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# Vegetative and reproductive innovations of early land plants: implications for a unified phylogeny

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As the oldest extant lineages of land plants, bryophytes provide a living laboratory in which to evaluate morphological adaptations associated with early land existence. In this paper we examine reproductive and structural innovations in the gametophyte and sporophyte generations of hornworts, liverworts, mosses and basal pteridophytes. Reproductive features relating to spermatogenesis and the architecture of motile male gametes are overviewed and evaluated from an evolutionary perspective. Phylogenetic analyses of a data set derived from spermatogenesis and one derived from comprehensive morphogenetic data are compared with a molecular analysis of nuclear and mitochondrial small subunit rDNA sequences.

Although relatively small because of a reliance on water for sexual reproduction, gametophytes of bryophytes are the most elaborate of those produced by any land plant. Phenotypic variability in gametophytic habit ranges from leafy to thalloid forms with the greatest diversity exhibited by hepatics. Appendages, including leaves, slime papillae and hairs, predominate in liverworts and mosses, while hornwort gametophytes are strictly thalloid with no organized external structures. Internalization of reproductive and vegetative structures within mucilage-filled spaces is an adaptive strategy exhibited by hornworts. The formative stages of gametangial development are similar in the three bryophyte groups, with the exception that in mosses apical growth is intercalated into early organogenesis, a feature echoed in moss sporophyte ontogeny.

A monosporangiate, unbranched sporophyte typifies bryophytes, but developmental and structural innovations suggest the three bryophyte groups diverged prior to elaboration of this generation. Sporophyte morphogenesis in hornworts involves non-synchronized sporogenesis and the continued elongation of the single sporangium, features unique among archegoniates. In hepatics, elongation of the sporophyte seta and archegoniophore is rapid and requires instantaneous wall expandability and hydrostatic support. Unicellular, spiralled elaters and capsule dehiscence through the formation of four regular valves are autapomorphies of liverworts. Sporophytic sophistications in the moss clade include conducting tissue, stomata, an assimilative layer and an elaborate peristome for extended spore dispersal. Characters such as stomata and conducting cells that are shared among sporophytes of mosses, hornworts and pteridophytes are interpreted as parallelisms and not homologies.

Our phylogenetic analysis of three different data sets is the most comprehensive to date and points to a single phylogenetic solution for the evolution of basal embryophytes. Hornworts are supported as the earliest divergent embryophyte clade with a moss/liverwort clade sister to tracheophytes. Among pteridophytes, lycophytes are monophyletic and an assemblage containing ferns, *Equisetum* and psilophytes is sister to seed plants. Congruence between morphological and molecular hypotheses indicates that these data sets are tracking the same phylogenetic signal and reinforces our phylogenetic conclusions. It appears that total evidence approaches are valuable in resolving ancient radiations such as those characterizing the evolution of early embryophytes. More information on land plant phylogeny can be found at: http://www.science.siu.edu/landplants/index.html.

**Keywords:** bryophytes; embryophytes; gametophyte; land plant phylogeny; morphogenesis; spermatogenesis

## 1. INTRODUCTION

The early evolution of land plants was characterized by high rates of morphological and reproductive innovations that were fuelled by stochastic genetic changes and culled by natural selection (Niklas 1997; Bateman *et al*. 1998). Poised at the extremity of an uninhabited landscape, transitional streptophytes (green plants) experienced a burst of diversification that resulted in radiation into and exploitation of a variety of new terrestrial sites. Repeated

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and simultaneous patterns of extensive morphological diversification followed by widespread decimation presumably characterized these early stages of land colonization (Gould 1989; Kenrick & Crane 1997*a*). Of the magnitude of morphological experiments that were attempted, only limited fragments have persisted through the millennia, including the three lineages of bryophytes (Stewart & Rothwell 1993; Taylor & Taylor 1993).

Given the time since divergence, the depauperate fossil record and the vastness of the newly inhabited landscape, it is not surprising that the early phylogenetic history of land plants remains one of the major unresolved problems in evolutionary biology. Axiomatic to current evolutionary thought is the concept that embryophytes are monophyletic, that bryophytes represent the basal clades and that Charophyceae, especially Coleochaetales and Charales, are the closest living algal relatives of archegoniates (McCourt 1995). Beyond these accepted maxims there is no consensus as to interrelationships among bryophytes and basal pteridophytes. Indeed, virtually every conceivable hypothesis regarding bryophyte phylogeny has been proposed: from paraphyletic in a variety of branching orders, to hornworts basal and mosses and liverworts monophyletic, to a completely monophyletic bryophyte assemblage (Bopp & Capesius 1996; Capesius & Bopp 1997; Bremer *et al*. 1987; Garbary *et al*. 1993; Garbary & Renzaglia 1998; Hedderson *et al*. 1998; Lewis *et al*. 1997; Kenrick & Crane 1997*a*; Malek *et al*. 1996; Mishler & Churchill 1984, 1985; Mishler *et al*. 1994; Duff & Nickrent 1999; Nishiyama & Kato 1999). Clearly, new data or perhaps new insights on analysing existing data are required to resolve this phylogenetic dilemma.

By nature of their antiquity, liverworts, hornworts, mosses and basal pteridophytes represent relics of a once more diverse flora, and, as the oldest living remnants of early land colonization, these organisms provide a living laboratory in which to examine early morphological adaptations to existence on land. In this paper, we explore morphological and developmental features of bryophytes and basal pteridophytes at the cell, tissue, organ and whole organism levels. Whenever possible, evidence is presented from more informative basal representatives of the major clades. This comparative approach is designed to identify successful phenotypic innovations of both the gametophyte and sporophyte, which enabled these plants to optimize vegetative and reproductive activities in a terrestrial setting. In addition to illuminating phylogenetic affinities, contemplation of morphological and morphogenetic strategies provides valuable insight into the direction and sequence of character transformation.

