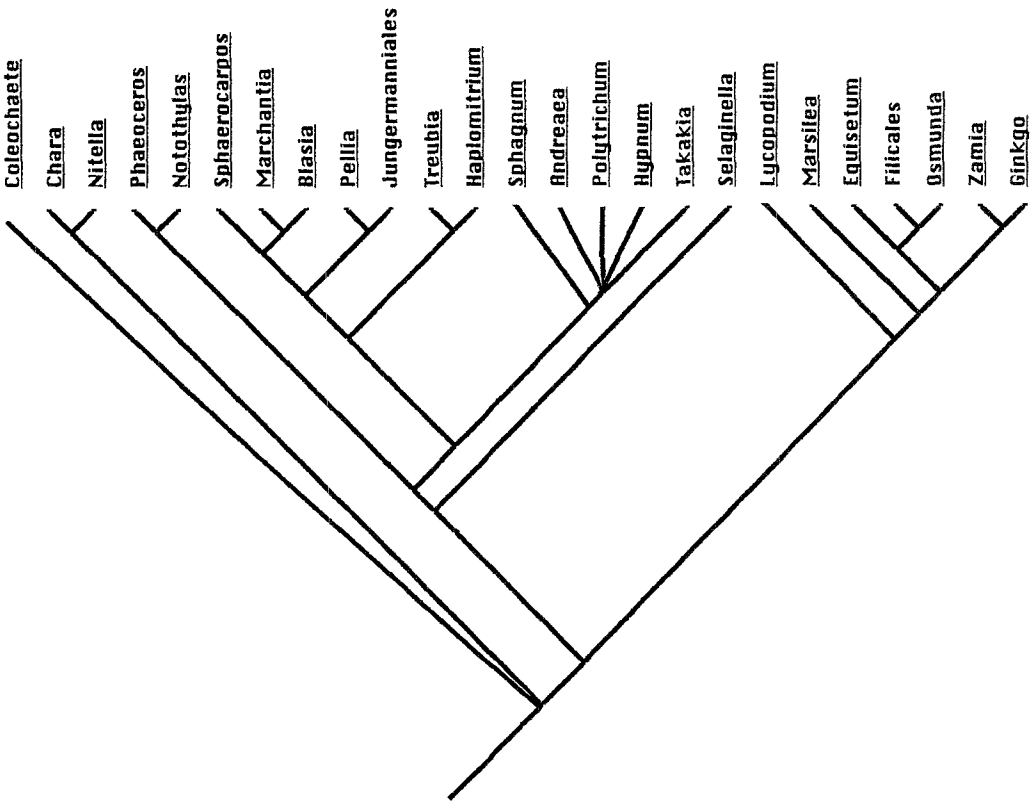

Fig. 2. The phylogeny of land plants. Strict consensus tree of seven most parsimonious cladograms produced in HENNIG 86

is growth of the LS (33), (5) there is a 45° orientation of the spline/LS (35), and (6) there are no flagellar scales (54).

In all of the most parsimonious cladograms bryophytes were monophyletic and *Selaginella* was the sister group (Figs. 1, 2). This combined clade was supported by five characters: occurrence of ventral or ventral/dorsal extensions to the anterior basal body (21) (also present in to a limited degree in *Osmunda* and *Filicales*), partial regression of the LS in all taxa except *Sphagnum* (27), maturational elongation of AM (44) (however, this is absent in liverworts); the occurrence of only two mitochondria in the sperm (78), and the change in structure of cristae in the mitochondria from sacs to baffles (79). *Selaginella* has three autapomorphies in which lipids are present in young spermatids (13) (shared with mosses), there is a mottled matrix around the BBs (52) (shared with *Lycopodium*), and the condensed chromatin strands are in the form of "spikes" (66).

The bryophyte clade. The cladistic analysis strongly supported the notion of bryophytes as a monophyletic group based on five clear synapomorphies: the arrangement of spermatogenous cells in blocks (4), the presence of an antheridial stalk (6) (shared with *Chara*/*Nitella*), anterior/posterior growth of the spline (43), the basal body triplets becoming impregnated with matrix at maturity (51), and the occurrence of a plastid/nuclear association in mid-stage spermatids (82).

Table 3. Apomorphy hypotheses for internal nodes of land plant phylogeny cladogram in Fig. 1. Unless otherwise indicated, character state changes are from 0 to 1

Node	Character number
1	1, 45, 57, 58, 81
2	6, 74, 80
3	15, 32, 33 (0–> 4), 35, 54
4	21, 27 (0–> 2), 44, 78, 79
5	4 (3–> 2), 6, 43, 51 (0–> 3), 82
6	5, 19 (2–> 1), 22, 42, 61, 66, 70, 77, 83, 84, 90
7	21 (1–> 2), 24, 25, 26, 30, 46, 62
8	44 (1–> 0)
9	3 (1–> 2), 36, 37
10	63
11	31 (1–> 0), 41, 45
12	2, 11, 13, 14, 38, 64, 65 (1–> 2), 66, (0–> 2), 81 (1–> 0), 85 (0–> 2), 88
13	22, 27 (2–> 1), 39 (0–> 2), 48, 83
14	7 (0–> 3)
15	13, 52, 66 (0–> 4)
16	14, 28
17	29 (–> 3), 40, 45 (0–> 3), 52, 58 (1–> 0)
18	17, 18 (1–> 0), 33 (4–> 2), 34, 38 (0–> 2), 56, 73 (1–> 0), 80, 85
19	51 (0–> 2), 68, 69 (–> 2)
20	49, 50
21	40, 68 (0–> 3)
22	20 (0–> 2), 21, 23, 24 (1–> 2)
23	1 (1–> 2), 8, 10 (–> 4), 12 (0–> 1), 53, 55 (2–> 1), 56 (1–> 2), 57 (1–> 2), 58 (1–> 0), 63 (0–> 2), 74, 84 (1–> 0)

1) Hornworts. The hornworts were among the most highly supported clades in the cladogram with 11 synapomorphies: endogenous development of antheridia (5), the side-by-side position of basal bodies (19), the absence of a stellate transition region (22) (shared with *Sphagnum*), the lateral increase in of the LS beyond the spline (42), the occurrence of a median constriction in the nucleus (61), the occurrence of condensed chromatin perpendicular to the spline (66), the presence of a dense body in the anterior mitochondrion (70), the localization of osmiophilic material with the anterior mitochondrion (77), the presence of single starch grains in the plastid (83) (shared with *Haplomitrium* and *Sphagnum*), the contact of the sperm plastid with the nucleus (84) (also in *Chara/Nitella* and *Jungermanniales*), and the asymmetrical sperm (90).

2) Liverworts. Of the three bryophyte groups, liverworts were the least strongly supported and there is only one apparent synapomorphy supporting the clade: the absence of maturational elongation of the anterior mitochondrion (44). All mature sperms of liverworts have a fibrillenscheide (86), and this may be an additional defining feature for liverworts. However, fibrillenscheiden have not been documented in *Treubia* and *Haplomitrium* because only immature sperms have been studied. As shown by the consensus tree (Fig. 2) the most parsimonious cladograms

all identified three groupings of taxa: a) *Haplomitrium* and *Treubia*; b) *Marchantia, Blasia*, and *Sphaerocarpos*; and c) *Pellia* and *Jungermannia*. *Sphaerocarpos* was the sister group of a clade including *Marchantia* and *Blasia*.

The *Haplomitrium* and *Treubia* clade has three synapomorphies referring to the spline aperture being left of center (31), the increase in spline MTs behind the LS (41) and presence of spline widths of 50–110 microtubules in the mature cell (45). Liverworts are also characterized by the presence of a diverticulum during nuclear shaping (67), however, this feature has not been characterized in *Treubia* or *Haplomitrium*.

3) Mosses. Male gametogenesis in mosses is very uniform and the moss clade is supported by 11 synapomorphies: the presence of an apical cell during antheridial ontogeny (2), the rounded shape of late spermatogenous cells (11), the presence of lipids in young spermatids (13), the absence of a diagonal spindle in the final mitotic division of sperm formation (14), the LS is present only under the ABB (38), the synchrony of nuclear shaping with spline growth (64), nuclear compaction occurring at equal rates along entire length (65), chromatin condensation in a spiral/central strand (66), the association between plastid location (at the poles) and the polarity of cell division in the antheridia (81), the positioning of plastids in the posterior of the cell but not attached to the spline (85), and the presence of sheets of ER (88). The lack of a diagonal spindle is also shared with the *Lycopodium*/pteridophyte/seed plant line, and the sheets of ER are also found in the *Equisetum*/*Filicales*/*Osmunda* clade.