This review is not intended to be a comprehensive overview of morphology in bryophytes but rather the goal is to explore answers to the following questions related to evolutionary adaptations exhibited by early plant life on land. What are the vegetative and reproductive innovations that enabled the three bryophyte lineages to persist? How do these organisms cope with the constraints of terrestrial existence, especially desiccation and gravity? And finally, what can be gleaned from careful scrutiny of extant basal embryophytes about morphological character evolution, including divergences, convergences, parallelisms and homologies? These questions provide the context for comprehensive phylogenetic analyses in which we review and update two previously published morphological data sets (Garbary *et al*. 1993; Garbary & Renzaglia 1998) and a molecular data set that combines sequences of both nuclear and mitochondrial small subunit (SSU) rDNA.

## 2. CELL STRUCTURE

Among embryophytes, there is remarkable consistency in the ultrastructure of parenchyma cells. The 'typical' living photosynthetic cell contains a large central vacuole, a peripheral nucleus, mitochondria, endoplasmic reticulum and lenticular chloroplasts equipped with grana and scattered starch (Gunning & Steer 1996). The bryophytes and lycophytes exhibit the greatest diversity in organellar complement and fine structure, and these cellular features provide important clues as to evolutionary relationships (Duckett & Renzaglia 1988; Brown & Lemmon 1993). Solitary plastids (monoplastidy) are found in most vegetative cells of hornworts and characterize mitotic cells of representative taxa and/or tissues (especially spermatogenous tissue) of all bryophytes and lycophytes (Brown & Lemmon 1990*a*, 1993). Likewise, monoplastidic meiosis (discussed below) is distributed among representatives of all these clades. Based on the widespread occurrence of monoplastidy in charophycean algae and the exclusive distribution of this condition among basal embryophytes, it is safe to conclude that monoplastidy is plesiomorphic (Brown & Lemmon 1990*a*; Graham 1993). To ensure distribution of a plastid into daughter cells, the division cycle of plastids must be tightly linked to nuclear division in monoplastidic cell lineages. Plastids are intimately associated with microtubule-organizing centres (MTOCs) and the two form the focal points for production of spindle microtubules.

In liverworts, only spermatogenous cells are monoplastidic and vegetative cells are polyplastidic. During the mitotic cycle, well-defined, electron-dense aggregations, the so-called polar organizers, organize the spindles. These structures also characterize the monoplastidic dividing cells in spermatogenous tissue of liverworts. Endoplasmic reticulum within the polar organizer is connected to the nuclear envelope and in this regard resembles the nuclear envelope-based centrosome of mosses and tracheophytes (Vaughn & Harper 1998). Late spermatogenous cells of all archegoniates have centriolar centrosomes and this enables direct comparison with similar algal MTOCs (see below).

Examination of the internal structure of hornwort plastids provides evidence of further retention of algal features. Pyrenoids are found in most anthocerote taxa and, as in algae, these electron-dense intrachloroplast bodies are the site of localization of RUBISCO (Vaughn *et al*. 1990). Grana of anthocerotes lack end membranes typical of other embryophytes and, as in *Coleochaete*, thylakoids traverse and dissect the pyrenoid into subunits (Vaughn *et al*. 1992). Channel thylakoids, found in algae but not other land plants, further point to these organelles as primitive in structure. Physiological specializations apparently are associated with this chloroplast microanatomy, especially mechanisms concentrating $CO_2$, and these may have been beneficial in a land habitat (Smith & Griffiths 1986*a*,*b*).

Although chloroplast structure in hepatics is similar to that of mosses and tracheophytes, liverworts possess a unique and diagnostic single membrane-bound oil body. Oil bodies are not found in all liverworts but they are widespread in basal taxa, suggesting loss in more derived lineages. Originating in meristematic cells from endoplasmic reticulum (Duckett & Ligrone 1995), the oil body has no counterpart in any other plant group. The function of these organelles is problematic as they apparently are not used as a food reserve.

## 3. VEGETATIVE GAMETOPHYTES

The phrase 'gametophyte dominant' distinguishes bryophytes from all other embryophytes. With the sexual phase as the prominent persistent generation, modifications of the bryophyte gametophyte entailed adaptive walks that optimized vegetative elaboration while simultaneously facilitating water-dependent sexual reproduction. In this regard, bryophytes and pteridophytes are amphibious, i.e. they require uninterrupted access to water for reproductive success and continue with vegetative activities during times of water deprivation. Bryophytes differ from pteridophytes in that it is the gametophyte alone that contends with these often-conflicting roles of sexual reproductive and vegetative persistence and dispersal. In pteridophytes, the sporophyte is the phase responsible for production of biomass and colonization of new sites. It is not surprising therefore that from perusal of structural complexity in gametophytes among land plants, two features are evident: (i) this generation never attains great stature and (ii) bryophyte gametophytes are the most elaborate of the land plants. Thus, bryophytes provide an opportunity to evaluate the structural strategies that enabled robust gametophytes to endure in an environment that presented extreme selective pressures for a motility-based fertilization system. We acknowledge that physiological mechanisms for contending with life in the air, including elaborate biochemical pathways for desiccation tolerance and thermotolerance in many mosses and pteridophytes, played a significant role in plant evolution (Oliver & Wood 1997; Kenrick & Crane 1997*a*; Hanson *et al*. 1999). However, it is not within the scope of this review to discuss these physiological strategies.

Two fundamental growth forms are exhibited by bryophytes: a flattened prostrate thallus (hornworts and complex thalloid and simple thalloid hepatics) and an erect or creeping cylindrical leafy shoot (leafy liverworts, selected simple thalloid liverworts and mosses). These growth habits correspond to the optimal oblate spheroid and cylindrical life forms hypothesized for small multicellular terrestrial organisms in morphospace (Niklas 1997). Compared with spherical growth forms that are found in representative green algae, dorsiventrally compressed thalli reduce total surface area while providing maximum surface area to volume for gas exchange and light harvesting (Niklas 1997). Leafy forms segregate vegetative functions into specialized organs; flattened leaves maximize light capture and enhance photosynthetic capacity while the central cylindrical stem enhances water conservation and enables exploratory growth, including upright growth in some taxa. Thalloid genera rarely grow upright; interestingly, *Hymenophyton* and *Symphyogyna*, two notable exceptions in the simple thalloid hepatics, both possess well-developed internal water-conducting systems (Ligrone *et al*., this issue). The erect habit facilitates exploitation of air for exchange of gases and light trapping. It also provides a means for elevating the attached sporophyte and thus promoting spore dispersal. At issue for both the thalloid and leafy growth forms is water conservation and protection of vulnerable growing tissues and reproductive organs.

A single apical cell is responsible for growth of bryophyte gametophytes. Apical cell geometry may be one of four fundamental shapes: wedge-shaped (cuneate), lens-shaped (lenticular), tetrahedral (pyramidal) or hemidiscoid (Crandall-Stotler 1986; Renzaglia 1982). Pyramidal systems are typically associated with leafy habits, lenticular systems with highly flattened thalli, often with thickened midrib and monostromatic wings (e.g. *Metzgeria* and *Pallavicinia*), and wedge-shaped and hemidiscoid apical cells are responsible for thalloid growth forms, including those of complex thalloid liverworts and hornworts. In mosses, pyramidal systems predominate with the rare secondary derivation of a lenticular system (Crandall-Stotler 1984). Similarly, the wedge-shaped apical initial is widespread in hornworts with a developmental and evolutionary transformation to hemidiscoid-based generative cells in *Dendroceros* (Renzaglia 1978). Hepatics are the most diverse in that all four apical cell shapes occur in various taxa and developmental modifications of cell geometry are commonplace. Documentation exists for ontogenetic transformation of cell shape from tetrahedral to lens-shaped, from lens-shaped to tetrahedral or wedge-shaped, and from wedge-shaped to hemidiscoid (Fulford 1956; Renzaglia 1982; Renzaglia & Bartholomew 1985). Often these geometric transitions are associated with a change in gross morphology and/or orientation of growth. For example, the transition from juvenile to mature habit is accompanied by a transformation from lenticular to tetrahedral apical cell geometry in certain leafy hepatics (Fulford 1956). In *Fossombronia*, a tetrahedral apical cell forms immediately in the upright sporeling and limited segmentation produces three rows of leaves (Renzaglia & Bartholomew 1985). Further oblique divisions convert the tetrahedral apical cell to a lenticular cell and this change results in the mature thallus habit with two lateral leaf rows. Concomitant with this metamorphosis is a switch in orientation of growth from vertical to horizontal. The discussion above serves to illustrate that: (i) conversion of apical cell from one geometric shape to another is a cornerstone of normal developmental processes in bryophytes and is especially widespread in liverworts; (ii) changes in apical cell shape are accompanied by changes in both growth form and thallus/shoot orientation; and (iii) because of the 'ease' with which apical cell shape is modified and the profound influence this modification has on habit, this process alone may well have provided significant morphological variation for major evolutionary change.

Crandall-Stotler (1980, 1984, 1986) clearly documented a fundamental difference between most mosses and leafy liverworts (Jungermanniales) in segmentation of the pyramidal apical cell and subsequent leaf development. Spiralled, undivided leaves in mosses versus three rows of

bifid leaves are the rudiments of these two distinct ontogenetic patterns. As concluded by Crandall-Stotler (1980), these underlying developmental discrepancies support the concept of independent origin of leaves in mosses and liverworts. We further suggest that additional differences in developmental design provide evidence that leaves or leaf-like structures ('leaves') have evolved at least twice in mosses and perhaps multiple times in liverworts. Production of the dissected leaves (phyllids) of *Takakia* is decidedly different from leaf development in *Sphagnum* and bryopsid mosses (Crandall-Stotler 1986), suggesting that leaf evolution in *Takakia* was independent of that in other mosses. Because of differences in apical organization and the phyletic distance between taxa such as *Haplomitrium*, *Treubia*, *Fossombronia*, *Noteroclada*, *Sphaerocarpos* and *Blasia*, it is possible that 'leaf' acquisition is homoplastic in all of these taxa.

Leaves not only function as photosynthetic organs but they also provide protection for the fragile growing tips. Leaf primordia overarch and surround the apical cell and immediate derivatives. Controlled production of stalked mucilage-secreting hairs (slime papillae) accompanies leaf development in mosses and liverworts and further affords protection against damage and desiccation. In the absence of leaves, abundant uniseriate to branched slime papillae surround and protect the meristematic region of simple thalloid liverworts (Renzaglia 1982; Duckett *et al*. 1990). In addition to slime papillae, flattened multicellular scales with marginal papillae provide an alternative but equally effective protective structure in *Blasia*, *Cavicularia* and complex thalloid liverworts.

In contrast to mosses, liverwort evolution has involved selection towards dorsiventrality. Schuster (1979, 1984*a*,*b*) suggested that the ancestral liverwort gametophyte was a branched, leafless, upright axis, much like that of most Devonian gametophytes, but lacking conducting tissue and stomata (Remy *et al*. 1993). In extant hepatics, erect, radially symmetrical shoots with three equal rows of leaves are considered plesiomorphic, while procumbent thalloid or leafy forms with reduced to absent underleaves are viewed as specialized. Appression to the substrate maximizes contact with water sources and thus minimizes potential damage associated with desiccation. In leafy liverworts, prostrate habits have evolved as an adaptation to horizontal and vertical substrates. In general, incubous leaf insertions are associated with vertical substrates (e.g. tree trunks) while succubous phenotypes predominate on horizontal substrates.