Within the mosses, *Sphagnum* was always the sister group to the remaining taxa. *Sphagnum* has five apparent apomorphies (22, 27, 39, 48, 83). Two of these (22 and 83) occur in hornworts, and the character states are unknown for two more (27 and 39) in *Takakia*. Since information relating to four of the features providing synapomorphies for the entire moss clade (64–66 and 88) are also unknown in *Takakia*, its position in the tree might change as these data become available. The mosses other than *Sphagnum* are characterized by a single feature: the presence of more than two opercular cells in the antheridium (7). The absence of information for *Takakia* combined with the overall similarity of male gametogenesis in the mosses has resulted in a lack of resolution in this clade. Thus the seven parsimonious cladograms resulting from our analysis described rearrangements of the various moss genera. In the strict consensus tree (Fig. 2) this has resulted in a polytomy that includes *Takakia, Andreaea, Polytrichum*, and *Hypnum*.

The pteridophyte, seed plant clade. The pteridophyte/seed plant clade includes *Lycopodium* but not *Selaginella* (Figs. 1, 2). The pteridophyte clade is characterized by only two features: the absence of a diagonal spindle during the final mitotic division (14) and a perpendicular orientation of the LS/AM in relation to the longitudinal axis of the spermatid (28). The absence of bicentrioles (18) might also be a synapomorphy of such a lineage, however, information is lacking for *Selaginella*. Similarly, posterior growth of the LS (33) might also provide a synapomorphy for pteridophytes even though this feature is unknown in *Lycopodium* and *Selaginella*, and mosses and liverworts have different character states.

The remaining pteridophytes and seed plants form a strong monophyletic group based on the following nine characters: the presence of more than 2 BBs and flagella per sperm (17, 56), the absence of bicentrioles (18), the posterior growth of the LS

(33), the presence of a stratified plaque between nascent blepharoplast (34), the position of the LS under some BBs (38), the absence of a mitochondrial association with plastids in mature sperm (73), the absence of monoplastidic sperm (80), and the central location of plastids in the sperm (85). The absence of a mitochondrial association with plastids in young spermatids might be another synapomorphy, however, as it is currently coded, it could equally well be a plesiomorphic state retained from the algal ancestory.

Marsilea is well characterized with three apomorphies: the cartwheel of the BB matrix develops a plug of matrix at maturity (51), the loss of excess nuclear envelope after condensation (68), and the occurrence of more than three gyres of the nucleus in the sperm (69). It is of interest that two of these features (51 and 69) also occur in *Filicales*, and that this is not sufficient to unite these taxa.

The remaining plants in the cladogram (*Equisetum, Filicales, Osmunda*, seed plants) have two synapomorphies relating to the presence of an osmiophilic crest (49), and the presence of an anterior osmiophilic ridge (50). Character 49 is unknown for *Lycopodium*, and its determination might affect placement of this genus.

The *Equisetum, Osmunda, Filicales* clade was consistent in all our cladograms and was supported by two characters: presence of an accessory band of microtubules (40) and the gradual loss of nuclear envelope throughout the nucleus (68). Character 40, however, also occurs in *Lycopodium* and character 68 has not been determined for *Selaginella*. *Osmunda* and *Filicales* are closely related and this clade has four synapomorphies: the presence of short proximal extensions (20) that are ventral (21), the presence of a transient cartwheel extension in the central BB hub (23), and the presence of connecting fibers between the basal bodies in the form of fine filaments (24). These character states for 20 and 21 are not unique to *Osmunda* and *Filicales* and the homology with other organisms requires reassessment.

The seed plants were the most strongly supported clade in our trees with 12 synapomorphies (1, 8, 10, 12, 53, 55–58, 63, 74, 89). Of these characters, nine (all except 12, 58, 74) provide character states limited to this clade. The pairing of the nascent spermatids (12), the ovoid nuclear shape (58), and the absence of a specialized anterior mitochondrion (74) are shared with some other taxa. Closer study of these features may allow redefinition of the character states to remove these apparent convergences.

Other trees. As a test of congruence of the male gametogenesis data set used here with other analyses of relationships, our most parsimonious tree (Fig. 1, tree-length 243) was manipulated in HENNIG 86 to simulate various phylogenetic hypotheses. These trees and their calculated treelengths are shown in Fig. 3. Thus for phylogenetic hypotheses where *Lycopodium* and *Selaginella* were considered monophyletic, and this clade was attached to the base of either the bryophyte line or the pteridophyte line (Fig. 3 A and B), treelengths were increased to 257 and 266, respectively, i.e., an increase in 14 or 23 steps. The paraphyletic bryophyte phylogeny hypothesized by MISHLER & CHURCHILL (1984, 1985) is indicated with Fig. 3 C and D with the hornworts and liverworts being modelled as the basal dichotomies, respectively. The treelengths in these cases were raised to 248 and 250. If bryophytes are monophyletic and liverworts are modelled as the outgroup of a hornwort + moss clade, the treelength is much longer at 269 (Fig. 3 E). A cladogram was constructed with the *Charales* being derived from within the land

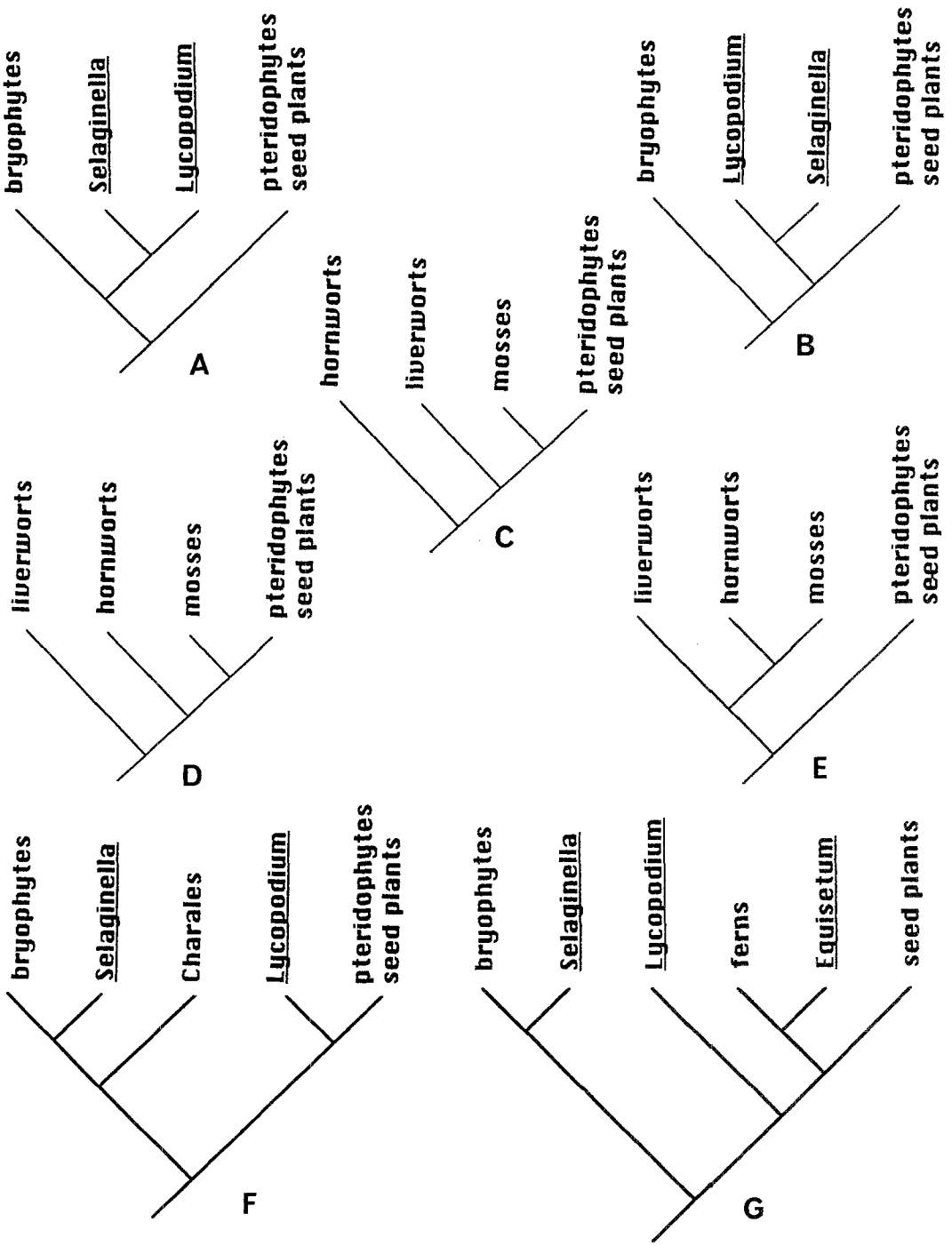

Fig. 3. Modifications of Fig. 1 constructed in MacClade and HENNIG 86 to reflect different phylogenetic hypotheses for land plants. *A* Lycopods monophyletic and on bryophyte line (257). *B* Lycopods monophyletic and on pteridophyte line (266). *C* Bryophytes paraphyletic with hornworts as initial sister group (248). *D* Bryophytes paraphyletic with liverworts as initial sister group (250). *E* Bryophytes monophyletic with liverworts as sister group (269). *F Charales* derived from land plant ancestor (247). *G* Ferns monophyletic (248)

plant assemblage and placed as the basal branch of the bryophyte lineage; this cladogram has a treelength of 248 (Fig. 3 F). A final tree incorporating a monophyletic fern assemblage (*Filicales, Osmunda, Marsilea*) has a treelength of 247 (Fig. 3 G) and places *Equisetum* as the sister group. These hypotheses (Fig. 3 A–G) are a minimum of four steps longer than our shortest cladogram.