While erect radial habits are exhibited by relatively few basal taxa of hepatics, isophyllous acrocarps comprise approximately half of all mosses (Crum & Anderson 1981). Evolutionary adaptations that have enabled mosses to grow upright and persist in relatively dry environments include the acquisition of more extensive and specialized conducting tissues (Hébant 1977; Ligrone *et al*., this issue), the thickening of cell walls and accumulation of polyphenolic compounds in these walls, and the exploitation of metabolic pathways that confer desiccation and thermal tolerance. Moreover, growth of multiple individuals in dense mats and tufts effectively facilitates absorption and retention of water in many mosses. Abundant uniseriate, branched to scale-like paraphyllia and pseudoparaphyllia are found on branches of some mosses and further serve to protect against desiccation and damage (Crum & Anderson 1981).

In mosses, branched filamentous protonemata develop upon spore germination, and unlike sporelings of liverworts and hornworts, these juvenile stages have the ability to produce and disperse multiple leafy shoots derived from a single spore. This latter feature is particularly adaptive in acrocarps that do not produce creeping shoots and rarely branch.

The exclusively thalloid hornworts do not produce leaves, slime papillae, scales or any organized external appendage. Indeed, these plants perform virtually all vegetative and reproductive functions in undifferentiated parenchyma cells or within internal thallus chambers (Renzaglia 1978; Renzaglia & Vaughn 2000). Perhaps one of the fundamental features that has been instrumental in the survival of anthocerotes is the ability to produce copious mucopolysaccharides (mucilage) in virtually any cell, apparently in response to environmental cues. Mucilages are carbohydrates that are concerned with imbibition and retention of water. Although mosses and liverworts produce mucilage, this substance, with rare exceptions (e.g. the ventral surface of the thallus of *Treubia* and the underground axes of Calobryales), is produced only in slime papillae. In hornworts, protection of the growing notch is afforded by mucilage secretion from epidermal cells of young apical derivatives. Scattered internal cells contain mucilage in most taxa and large mucilage-filled chambers are common in *Anthoceros* and selected species of other genera. During development, antheridial chambers contain mucilage and mucilage surrounds the exterior of archegonial necks. Capitalization on mucilage production and the ability to sequester this hydrophilic substance in internal chambers are integral processes that bestowed partial tolerance to drying conditions in these small thalloid taxa.

A further evolutionary innovation that almost certainly contributed to the vegetative and reproductive success of hornworts (as measured by length of time in existence) was the ability to form schizogenous spaces. Essential to this process is the separation of adjacent cells at the middle lamella followed by controlled division and expansion of cells around a chamber or canal. In addition to exclusively mucilage-containing cavities, hornworts sequester antheridia and *Nostoc* colonies in such chambers.

This motif of internalization coupled with cell separation and mucilage proliferation is embodied in the development of *Nostoc* colonies in anthocerotes (Renzaglia 1978). This symbiotic relationship between the hornwort and a nitrogen-fixing bacterium has enabled these plants to flourish under conditions of low nitrogen. The development of *Nostoc* colonies is ubiquitous in anthocerotes and begins near the apical cell with the apparently random separation between two adjacent epidermal cells in the ventral thallus. The resulting mucilage cleft provides a port through which soil-dwelling cyanobacteria may enter the thallus. With maturation of the thallus, a schizogenous mucilage-filled chamber forms internal to the cleft and expands with multiplication of cells of the *Nostoc* endosymbiont. Thallus cells elongate and grow among the cyanobacterium. The mature colony may be rather extensive and often forms a mound that projects from the ventral thallus.

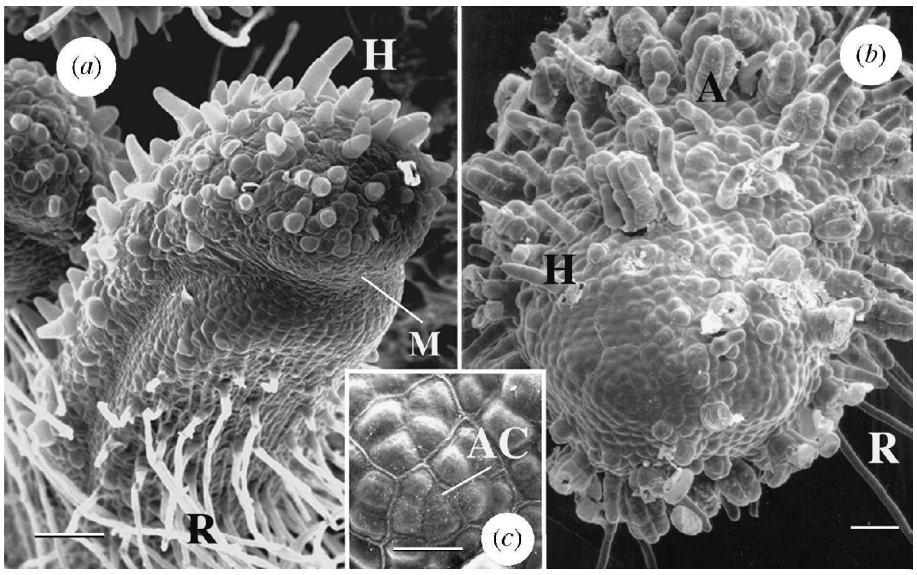


Figure 1. Scanning electron micrographs of cultured pteridophyte gametophytes provided by Dean P. Whittier. (*a*) Young gametophyte of *Huperzia lucidula*. Meristematic zone (M) in a groove that continues around laterally. Rhizoids (R) originate from ventral derivatives, while uniseriate hairs and gametangia (not produced yet) are formed on a dorsal crown. Bar = 0.1 mm. Micrograph by Angel R. Maden. (*b*,*c*) *Tmesipteris lanceolata*. (*b*) Cylindrical gametophyte with abundant uniseriate hairs (H), archegonia (A) and rhizoids (R). The growing tip appears to be unprotected. Bar = 0.1 mm. (*c*) Enlarged view of the growing tip showing triangular pyramidal apical cell (AC) surrounded by packets of cells. The prominent walls that outline cell packets (merophytes) represent original segmentation from the apical cell. Bar = 0.05 mm.

In hepatics, *Nostoc* colonies are restricted to two sister genera, *Blasia* and *Cavicularia* (Renzaglia 1982). A cursory comparison of *Nostoc* colony production in *Blasia* with that in anthocerotes exemplifies highly divergent developmental strategies for attaining functionally similar structures in the two bryophyte groups. In *Blasia*, *Nostoc* is housed in external 'organs' on the ventral thallus. This so-called auricle is produced in a controlled fashion from apical derivatives and originates as a mucilage hair, which undergoes extensive elaboration. *Nostoc* enters the auricle when it is a small, dome-shaped structure, and concomitant growth of *Nostoc* and thallus intermixes cells of the plant and prokaryote. Continued auricle expansion results in a massive external structure that superficially resembles the *Nostoc* colony of hornworts.

Having considered apical growth, protective structures and habit variability in the three bryophyte groups, comparisons with basal pteridophytes are in order. In general, gametophytes of pteridophytes are not as complicated or long-lived as those of bryophytes (Bierhorst 1971; Gifford & Foster 1988; Bell 1994; Whittier 1977, 1981, 1983; Whittier & Webster 1986; Whittier & Thomas 1993). Although variability exists among the genera, two fundamental growth forms predominate in basal tracheophytes: (i) green epiterrestrial forms with irregular upright lamellae and (ii) thick, fleshy subterranean forms with a fungal symbiont. The latter type, exemplified by *Huperzia* (figure 1*a*), *Lycopodium*, *Diphasiastrum*, *Phegmariurus*, Psilophytes (figure 1*b*), *Botrychium* and *Ophioglossum*, is generally considered ancestral. These gametophytes may persist for several years and they have the capacity to produce multiple sporophytes and form abundant regenerants. The gametophytes of *Phylloglossum* (Whittier & Braggins 1992) and *Huperzia* (A. R. Maden and D. P. Whittier, unpublished data) are non-photosynthetic when young and convert to green forms when exposed to light. Epiterrestrial gametophytes of *Palhinhaea*, *Lycopodiella*, *Pseudolycopodiella* and *Equisetum* typically survive for a single growing season but in some instances may bear several sporophytes (Bierhorst 1971; Duckett & Duckett 1980). Apical growth is accomplished by a meristematic zone in *Equisetum* (Duckett 1970) and in lycophytes (figure 1*a*) but involves a well-defined tetrahedral apical cell in *Psilotum* and *Tmesipteris* (figure 1*b*,*c*). Protection of the growing region and gametangia is accomplished by production of glandular and non-glandular hairs, rhizoids and limited mucilage production (Whittier & Peterson 1984). Often apices of subterranean gametophytes are not surrounded by protective structures (figure 1*b*,*c*). Although no leaves are produced by any tracheophyte gametophyte, the capacity to form abundant hairs and papillae is reminiscent of mosses and liverworts. However, conducting tissue, which is commonplace in mosses and more restricted in occurrence in liverworts, is a developmental anomaly in gametophytes of extant pteridophytes (Bierhorst 1971).

Fungal associations are widespread in gametophytes of pteridophytes, liverworts and hornworts, but are lacking in mosses (Duckett *et al*. 1991). Fungal hyphae facilitate absorption and translocation of minerals, water and organic molecules within the plant. In nature, heterotrophic subterranean gametophytes of pteridophytes rely exclusively on endophytic fungi for nutrient uptake. Fungal–plant symbioses were probably established in the primitive archegoniates and have developed repeatedly during the evolution of land plants (Pirozynski & Malloch 1975; Pirozynski 1981). For a discussion of symbiotic interactions among fungi and bryophytes see Read *et al*. (this issue).