Discussion

Sperm data and phylogeny. Our analysis has shown that information derived from spermatogenesis can be used as the basis for phylogenetic interpretations of land plants. Applications of spermiogenesis data (with or without cladistic analyses) have been carried out on various groups including seed plants (FRIEDMAN 1993), amniotes (JAMIESON & HEALY 1992), annelids (JAMIESON & ROUSE 1989), insects (JAMIESON 1987), gastropods (HEALY 1989), and fishes (MATTEI 1991). This approach to phylogeny has been termed spermiocladistics (JAMIESON 1987), and points to the significance of sperm data in phylogenetic reconstruction in general.

The origin of land plants. Over the last 20 years ultrastructural studies have provided conclusive evidence that land plants were derived from green algae in the charophycean lineage (PICKETT-HEAPS 1972, STEWART & MATTOX 1975). A primary source of information from these investigations is derived from spermatogenesis. Thus the existence of an MLS in the spermatid of the biflagellated condition and monoplastidy in sperm cell lineages all unequivocally link the charophytes with the bryophytes and lycopsids. Based on ultrastructural evidence, GRAHAM & al. (1991) were able to hypothesize a single species of *Coleochaete, C. orbicularis*, as being closer to the land plants than other species of *Coleochaete*, the *Charales* or the *Zygnematales*. Male gametogenesis has not been evaluated in *C. orbicularis*, however, the general acceptance of this conclusion provides a reasonable basis for utilizing *Coleochaete* as the outgroup in our phylogenetic analysis.

Molecular evidence relating to the origin and early diversification of land plants is not always consistent, and has not yet provided the same resolution as the corresponding ultrastructural studies. The *tuf*A gene is nuclear encoded in land plants and *Zygnematales* whereas in *Charales, Coleochaete*, and other green algae it is associated with chloroplast DNA (BALDAUF & al. 1990). In contrast, intron distribution for $tRNA^{Ala}$ and $tRNA^{Ile}$ suggests that *Charales*/*Coleochaete* are closer to land plants than *Spirogyra* (MANHART & PALMER 1990). The sequence data of HORI & al. (1985) for 5s rRNA are also problematic. Their "phylogenetic" tree does show charophytes as the sister group to land plants, however, the position of *Spirogyra* in a branch that includes *Ulva* and *Chlorella* is highly anomalous. BREMER & al. (1987) reanalyzed the rRNA sequences of HORI & al. (1985) using cladistic methods (rather than the original cluster analysis), and produced tree topologies for *Spirogyra* and *Nitella* consistent with the ultrastructural data, although other anomalies appeared in the positions of *Ulva, Chlamydomonas*, bryophytes, and pteridophytes. This problem raises serious questions about the appropriateness of the 5s rRNA sequences for determining relationships at the base of the charophycean/land plant lineage (also see DEVEREUX & al. 1990 for similar conclusion). On the other hand, our primary cladograms are virtually congruent with the phylogenetic tree of VAN DE PEER & al. (1990) based on a more complete analysis of 5s rRNA sequences. MISHLER & al. (1992) analyzed sequences of chloroplast-

encoded 16s and 23s rRNA genes. Separately the two genes provide very little resolution of taxonomic structure and the 16s rRNA gene does not even support the notion of land plants being monophyletic. The combined data from the two genes provides a better picture, however, the individual branches defining the primary divergences are generally poorly supported based on the "decay index" (MISHLER & al. 1991). Analysis of molecular data has problems of homology and data handling comparable to morphological data, and this needs to be kept in mind when evaluating the results of molecular systematic work (WILLIAMS 1992).

The discrepancies among the molecular data suggest that the analysis of morphological and ultrastructural features (e.g., male gametogenesis, spermiocladistics) has a valuable role in the development of concrete hypotheses relating to the evolution and early radiation of land plants. Our studies confirm the notion that land plants are monophyletic, and six additional steps (cf. Figs. 1, 3 G) were required to derive *Charales* from a land plant ancestor, a notion discussed by STEBBINS & HILL (1980). It is of particular interest that this tree was more highly supported than the cladograms based on a paraphyletic bryophyte assemblage.

Bryophyte phylogeny. The most parsimonious cladograms that we obtained differ from those produced by previous phylogenetic analyses in two fundamental respects: (1) bryophytes are resolved as monophyletic, and (2) mosses and liverworts are shown to be sister groups. Both of these are consistent with the 5s rRNA analyses of VAN DE PEER & al. (1990). Thus bryophytes are not considered as a paraphyletic assemblage as concluded by MISHLER & CHURCHILL (1985), SLUIMAN (1985), BREMER (1985), BREMER & al. (1987), MISHLER & al. (1992), and WATERS & al. (1992). Some of these studies are based on analyses with poor choices of outgroup or with low taxon numbers (e.g., MISHLER & al. 1992), and these factors are known to distort cladograms. MISHLER & CHURCHILL (1985) suggested that liverworts were the sister group of other bryophytes and that hornworts and mosses formed a monophyletic assemblage that included vascular plants. Our cladograms do not support this conclusion, and in trees where we model bryophytes as paraphyletic (i.e., Fig. 3 C and D), shorter trees result with hornworts as the basal group rather than liverworts. GRAHAM & WEDEMEYER (1984) and RENZAGLIA & DUCKETT (1988) note similarities between hornwort sperm and *Coleochaete* and we suggest that this is the plesiomorphic condition for land plants. Similarly, the presence of pyrenoids in hornworts is most realistically viewed as plesiomorphic (VAUGHN & al. 1990, 1992). A number of the characters supporting the moss plus vascular plant clade have been questioned (e.g., KENRICK & CRANE 1991) and additional study is required before this phylogenetic interpretation should be accepted as a "fact" of land plant evolution.

WATERS & al. (1992) made phylogenetic inferences for bryophytes and their relationships with vascular plants from sequences of nuclear-encoded rRNA. Their tree confirmed the monophyletic nature of mosses, liverworts, and hornworts, but bryophytes were paraphyletic with liverworts as the sister group to the remaining plants. Only one additional step was required to make the three bryophyte groups completely paraphyletic with the mosses as the sister group to vascular plants, a hypothesis congruent with the MISHLER & CHURCHILL (1984, 1985) phylogeny for land plants. Limitations of this work are that only three liverworts were used in the analysis, and all of these were from the putatively derived order *Marchantiales*

(SCHUSTER 1984); inclusion of more "primitive" genera from *Calobryales* or *Metzgeriales* (e.g., *Haplomitrium* and *Treubia*) might have yielded a result closer to our cladogram in Fig. 1. In addition, the "decay index" (MISHLER & al. 1991, see account by KÄLLERSJÖ & al. 1992, as "Bremer support") utilized by WATERS & al. (1992) suggests that the relaxation of parsimony by only single steps disrupted key clades on their tree. By comparison, the cladograms that we present in Figs. 1–3 are more robust, considering the number of additional steps required to model different phylogenetic proposals (i.e., Fig. 3).

Evolution of liverworts. Among the bryophytes, the hepatics are unquestionably the most diverse group (SCHUSTER 1984, RENZAGLIA 1982). Despite recent ultrastructural and molecular advances, the interrelationship between the two major liverwort lineages: complex thalloids and leafy/simple thalloids, currently remains unclear. SCHUSTER (1984) provided an extended discussion of liverwort evolution. He concluded that the primitive liverwort had an erect axis and that the *Calobryales* were the most primitive group. These conclusions were not supported by the cladistic analyses of MISHLER & CHURCHILL (1985) who concluded that the *Sphaerocarpales*, a thallose order, were the sister group to the remaining liverworts. *Jungermanniales* were resolved as among the most derived groups, being the sister group of the *Metzgeriales* (MISHLER & CHURCHILL 1985). None of our most parsimonious trees support the cladogram of MISHLER & CHURCHILL (1985) which suggested that *Sphaerocarpales* were the sister group for the remaining liverworts.