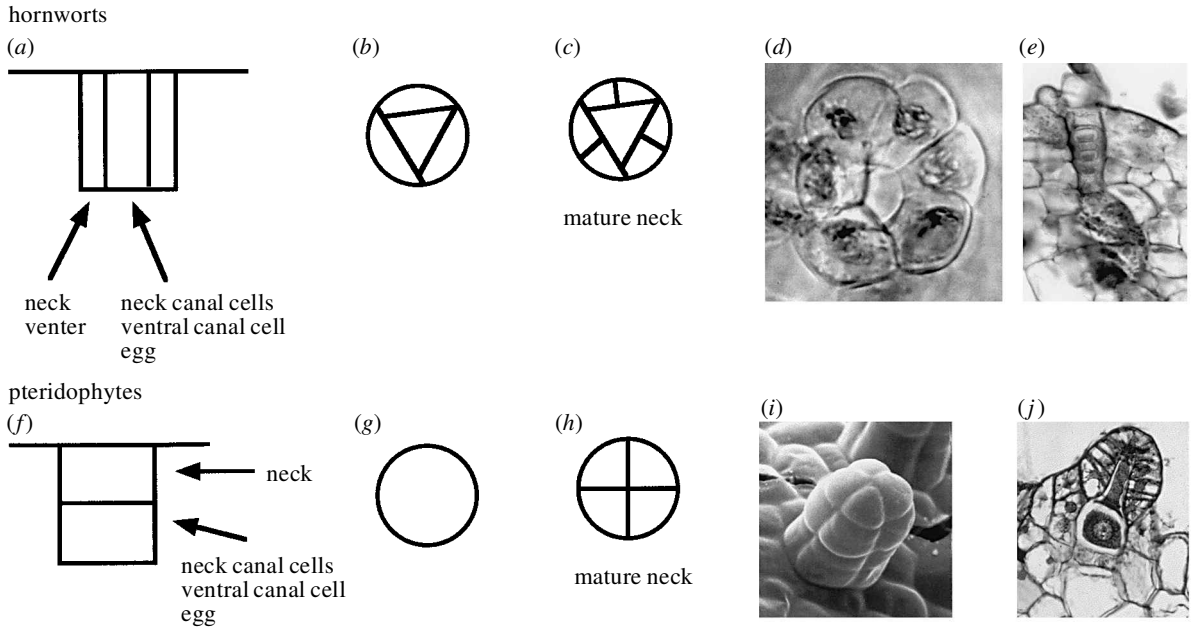


Figure 2. Comparison of archegonial development in hornworts (*a–e*) and pteridophytes (*f–j*). (*a*) Longitudinal view and (*b*) cross-sectional view of archegonial initial traversed by three anticlinal walls that delimit a central axial cell and three peripheral cells. The axial cell gives rise to the neck canal cells, ventral canal cell and egg while the peripheral cells form sterile outer layers. (*c*) As seen in cross-section, three additional segments in the peripheral cells form six rows of neck cells. (*d*) Light micrograph surface view of six rows of neck cells in *Phaeoceros laevis*. (*e*) Longitudinal section of the sunken archegonium of *Phaeoceros laevis*. (*f*) Longitudinal view and (*g*) cross-sectional view of archegonial initial traversed by single periclinal wall that delimits an outer cell that forms the neck and an inner cell that gives rise to neck canal cells, ventral canal cell and egg. (*h*) As seen in cross-section, successive anticlinal divisions in the outer cell form four rows of neck cells. (*i*) Scanning electron micrograph of archegonial neck with four rows of cells in *Psilotum nudum*. (*j*) Longitudinal section of sunken archegonium of *Pteridium aquilinum*.

## 4. REPRODUCTIVE GAMETOPHYTES

Selective pressures in a terrestrial environment for reproductive processes that require uninterrupted access to water are indeed extreme. The transmigration of algae to land and the subsequent evolution of embryophytes necessitated the evolution of multicellular sex organs (Niklas 1997). These organs not only served to protect vulnerable developing gametes but also the origin of multicellular female sex organs (archegonia) was a requisite for embryo development, a universal feature of land plants. The evolution of gametangia in archegoniates undoubtedly was a complex historical process that required coordination of morphogenetic with physiological and ecological signals. For example, production of sex organs must be timed appropriately and must occur rapidly in response to fluctuating seasonal conditions. Gametangia must be strategically placed on the plant and protective structures amply developed. In gametophyte-dominant, land-dwelling plants the compromise between optimization of vegetative growth and the constraints of sexual reproduction often resulted in the abandonment or reduced emphasis on genetic exchange (i.e. elaboration of mechanisms for asexual reproduction and self-fertilization). Such strategies are also common in algal groups but evolved in response to different selective pressures.

In an attempt to understand adaptive strategies and character evolution in the sexual phase of early archegoniates, we will briefly examine and compare: (i) sex organ ontogeny and (ii) spermatogenesis among basal bryophytes and seedless tracheophytes.

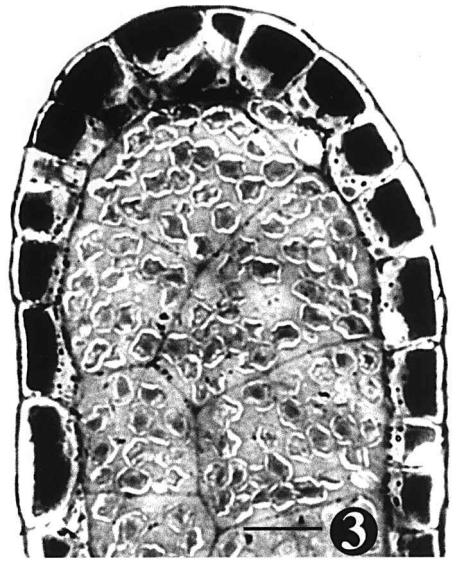


Figure 3. Light micrograph of antheridium of *Takakia ceratophylla*. Longitudinal section of anterior of developing, elongated antheridium. Cells are in packets arranged in two rows that represent the original biseriate filament produced by left–right divisions from an apical cell. The outline of the apical cell is visible as a triangular wall containing developing sperm cells at the antheridial tip. Bar = 10.0 μm.

Unlike those of pteridophytes, gametangia of mosses and liverworts are stalked and extend from the epidermal surface (Bold *et al*. 1987). In leafy taxa, gametangia are either associated with leaves or leaves surround a terminal

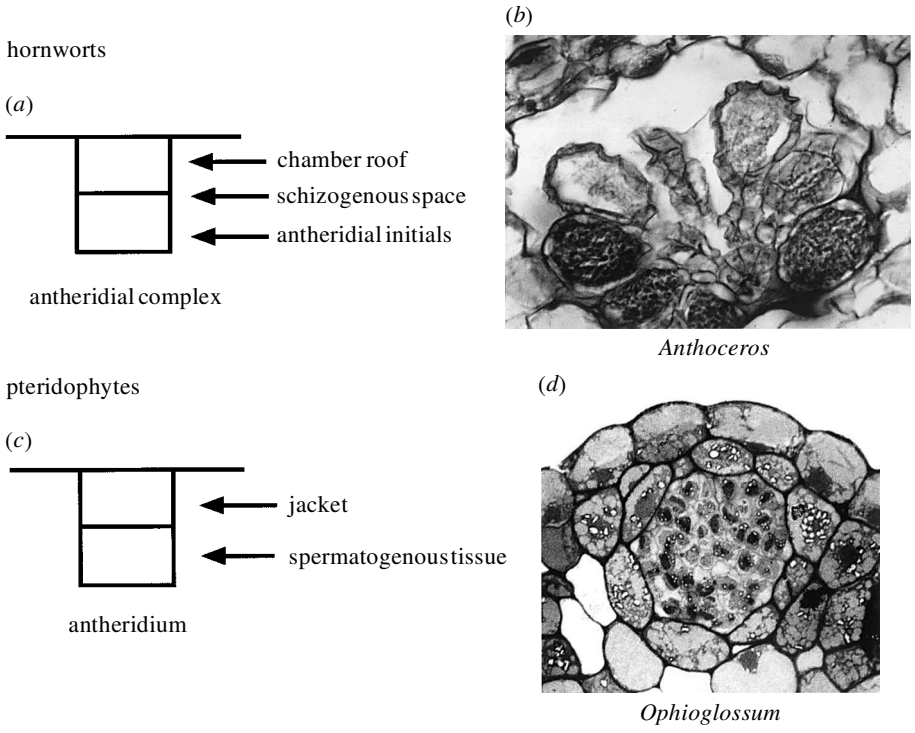


Figure 4. Comparison of antheridial development in hornworts (*a*,*b*) and pteridophytes (*c*,*d*). (*a*) In hornworts, a periclinal division in a superficial cell delimits a subepidermal cell that gives rise to up to 25 antheridia and an epidermal cell that produces a chamber roof two cells thick. A schizogenous space forms the cavity that houses the antheridia. Antheridial development from initials at the base of the cavity follows a developmental pathway similar to other bryophytes. (*b*) Light micrograph of antheridial complex in *Anthoceros agrestis*. (*c*) In pteridophytes, a periclinal division in a superficial cell delimits a subepidermal cell that gives rise to the spermatogenous tissue and an epidermal cell that forms the sterile jacket. (*d*) The single antheridium that develops from this process in *Ophioglossum engelmannii*.

cluster of sex organs. Abundant papillae, hairs and paraphyses are intermixed among gametangia and provide further protection. Thalloid liverworts have evolved a vast array of structures and mechanisms for protecting gametangia, including inrolled ventral branches (*Metzgeria*), secondarily sunken chambers (*Pellia*, *Noteroclada* and complex thalloid liverworts) and lamellar outgrowths of the thallus (Renzaglia 1982). In contrast, gametangia in hornworts are produced and maintained within the confines of the thallus and as such superficially resemble those of pteridophytes. Gametangia of basal pteridophytes are never stalked and are either entirely sunken within the thallus or form conspicuous epidermal mounds (Bierhorst 1971).

To evaluate homology of gametangia, it is critical to examine cell division patterns and especially the initial formative division sequences during which the developmental fate of subsequent cell lineages is determined (Roux 1895; Niklas 1997). These primary divisions are genetically controlled and provide the blueprint for organogenesis. At first glance, there appears to be no similarity in archegonial ontogeny among liverworts, hornworts and mosses. Archegonial development in mosses uniquely involves the initial production of an apical cell that segments left and right forming a biseriate filament (Schofield 1985; Smith 1955). The archegonium proper forms from the terminal cell in the filament after the apical cell ceases its patterned mitotic divisions. Hornwort and liverwort archegonia develop without elongation from an apical cell. In mosses and liverworts, the archegonial initial elongates and divides above the epidermal surface, while in hornworts, the developing archegonium remains surrounded by thallus tissue. This mosaic of characters related to archegonial ontogeny provides evidence of divergent developmental pathways in the bryophyte clades. However, when the formative division sequences that establish the archegonium proper are examined, a fundamental pattern emerges that is diagnostic of bryophytes (Schuster 1966). In all three groups, the process involves three longitudinal divisions that form a central triangular axial cell surrounded by three peripheral cells (figure 2*a*,*b*). The peripheral cells form the neck and venter while the axial cell gives rise to the neck canal cells, ventral canal cell and egg. Further divisions in the peripheral cells typically result in a neck of five or six cell rows (figure 2*c*,*d*) (Renzaglia 1982).

This division pattern is markedly different from that of pteridophytes, in which the single epidermal initial first divides transversely (periclinal division). The outer cell then further divides to form the neck while the inner cell divides periclinally to produce neck canal cells, ventral canal cell and egg (figure 2*f*,*g*,*j*) (Bierhorst 1971; Kenrick & Crane 1997*a*). With expansion, the archegonium projects from the epidermal surface. The neck invariably consists of four vertical rows of cells (figure 2*h*,*i*). Clearly, there are no parallels in development between the sunken archegonia of hornworts (figure 2*e*) and similar embedded archegonia in pteridophytes (figure 2*j*).