The previous lack of resolution of hepatic phylogeny is largely due to the virtual neglect in phylogenetic analyses of representatives of diverse hepatic elements. Several unique, ancient lineages (each containing only two or more taxa) are extant. These represent the remnants or the early diversification of the hepatic clade and include such families as *Monocleaceae, Blasiaceae, Treubiaceae, Haplomitriaceae,* and *Fossombroniaceae.* Analysis of spermatogenesis in representatives of three of these families has indeed provided interesting interpretations of affinities within hepatics. For example, *Blasia*, a genus that has traditionally been included in the leafy/simple thalloid line of evolution (RENZAGLIA 1982 and literature cited therein), clearly has marchantialean sperm cells (also noted in RENZAGLIA & DUCKETT 1987 a, b, 1988). Spermatozoids of *Haplomitrium* and *Treubia* share peculiarities that isolate them from the remaining liverworts as well as certain features similar to pteridophyte taxa. Interestingly, *Haplomitrium* and *Blasia* are among the three hepatic genera in which monoplastidic meiosis was recently discovered (RENZAGLIA & al., unpubl.). Monoplastidic cell division characterizes sperm development and sporogenesis in all bryophyte groups investigated to date, as well as the lycophytes, but has not been described in other land plant groups; this is clearly a symplesiomorphy between these plants and their green algal ancestors. Thus, there is a pressing need to examine sperm ontogeny in these basal lineages of archegoniates and especially the phylogenetically significant liverwort genera, including *Monoclea, Phyllothallia,* and *Fossombronia.*

In all 14 of the most parsimonious trees (e.g., Figs. 1, 2) *Haplomitrium* and *Treubia* formed a clade that was the sister group of the remaining liverworts. The consensus tree (Fig. 2) identified three clades of liverworts and there is little congruence between this phylogenetic hypothesis, the classical taxonomic interpreta-

tions of SCHUSTER (1984) and the cladistic evaluation of morphological features by MISHLER & CHURCHILL (1985).

The evolution of mosses. Although mosses were resolved as strongly monophyletic and *Sphagnum* was resolved as the sister group of the remaining mosses, *Musci* do not show extensive variation in male gametogenesis. Thus, additional features are needed to distinguish the various groups. Our cladograms strongly support the notion that *Takakia* is a moss, an association that has been the source of intense debate over the last 40 years (SCHUSTER 1984, SCHOFIELD 1985, CRANDALL-STOTLER 1986, MURRAY 1988). The recent discovery of male plants and sporophytes of *T. ceratophylla* (SMITH & al. 1990, RENZAGLIA & al. 1991) unequivocally identifies this unique plant as a member of the moss clade. Sporophytic evidence points to a close affinity between *Takakia* and the andreaopsid mosses which share eperistomate capsules that dehisce by longitudinal sutures. The single dehiscence line in the capsule of *Takakia* is also found in "primitive" archegoniates (e.g., *Lycopodium*) and certain liverworts (e.g., *Monoclea* and *Haplomitrium*) and therefore may represent a symplesiomorphy of land plants. The simple conducting cells found in both the gametophyte and sporophyte of *Takakia* (RENZAGLIA & al. 1991) are remarkably similar to those of certain thalloid liverworts as well as early fossil pteridophytes (KENRICK & CRANE 1991). Moreover, unlike liverworts and the remaining mosses, *Takakia*, with its combination of plesiomorphic characters shared with various plant groups, is particularly important in establishing the early divergence of embryophytes. Further resolution of the phylogenetic position of *Takakia* in the moss clade will require completion of the data set presented here and the analysis of additional characters. Although MISHLER & al. (1992) did not include *Takakia* in the rRNA analysis, our studies are consistent in recognizing *Sphagnum* as the outgroup of remaining mosses.

What is the sister group of the fern/seed plant lineage? Our analyses are unable to answer this question. It has been tacitly assumed that among extant plants lycopsids were the closest relatives to such an assemblage, i.e., that *Lycopsida* are "the sister-group of the remainder of the post-Silurian tracheophyte lineages" (BATEMAN 1990). In our most parsimonious cladograms this assumption was not supported and lycopsids are paraphyletic if not polyphyletic (Figs. 1, 2). The unresolved nature of this part of the cladogram where so few taxa have been included, suggests that the inclusion of additional taxa might produce a cladogram with quite different topologies. Critical genera include *Phylloglossum, Isoëtes, Psilotum, Tmesipteris*, and *Ophioglossum*. Until a data matrix is available that includes these and other genera of diverse pteridophyte lineages, it will not be possible to resolve this issue. It should also be stressed that our analysis of lycopsids is based on data from one species in each of *Lycopodium* and *Selaginella*; additional species should be studied in these genera to determine the extent of intrageneric variation. The extreme age of these lineages, i.e., from the Carboniferous (THOMAS 1992), points to the likelihood of such variability.

Recent studies of plants from the Devonian suggest that an assemblage of land plants had evolved that were neither algal nor clearly vascular in structure (e.g., *Aglaophyton* and *Catenalis*, EDWARDS 1986, SHOUGANG & BECK 1991). These organisms might represent either forms intermediate between the *Chara/Nitella* clade and the land plants included in our analysis, or primitive members of either the

bryophyte or pteridophyte clades. KENRICK & CRANE (1991) refer to this assemblage as the "protracheophyte" grade. This is further complicated by studies of REMY (1982) and REMY & HASS (1991) on pteridophytes from the Rhynie Chert which indicate that lower Devonian gametophytes produced moss or liverwort-like antheridia in clusters at the tips of upright shoots. Each antheridium contained thousands of tiny coiled spermatozoids, also reminiscent of bryophytes. It should be noted that these organisms have highly developed, branched sporophytes.

The relationships of *Equisetum*. Morphological evidence provides only fragmented clues as to the relationships of the class *Equisetopsida* (or *Sphenopsida*) and other archegoniates (e.g., SCAGEL & al. 1984, BOLD & al. 1987). Based on male gametogenesis, *Equisetum* is resolved as the sister group to the *Filicales* and *Osmunda* clade (Figs. 1, 3). The 5s rRNA sequence analysis of HORI & al. (1985) (confirmed with the cladistic analysis of this data set by BREMER & al. 1987) and VAN DE PEER & al. (1990) also resolve this relationship. It is therefore of considerable interest that our cladograms also reconstruct this relationship using only information from male gametogenesis. The congruence of these data sets suggests that the determination of sequences of 5s rRNA in additional species may therefore be extremely useful in determining phylogenetic structure among ferns and various pteridophyte groups, even though it appears less conclusive at resolving deeper phylogenetic relationships.

Taxonomic implications. Cladistic methodology is designed specifically for producing nested sets of relationships among organisms that can be interpreted as phylogenies. A second part of cladistic analysis involves the establishment of classifications that are consistent with the phylogenetic hypotheses of the cladograms. This is among the most controversial aspects of cladistic taxonomy, and it is not unusual to find cladistic methodology accepted as a method for reconstructing phylogenies, but to have the taxonomic implications rejected in favor of classifications based on other criteria (e.g., CRONQUIST 1987), usually involving evolutionary grades or levels of organization (see discussion in DONOGHUE & CANTINO 1988). Our analysis of land plant evolution has important taxonomic implications from kingdom to class level. At this time, however, we will restrict our discussion to the implications of our analysis, and we will wait until additional critical taxa have been examined before making any formal taxonomic proposals.

Subdivision to class. Previous cladistic analyses of land plant evolution suggested a phylogeny of land plants with numerous paraphyletic groups (including hornworts, liverworts, and mosses), each of which could be recognized as classes (MISHLER & al. 1985, BREMER 1985). The fact that sperm data resolve bryophytes as monophyletic, suggests that these organisms should be included in a single taxon. Four groups might be recognized among extant organisms, possibly at subdivision rank for (1) bryophytes, (2) pteridophytes plus seed plants, (3) *Selaginella*, and (4) *Lycopodium*. The lycophytes appear for the present to be polyphyletic, however, additional taxa need to be investigated before any taxonomic formalization of this phylogeny is proposed. Further taxa at the same rank would be required among fossil forms for the "protracheophyte" grade and the *Rhyniaceae* as outlined by KENRICK & CRANE (1991). A formal presentation of a taxonomic scheme for all extant land plants requires more detail in relationships of spore-bearing vascular plants, and these studies are currently underway.

The phylogenetic position that we present for *Selaginella* (Figs. 1–3) is counter to all previous interpretations of land plant evolution. It is difficult to account for the fact that the features of gametogenesis could provide on the one hand, several highly plausible evolutionary scenarios for virtually all land plants being considered, and on the other hand, provide such an apparently anomalous placement of *Selaginella*. Neither of these conclusions is satisfactory, and we must await more critical ultrastructural and molecular studies on *Selaginella, Lycopodium, Isoëtes, Phylloglossum*, and *Psilotum* (spermatogenesis in the latter three genera has not yet been studied) to resolve this issue. It is of interest that the similarities between sperm of bryophytes and *Selaginella* discovered by ROBERT (1974), and discussed by DUCKETT & CAROTHERS (1982) and DUCKETT & al. (1982), have not been emphasized in subsequent phylogenetic analyses.

Our cladistic analyses have suggested that, based on male gametogenesis, *Selaginella* is distantly related to *Lycopodium* with which it is usually given class rank in the *Lycopodiopsida* (e.g., DIMICHELE & SKOG 1992). Some authors (e.g., BOLD & al. 1987) segregated *Selaginella* and *Isoëtes* in the class *Glossopsida*, and placed *Lycopodium* in the *Aglossopsida* in the division *Microphyllophyta*. Although sperm of *Isoëtes* and *Phylloglossum* are incompletely characterized, the former has 14–20 flagella (THOMAS 1976). When *Phylloglossum* and *Isoëtes* have been adequately characterized in terms of spermatogenesis they may provide features that further link *Lycopodium* and *Selaginella*. *Lycopodium* itself is a taxonomically diverse group, and a number of generic segregates have been recognized (WAGNER & BEITEL 1992). As more taxa in the entire assemblage are investigated in terms of spermatogenesis, this could result in the delineation of a monophyletic lycophyte clade as proposed by paleobotanical evidence (e.g., KENRICK & CRANE 1991, DIMICHELE & SKOG 1992, THOMAS 1992).

What about the ferns? Widely divergent classifications have been proposed for the ferns. At one extreme is the tendency to include both the homosporous and the heterosporous ferns in a single class *Filicopsida* (e.g., SCAGEL & al. 1984). The opposite extreme is the tendency to recognize at least three or four classes (BIERHORST 1971, BOLD & al. 1987). Our analysis suggests that the *Filicopsida* (sensu SCAGEL & al. 1984) is polyphyletic, with *Equisetum* being the sister group to the *Osmunda* plus *Filicales* clade. Our work raises the possibility that if class rank is to be maintained for *Equisetum*, then *Marsilea* should also be in a class distinct from *Osmunda* and *Filicales*.

BIERHORST (1971) and BOLD & al. (1987) used a classification for vascular plants that included the classes *Filicopsida, Ophioglossopsida*, and *Marratiopsida*. So few taxa in this assemblage (especially eusporangiate forms) have been studied from the perspective of spermiocladistics that our analysis at this time helps to define problems rather than come to definitive conclusions. Of particular importance are members of the *Ophioglossaceae* that KATO (1990, 1991) considers as living progymnosperms and part of an evolutionary line leading to flowering plants. We are currently targeting eusporangiate ferns in our studies of spermatogenesis. Undoubtedly, inclusion of these taxa in our analyses will clarify evolutionary relationships among the ferns.

Correlation with other characters. MISHLER & CHURCHILL (1984, 1985) have summarized most of the morphological evidence relating to the early radiation of

land plants, and we do not wish to repeat this material. Two other anatomical/developmental features have recently been discussed as possible indicators of phylogenetic position. The first of these is monoplastidic cell division which is characteristic of bryophytes, *Selaginella, Isoëtes*, and *Lycopodium* (BROWN & LEMMON 1990 a, RENZAGLIA & al. 1993), but is unknown in other pteridophyte and seed plant groups. This feature is consistent with our primary cladogram and suggests that polyplastidic cell division became "locked in" as the only pattern throughout the entire life histories of the fern plus seed plant clade. In bryophytes monoplastidy is characteristic of all dividing cells of hornworts, however, it becomes more restricted in mosses and liverworts where it is usually associated with sperm development or meiosis (DUCKETT & RENZAGLIA 1988 a, b) (an exception is *Takakia* where it is consistently associated with mitosis). BROWN & LEMMON (1990 b) have also studied microtubular systems associated with the meiotic spindle in hornworts, and additional phylogenetic inferences may be possible using this feature. However, too few organisms have been characterized to evaluate this character at present.

PHILIPSON (1990) described two kinds of apical meristems that he considered diagnostic for particular land plant groups. Accordingly, bryophytes, ferns, *Equisetum, Psilotum, Tmesipteris, Selaginella* have one type of shoot apex without periclinal divisions of the apical cell. This is contrasted with the seed plant apex diagnostic of angiosperms, gymnosperms, *Lycopodium, Phylloglossum, Isoëtes*, and *Stylites*. There is considerable congruence between this feature and our most parsimonious cladogram. Of particular note are the separation of *Selaginella* and *Lycopodium*, and the association of *Equisetum* with the fern lineage. PHILIPSON's (1990) hypothesis and our cladogram are not fully congruent, however, and it is likely that the expression of the seed plant apex evolved several times in different clades.

Early evolution of land plants. It is conceptually appealing to visualize the phylogeny of a group of organisms as occurring as a "ladder" with the slow evolution of incrementally more advanced taxa. This is essentially the picture resulting from previous phylogenetic analyses (MISHLER & CHURCHILL 1985, BREMER & al. 1987, SLUIMAN 1985) and the summary tree presented by BREMER (1985: his fig. 2). The phylogenetic hypothesis derived from the male gametogenesis data suggests a very different picture. If the bryophyte clade did branch off from the pteridophyte plus seed plant clade very early in the origin of land plants, then the "ladder"-like aspect disappears, and one is left (using extant organisms) with a dichotomy at the base of the tree. This is more akin to the model of land plant evolution presented by TAYLOR (1988) and SELDON & EDWARDS (1990) with a basal "shrub" rather than a ladder-like arrangement, as well as the notion of evolutionary divergence propounded by GOULD (1989) in his commentary on the Burgess Shale fauna. Accordingly, once land plants evolved, there very quickly arose a number of evolutionary lines each with several different body plants. If they were to exist, the inclusion of these original land plants in our analysis would certainly yield a more complex set of relationships. However, information on sperm architecture is virtually absent from the fossil record.

Our cladogram for the evolution of bryophytes provides a perspective on this issue very different from previous studies. Far from evolving by means of the accumulation of additional levels of anatomical complexity, the primary events

seem to be a reduction series from possibly vascular plant ancestors where sporophytic complexity was lost (e.g., branching, conducting cells, stomata in liverworts). Such an argument would be strengthened if *Selaginella* (and *Isoëtes*) were indeed the sister group to the bryophyte clade. These questions can only be resolved by the acquisition and analysis of new data, especially molecular data using 16s or 18s rRNA and ultrastructural data on spermatogenesis in a wider range of organisms. Supporting evidence for our conclusions has come in the form of the structure of the chloroplast genomes among land plants. RAUBESON & JANSEN (1992) show that a gene inversion is present in the cpDNA of two bryophytes (*Marchantia* and *Physcomitrella*) and lycopsids (*Selaginella, Lycopodium*, and *Isoëtes*) and is absent in other vascular plants. This is consistent with the basal polytomy in our land plant clade (Fig. 2). A note of caution is required in interpreting such studies. Chloroplast genomes of liverworts are variable (PIKE & al. 1992), and conclusions based on a single taxon (i.e., *Marchantia*) may not be definitive.

Why is our cladogram so different from previous ones? The basic phylogeny of land plants as derived from our analyses provides a hypothesis very different from that found in previous analyses. Since our cladograms are clearly a reflection of the male gametogenesis data that we used, it becomes a critical question as to whether or not such data are more appropriate for this kind of study than the general ultrastructural features used by SLUIMAN (1985) or the mixture of morphological and cytological features used by MISHLER & CHURCHILL (1985), BREMER (1985), and BREMER & al. (1987). These authors used a wide diversity of organisms or extremely generalized features in their studies. We suggest that the homologies of characters among the different taxa were perhaps questionable, resulting in considerable convergence or homoplasy in their data sets. On the one hand, this is much less likely in our data set, where there is little doubt that the cells involved in male gametogenesis are homologous in all the organisms. On the other hand, our data set may be prone to extensive character correlation, and this may have have resulted in a misrepresentation of phylogenetic structure.

Our results suggest that there are two fundamentally different ways of making sperm as reflected in the bryophytes (+ *Selaginella*) and the pteridophyte plus seed plant clade. It is possible that these sperm architectures are a reflection of different development constraints. A danger in our analysis is the possibility that these unrecognized "constraints" are polyphyletic, and have canalized sperm morphology and ontogeny into two types that may only partly be based on cladistic pattern (also see DUCKETT & RENZAGLIA 1988 a). In addition, some of the processes involved in spermatogenesis from which our characters are derived may not be homologous (e.g., RENZAGLIA & DUCKETT 1988) and it would be useful to have biochemical data associated with dynamic events. The position of *Selaginella* at the base of the bryophyte clade might be explained in such a way. What is required at this juncture is the development and analysis of data sets equivalent to ours, using different kinds of developmental, ultrastructural, biochemical or molecular information, so that congruence among the various data sets can be evaluated. In addition, details of male gametogenesis need to be resolved in as yet unstudied genera. Spermiocladistics has proven an extremely useful tool in phylogenetic interpretation of other organisms; our analyses show that this is also the case with respect to the phylogeny of land plants.

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Appendix 1. Characters used in cladistic analysis of land plants with character state codes. See Table 2 for data matrix.

Abbreviations used in Appendix and text:
ABB (anterior basal body)
AM (anterior mitochondrion)
BB (basal body)
ER (endoplasmic reticulum)
LS (longitudinal spline)
MLS (multilayered structure)
MT (microtubules)
NA (not applicable)

Antheridium development

1. Multicellular antheridia: 0 = absent; 1 = present.
   Based on the development of the male structure in *Chara* (PICKETT-HEAPS 1968), we consider the male reproductive organs of *Chara* and *Nitella* to be true antheridia. Because of the distinct jacket cells in *Selaginella* and *Marsilea*, these microgametophytes are regarded as single reduced antheridia (BIERHORST 1971). Undoubtedly, multicellular antheridia were lost in the evolution of pollen, and their absence in the seed plants and the green algae is not comparable.
2. Apical cell in antheridial ontogeny: 0 = absent; 1 = present.
   An apical cell is present in developing antheridia of *Takakia* (RENZAGLIA, unpubl.) as it is in other mosses (SMITH 1955, SCHOFIELD 1985).
3. Division patterns in the young antheridia: 0 = four-celled pattern; 1 = two-celled pattern.
   This refers to the series of cell divisions that delimit the primary androgones in cross-sectional view of the antheridial body initial. In the two-celled pattern, two androgones originate with the peripheral segmentation of four jacket cells. In the four-celled pattern, quadrants of cells are delimited and two jacket cells then form in each quadrant; the four primary androgones are therefore surrounded by eight jacket cells.
4. Arrangement of spermatogenous cells: 0 = single; 1 = filaments; 2 = blocks; 3 = random.

Blocks of spermatogenous cells that represent the initial cell divisions in organogenesis are characteristic of bryophyte antheridia and are most prominent in the mosses. This is most certainly a function of the relatively large antheridia and the immense numbers of minute spermatozoids (tens of thousands per organ) produced by these plants. Antheridia of lycopods and horsetails each contain hundreds of angrogones, but these do not appear to be aggregated in cellular blocks. It is interesting to note that the antheridia of the oldest known land plant gametophytes (from the Rhynie Chert) produce extremely small spermatozoids that are arranged in well defined blocks (REMY & HASS 1991).

5. Endogenous antheridium: 0 = absent; 1 = present.
   Only the hornworts have truly endogenous antheridia, and the entire antheridium is derived from a subepidermal cell (RENZAGLIA 1978).
6. Antheridial stalk: 0 = absent; 1 = present.
   The large basal cells of the antheridium in the *Charales* is interpreted as a stalk.
7. Operculum cells: 0 = absent; 1 = single; 2 = paired; 3 = > 2; 4 = other (variable).
8. Sperm in pollen tube: 0 = absent; 1 = present.
9. Jacket cells with chromoplasts: 0 = absent; 1 = present.
10. Number of sperm per male structure: 0 = 1; 1 = 16–64; 2 = 100–1000; 3 = > 1000; 4 = 2.
    Bryophyte antheridia are relatively large, each producing thousands of minute spermatozoids. This condition is strikingly similar to that recently described in the earliest fossil land plant gametophytes (REMY & HASS 1991). Ferns typically produce from 16–64 sperm per antheridium, with 32 being the norm (KOTENKO 1990, HEPLER 1976, MARC & GUNNING 1986, DOONAN & al. 1986). There are up to 256 spermatozoids per microgametophyte in *Selaginella* (ROBERT 1974), while in *Equisetum* and *Lycopodium* sperm production varies considerably, but is typically in the hundreds per antheridium (DUCKETT 1973, 1975; BIERHORST 1971).

Spermatogenous cells

11. Late spermatogenous cells shape: 0 = angular; 1 = rounded; 2 = intermediate.
    The polygonal shape of spermatocytes is much more pronounced in the marchantialean hepatics, *Blasia* and the hornworts than in the remaining liverworts. In mosses, late spermatogenous cells are readily distinguished because they become spherical and widely separate from each other. *Takakia* spermatid mother cells are distinctly moss-like in this character (RENZAGLIA, unpubl.). Although rounding of cells is completed after the final mitotic division of pteridophytes, the late spermatogenous cells do not exhibit the extreme angularity typical of liverworts and hornworts (DUCKETT 1973, KOTENKO 1990).
12. Nascent spermatids: 0 = paired; 1 = not paired.
    This character refers to the pairing of the spermatids immediately following the final mitotic division (see RENZAGLIA & DUCKETT 1987a, for a description in *Blasia*).
13. Lipids in young spermatids: 0 = absent; 1 = present.
    There is an aggregation of lipid droplets in close proximity to the developing blepharoplast of mosses (RENZAGLIA & DUCKETT 1988, BERNHARD & RENZAGLIA 1991). These likely function as a food reserve. Lipid droplets are also visible in spermatids of *Selaginella* (ROBERT 1974) and have been coded as present.
14. Diagonal spindle in the final mitotic division: 0 = present; 1 = absent.
    Species of *Coleochaete* are variable in this feature: some exhibit diagonal final divisions while others do not (GRAHAM & al. 1991). GRAHAM & al. (1991) cite this as evidence that *Coleochaete* is not monophyletic. Charalean genera do not display a diagonal ultimate division but diagonal spindles are characteristic of earlier spermatogenous mitoses (PICKETT-HEAPS 1968, GRAHAM & al. 1991).

15. Replication of the centrioles: 0 = present; 1 = absent.
In land plants, the centriolar pairs form de novo in the spermatid mother cells (SMCs) and are distributed into the young spermatids; they never replicate. In the charophytes, paired centrioles occur in vegetative cells and replicate at right angles to one another prior to each mitotic cycle.
16. Time of origin of centrioles or centriole-generating organelles: 0 = always present; 1 = spermatid mother cells; 2 = spermatid mother cell progenitor; 3 = earlier (in spermatogenous tissue).
The spherical blepharoplast of the ferns included in this analysis first appears in the generation prior to the spermatid mother cell. It disappears and reforms in the spermatocyte (DOONAN & al. 1986).
17. Basal bodies and flagella: 0 = two; 1 = more than two.
18. Bicentrioles: 0 = present; 1 = absent.
The centrioles originate as bicentrioles in spermatid mother cells of archegoniates with biflagellated sperm cells. In land plants with multiflagellated gametes the procentrioles/centrioles originate around the periphery of a spherical organelle, the blepharoplast. The origin of the centrioles in *Charales* has not been observed, thus it remains uncertain as to whether or not bicentrioles occur in these algae. Replication of centrioles, on the other hand is orthogonal in other charophytes as it is in most protists and animals (PICKETT-HEAPS 1975 b).

Flagellar apparatus and cytoskeleton

19. Basal body position: 0 = at right angles (at least during early development), 1 = side-by-side; 2 = staggered/anterior/posterior; 3 = staggered/continuous; 4 = staggered laterally.
This feature isolates *Lycopodium* from the remaining archegoniates and links *Selaginella* with the liverworts and hornworts. Additional developmental data on basal body migration and repositioning will provide definitive evidence regarding the evolutionary significance of this character.
20. Proximal extensions A: 0 = absent; 1 = long; 2 = short.
Only in bryophytes are the proximal extensions well developed. If triplet extensions occur in pteridophytes, they are extremely short as in *Pteridium* (DUCKETT 1975, DUCKETT & CAROTHERS 1982) and *Onoclea* (KOTENKO 1990). Micrographic evidence in ROBERT (1974) shows short ventral triplets extending in front of the PBB of *Selaginella*.
21. Proximal extensions B: 0 = absent (criterion not applicable because extensions do not occur); 1 = ventral; 2 = ventral/dorsal.
Dorsal triplet extensions are unique to the anterior basal body of mosses and liverworts, while the posterior basal body of these taxa comprises primarily ventral extensions. According to CAROTHERS & RUSHING (1988), both basal bodies of *Haplomitrium gibbsiae* possess ventral proximal triplets. The ABB of *H. hookeri*, in contrast, is reported to consist of dorsal triplets as in other hepatics (CAROTHERS & DUCKETT 1979).
22. Stellate transition: 0 = present; 1 = absent.
Stellate patterns are universally found in the transition zone of the flagella of chlorophyll a- and b-containing eukaryotes, with the notable exception of the hornworts and *Sphagnum*.
23. Transient cartwheel extension: 0 = absent; 1 = present.
In those pteridophytes with proximal extensions of the BB triplets, the central BB hub is characterized by a transient period of elongation (DUCKETT & CAROTHERS 1982).
24. Connecting fibers between the basal bodies: 0 = present; 1 = absent; 2 = fine filaments.
This feature unifies the charophytes and hornworts (GRAHAM & WEDEMAYER 1984).

Dense fibrillar material likewise connects the two basal bodies in the young spermatids of *Selaginella* (ROBERT 1974). Fine filaments are evident between the BBs in young spermatids of *Pteridium* and *Osmunda* (MILLER & al. 1985).

25. Basal body structure: 0 = monomorphic; 1 = dimorphic.
Dimorphic basal bodies are a diagnostic feature of the moss and liverwort clades.
26. BB staggering associated with growth of MT triplets: 0 = absent; 1 = present.
This is a diagnostic feature of moss and liverwort blepharoplasts only.
27. Regression of LS: 0 = absent; 1 = partial; 2 = complete.
The regression of the LS in later stages typifies sperm differentiation in the archegoniates with anterior/posterior staggering of the basal bodies, i.e., in bryophytes and *Selaginella*. Only in *Sphagnum* is the regression complete, i.e., no remnant of the LS exists in the mature sperm. A full LS remains in the spermatozoids of pteridophytes (except *Selaginella*) and seed plants. The fate of the LS is unknown for *Nitella, Treubia*, and *Takakia*.
28. LS/AM elongation in relation to the longitudinal axis of the spermatid: 0 = parallel; 1 = perpendicular.
Elongation of the lamellar strip and associated anterior mitochondrion is parallel to the spline MTs and to the longitudinal axis of the cell in biflagellated, streamlined sperm cells. In multiflagellated gametes and the ovoid biflagellated gametes of *Lycopodium*, the AM and LS are elongated along the transverse axis at the apical end, i.e., perpendicular to the longitudinal axia and at a 45° angle to spline MTs.
29. Change in the substructure of the LS at maturity: 0 = absent; 1 = partially occluded; 2 = general loss of plate clarity; 3 = S_2 occluded; 4 = S_3 occluded; 5 = S_4 occluded (MILLER & al. 1985).
With the localization of centrin in the LS of two bryophytes and a fern (VAUGHN & al. 1993), it may now be possible to determine the functional significance of changes in substructure of the lamellar plates and thereby elucidate the evolution of variations in microanatomy of this structure.
30. Spline aperture: 0 = absent; 1 = present.
We consider the presence of a spline aperture directly subtending the ABB to be one of the major synapomorphies linking mosses and liverworts (RENZAGLIA & DUCKETT 1991).
31. Spline aperture location: 0 = left of center; 1 = right of center.
32. Position of developing MLS: 0 = adjacent to BBs; 1 = beneath BBs.
GRAHAM & REPAVICH (1989) note that in the charophytes the MLS originates and develops adjacent to the BBs, while in the embryophytes the MLS always subtends the BBs.
33. Growth of LS: 0 = no growth; 1 = anterior; 2 = posterior, 3 = anterior and posterior.
This information is summarized in RENZAGLIA & DUCKETT (1991).
34. Stratified plaque between nascent blepharoplast: 0 = absent; 1 = present.
A multilayered plaque exists between forming blepharoplasts of pteridophytes (DOONAN & al. 1986, HEPLER 1976).
35. Spline/LS orientation: 0 = 90°; 1 = 45°.
The 45° orientation is a synapomorphy in the archegoniates.
36. Left-hand taper to spline: 0 = absent; 1 = present.
This feature is found only in the complex thalloid hepatic lineage (RENZAGLIA & DUCKETT 1987 a, b).
37. Posterior notch to LS: 0 = absent; 1 = present.
The presence of features 35 and 36 are synapomorphies linking the marchantialean liverworts and the enigmatic genus *Blasia* (RENZAGLIA & DUCKETT 1986, 1987 b).
38. LS position: 0 = under all BBs; 1 = under ABB only; 2 = later some BBs.

39. Stray spline MT: 0 = absent; 1 = present; 2 = develops late.
Because of developmental differences between the stray MT in *Sphagnum* and those of other mosses, we are interpreting the occurrence of this character in *Sphagnum* and bryopsid mosses as a homoplasy.
40. Accessory band of MTs: 0 = absent; 1 = present.
To date an accessory band of MTs has been identified only in the pteridophytes and *Haplomitrium* (DUCKETT & CAROTHERS 1979). Variations in the number of MTs in the band likely reflect evolutionary relationships.
41. Increasing spline MTs behind LS: 0 = absent; 1 = present.
The outline of the LS and the shape of the spline contribute to the unique blepharoplast morphology of *Haplomitrium* (CAROTHERS & RUSHING 1988) and *Treubia* (CAROTHERS & RUSHING 1990).
42. LS wider than spline: 0 = absent to slight; 1 = extensive.
The lateral elongation of the LS beyond the spline MTs is a derived feature of the hornwort blepharoplast.
43. Spline growth: 0 = posterior; 1 = anterior/posterior.
Spline growth in liverworts and mosses is initially anterior; once the LS and ABB have elongated, the remaining increase in MT length is in a posterior direction (RENZAGLIA & DUCKETT 1987 a, 1991). In hornworts, anterior growth of the spline MTs occur in register with elongation of the AM in the final stages of sperm maturation (RENZAGLIA & DUCKETT 1991). In ferns and *Equisetum*, the spline begins as a short anterior piece and it elongates in a posterior direction only (MARC & GUNNING 1986).
44. Maturational elongation of AM: 0 = absent; 1 = posterior.
For a review of this character in bryophytes refer to RENZAGLIA & DUCKETT (1991).
45. Spline widths (in the mature cell): 0 = 40; 1 = 50–110; 2 = 150–180; 3 = approx. 200; 4 = approx. 300; > 1000.
Spline width in the developing spermatid of *Lycopodium* is considerably less than in the mature cells; the addition of spline MTs in the late stages is associated with nuclear and cell shaping, not the ontogeny of the locomotory apparatus. As developmental data are accumulated, it would seem to be informative to compare the width of the spline associated only with the MLS, in the mid-stage spermatid.
46. Spline shank: 0 = tapering uniformly; 1 = tapering right side.
47. Spline shank: 0 = wide; 1 = less than 4 tubules.
The *Sphaerocarpales* are characterized by the possession of a narrow spline shank (BROWN & al. 1983, CAROTHERS & al. 1983).
48. Fibrous sheath at maturity: 0 = absent; 1 = present.
The existence of a sheath of fibers subjacent to the plasma membrane and approximately the same length as the nucleus is a feature peculiar to *Sphagnum* (MANTON 1957, DUCKETT & al. 1984, MILLER & al. 1983).
49. Osmiophilic crest: 0 = absent; 1 = present.
A prominent osmiophilic crest, overlying the anterior rim of the spline, is seen in spermatids of many multiflagellated pteridophytes, the liverwort *Haplomitrium, Ginkgo*, and *Zamia*. Until cytochemical studies of this structure are undertaken, it is impossible to determine the homology of this feature in the groups in which it occurs. However, in the ferns and *Equisetum* it has a unique banded substructure, similar to the centrin-containing rhizoplast of some algae.
50. Anterior osmiophilic ridge: 0 = absent; 1 = present.
The osmiophilic ridge extends from the inner anterior tip of the LS inwards, between the AM and plasmalemma. Unlike the osmiophilic crest it is rapidly broken down in the egg cytoplasm after fertilization (DUCKETT & BELL 1972, BELL & DUCKETT 1976).
51. Changes in BBs at maturity: 0 = absent; 1 = dense material at extreme tip; 2 = BB cartwheel with plug of matrix; 3 = basal body triplets impregnated with matrix.

52. Matrix around BBs: 0 = homogeneous; 1 = mottled.
53. Position of the stellate pattern: 0 = extracellular or partially so; 1 = entirely intracellular.
    The long stellate transition region of the spermatozoids of *Ginkgo* and *Zamia* (NORSTOG 1976, LI & al. 1989) are entirely intracellular, only the axoneme extends from the cell. In the remaining organisms, the transition regions are shorter and are external to the cell over most or all of their lengths.
54. Flagellar scales: 0 = present; 1 = absent.
    Flagellar scales are confined to the green algae.
55. Late blepharoplast with transient core: 0 = NA; 1 = yes; 2 = no.
    This feature is unique to gymnosperms.
56. Flagellar number: 0 = 2; 1 = 40–150; 2 = > 1000.
57. Direction of flagellar emergence: 0 = toward side; 1 = toward rear; 2 = toward anterior.

Nucleus

58. Nuclear shape at maturity: 0 = ovoid; 1 = elongate.
    The only land plants with ovoid spermatozoid nuclei are *Lycopodium* and the gymnosperms.
59. Nuclear posterior shape: 0 = not expanded; 1 = expanded.
    Streamlined spermatozoids of most pteridophytes possess a nucleus that expands in diameter posteriorly. Notable exceptions are *Selaginella* (ROBERT 1974, DRACINSCHI 1932, BELAJEFF 1885, YUASA 1933) and *Marsilea*.
60. Condensed chromatin: 0 = homogeneous; 1 = heterogeneous; 2 = absent.
    Patterns of nuclear condensation are outlined in RENZAGLIA & DUCKETT (1988).
61. Median constriction: 0 = absent; 1 = present.
    Only in the hornwort spermatozoid is the elongated, condensed nucleus distinctly narrower in diameter in its mid-region than on either end (RENZAGLIA & DUCKETT 1989).
62. Overlap of AM and nucleus: 0 = absent; 1 = present.
    Of the streamlined spermatozoids, only those of the charophytes, hornworts and *Selaginella* exhibit an elongated AM that abuts the nucleus but does not overlap it.
63. Spline attached to nucleus: 0 = attached at maturity; 1 = detached at maturity; 2 = never attached.
    A unique feature of jungermanniopsid and metzgerialian liverworts is the detachment and tangential displacement of the spline from the nucleus in the final stages of spermiogenesis (DUCKETT & al. 1983). The attached character state for *Coleochaete* was described by GRAHAM & WEDEMAYER (1984).
64. Spline growth and nuclear shaping: 0 = absent; 1 = present.
    Growth of the spline is synchronized with the elongation and shaping of the nucleus in mosses (RENZAGLIA & DUCKETT 1988).
65. Direction of nuclear compaction: 0 = outer shell; 1 = anterior to posterior; 2 = at equal rates along nucleus; 3 = general increase in density.
    Nuclear compaction for bryophytes and pteridophytes is outlined in RENZAGLIA & DUCKETT (1988) and DUCKETT (1975).
66. Condensed chromatin strands: 0 = spaghetti-like; 1 = perpendicular to spline; 2 = spiral/central strand; 3 = general compaction; 4 = spikes; 5 = irregular plates; 6 = solid mass from anterior tip.
    For a review of this character refer to RENZAGLIA & DUCKETT (1988).
67. Diverticulum during shaping: 0 = absent; 1 = present.
    A diverticulum is a diagnostic feature of nuclear metamorphosis during spermatogenesis in liverworts (RENZAGLIA & DUCKETT 1987 a).

68. Excess nuclear envelope loss: 0 = absent; 1 = after condensation; 2 = gradual posteriorly; 3 = gradual throughout.
69. Number of gyres of nucleus: 0 = not coiled; 1 = 0.5–3; 2 = greater than 3.
Coiling of the nucleus over three gyres is found in the highly coiled sperm cells of some ferns (DUCKETT 1975, MYLES & HEPLER 1977, KOTENKO 1990), *Osmunda* being the notable exception (MILLER & al. 1985).

Mitochondria

70. Dense body in anterior mitochondrion: 0 = absent; 1 = present; 2 = NA.
A distinct electron opaque body is found in the AM of hornworts (RENZAGLIA & DUCKETT 1989).
71. Mitochondria associated with plastids in spermatogenous tissue: 0 = absent; 1 = present.
Mitochondria are physically associated with the plastids during antheridial development only in mosses (DUCKETT 1975, RENZAGLIA & DUCKETT 1988, BERNHARD & RENZAGLIA 1991).
72. Mitochondria with plastids in young spermatids: 0 = absent; 1 = present.
In bryophytes (RENZAGLIA & DUCKETT 1988) and *Selaginella* (ROBERT 1974), the numerous small mitochondria in the nascent spermatid aggregate around the plastid. After fusion of these organelles, the resulting large mitochondrion (two in liverworts and *Selaginella*) retains its association with the plastid.
73. Mitochondria with plastids in mature sperm: 0 = absent; 1 = present.
Nitella possess mitochondria only in the mid-region of the spermatozoid and not in association with the plastids (TURNER 1968). *Chara* spermatozoids, in contrast, contain mitochondria in the mid-region as well as in association with the posterior plastids (PICKETT-HEAPS 1968). Bryophytes and lycopods have either one or numerous mitochondria in association with the single plastid. The remaining organisms lack this organellar association.
74. Specialized anterior mitochondrion: 0 = present; 1 = absent.
Of the charophycean algae, only *Coleochaete* has been shown to possess an anterior mitochondrion associated with the LS (GRAHAM & REPAVICH 1989).
75. Additional mitochondria in anterior of cell: 0 = absent; 1 = row of mitochondria behind AM; 2 = numerous unspecialized.
76. Origin of AM: 0 = fusion; 1 = elongation.
77. Osmiophilic material with AM: 0 = absent; 1 = present.
This is a characteristic feature of hornwort spermatozoids.
78. Number of mitochondria in sperm: 0 = many; 1 = two.
79. Cristae sacs to baffles: 0 = absent; 1 = present.
Changes in mitochondrial substructure are evident during spermatogenesis in bryophytes and *Selaginella*.

Plastids

80. Monoplastidic sperm: 0 = present; 1 = absent.
Numerous plastids are aggregated at the posterior end of the cell in *Nitella* (TURNER 1968) and *Chara* (PICKETT-HEAPS 1968). *Coleochaete* is monoplastidic as are all bryophytes. There are multiple plastids in the young sperms of *Lycopodium* and only one in the mature cell. *Selaginella* possesses a single plastid throughout spermiogenesis. The remaining organisms produce polyplastidic spermatozoids.
81. Plastid determines division polarity: 0 = present (at poles); 1 = present (asymmetrical); 2 = absent.
The two plastids cap the poles during mitotic divisions in the antheridia and spermatid

generating cell of *Coleochaete* and mosses. Although the liverworts and hornworts are also monoplastidic, the plastids are not positioned directly at the poles, rather they occupy a lateral location, adjacent to the spindle focal points (RENZAGLIA & DUCKETT 1987a, 1988).

82. Plastid/nuclear association: 0 = absent; 1 = present.
This refers to an association between the nucleus and plastid at mid-stage spermatids, a characteristic peculiar to bryophyte spermatids.
83. Starch grains in single plastid: 0 = more than one; 1 = one.
Only *Sphagnum, Haplomitrium,* and hornworts possess single, large starch grains in their solitary plastids.
84. Sperm plastid contacting nucleus: 0 = absent; 1 = present; 2 = present via chloroplast extension.
In the final stages of sperm maturation, the chloroplast in some liverworts forms an extension over the mitochondrion and cytoplasmic remnant and establishes contact with the nucleus (RENZAGLIA & DUCKETT 1987a).
85. Location of plastids: 0 = posterior; 1 = central; 2 = posterior but not attached to spline.
The plastid is located in the posterior or the cell in *Chara, Nitella,* the hornworts, *Selaginella, Lycopodium* and the liverworts. In mosses the plastid is in a posterior location, up against the inner surface of the nucleus and not near the spline.
86. Fibrillenscheide: 0 = absent; 1 = present.
This is a feature unique to liverworts.

Endomembrane system

87. ER/Plastid association: 0 = present; 1 = absent.
A distinct feature of young to mid-stage spermatids of some pteridophyte and all mosses examined to date is the occurrence of sheets of RER around the plastid at the time of starch accumulation.
88. Sheets of ER: 0 = absent; 1 = present.
This is a definitive feature of some moss and some pteridophyte spermatids.

Sperm maturation

89. Cytoplasmic loss: 0 = absent; 1 = partial; 2 = complete or with tiny remnant.
90. Sperm symmetry: 0 = longitudinal symmetry; 1 = asymmetrical.

Addresses of the authors: DAVID J. GARBARY, Department of Biology, St. Francis Xavier University, Antigonish, Nova Scotia, B2G 1C0, Canada. – KAREN S. RENZAGLIA (reprint requests), Department of Biological Sciences, East Tennessee State University, Johnson City, Tennessee 37614, U.S.A. – JEFFREY G. DUCKETT, School of Biological Sciences, Queen Mary and Westfield College, Mile End Road, London E1 4NS, U.K.

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