Bryophyte antheridia contain well-developed stalks and globose to elongated antheridial bodies. As in archegonial development, antheridial ontogeny in mosses is peculiar in that an apical cell is responsible for early elongation (Smith 1955). This feature alone, i.e. antheridial development involving an apical cell, enables the identification of *Takakia* as a moss (figure 3) (Smith & Davison 1993). The genesis of hornwort antheridia within an internal thallus chamber has been cited as a fundamental departure in morphogenetic design from all other archegoniates (Crandall-Stotler 1980; Bold *et al*. 1987). However, with closer scrutiny, a single underlying pattern of development is manifest in antheridial development of mosses, hornworts and liverworts. In all three groups, the antheridial initial elongates and similar division cycles form either two primary spermatogones with four surrounding jacket initials (mosses and leafy/simple thalloid liverworts) or four primary spermatogones with eight peripheral jacket initials (hornworts and complex thalloid liverworts). These similarities must represent plesiomorphies of the land plant clade. The difference in hornworts is that the antheridial initial is located at the base of the schizogenous antheridial chamber and not at the thallus surface as in other bryophytes. Apparently, in hornworts, an evolutionary shift in developmental potential has occurred from epidermal (layer surrounding the external surface) to epithelial (layer surrounding an internal space) cells, both of which are surface cells that enclose tissue. The designation of hornwort antheridia as endogenous refers only to the location of development and not to an inherently different developmental pathway.

An initial periclinal division in a superficial cell in hornworts mimics early antheridial development in pteridophytes (cf. figure 4*a*,*c*). However, unlike in pteridophytes, where this initial division gives rise to a single antheridium (see below), in hornworts extensive cell divisions in the resulting two cells produce an entire antheridial complex consisting of a sunken chamber with overlying two-layered roof and up to 25 enclosed antheridia (figure 4*a*,*b*).

Antheridial development is essentially identical to archegonial development in pteridophytes (figure 4*c*) (Bierhorst 1971). The process begins with a transverse division in an epidermal cell. The outer cell gives rise to a sterile jacket and the inner cell forms the spermatogenous tissue. So similar are these division patterns in the two sex organs of pteridophytes that it is virtually impossible to differentiate antheridia from archegonia in early stages of organogenesis. Moreover, in protandrous lycophytes, when the transition between male and female sex organs occurs, it is possible to find bisexual gametangia that contain developing sperm in the 'neck region' and an egg cell at the base (figure 5) (K. S. Renzaglia and D. P. Whittier, unpublished data).

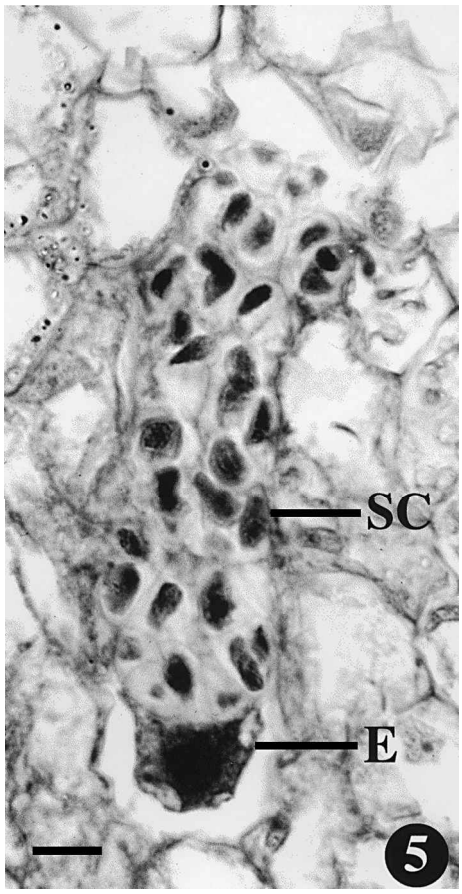


Figure 5. Longitudinal section of *Phylloglossum drummondii* bisexual gametangium. In the region of the mature gametophyte where antheridial production shifts to archegonial production, bisexual gametangia are common. This gametangium has developing sperm cells (SC) in the 'neck region' that overlie a mature egg cell (E). Bar = 20 μm.

## 5. SPERMATOGENESIS

Sperm cells of archegoniates are propitiously constructed for optimal swimming efficiency in a terrestrial environment. As the most complicated cells produced by archegoniates, motile sperm cells provide a wealth of developmental and phylogenetic data (Renzaglia & Duckett 1988, 1991; Garbary *et al*. 1993). During spermatogenesis, undifferentiated parenchyma cells are progressively transformed into streamlined, coiled cells containing minimal organelles. The process involves the origin and development of an elaborate locomotory apparatus, a structure that enables unparalleled comparisons with motile algal cells. A unifying feature of motile gametes of archegoniates is the *de novo* origin of centrioles in late spermatogenous tissue (Vaughn & Harper 1998; Vaughn & Renzaglia 1998). In plants with biflagellated sperm cells, centrioles originate as bicentrioles, structures that are composed of two centrioles attached end to end. The bicentriole is the land plant analogue of the orthogonal centriolar pair that is typical of protists and animals. In multiflagellated gametes, centrioles originate as more complicated spherical organelles known as blepharoplasts. In addition to origin and development of the locomotory apparatus, histogenesis of male sex organs provides comparative data on cytoplasmic phenomena such as plastid behaviour and cellular polarity. Numbers of organelles and their spatial arrangements are established during the proliferative divisions of organogenesis and the nascent spermatid is organized for expeditious differentiation into a motile cell (Renzaglia & Duckett 1987; Bernhard & Renzaglia 1995; Renzaglia *et al*. 1994). The process of spermatogenesis and


Figure 6. Reconstructions of mature biflagellated spermatozoids of Charales and bryophytes. Note sinistral coiling of all but *Phaeoceros* (*b*), which exhibits a right-handed coil. Colour coding: red (pink) = flagella and basal bodies; blue = nucleus; brown = mitochondria; yellow = spline microtubules; orange = lamellar strip; green = plastid. (*a*) *Chara vulgaris*. Adapted from Duncan *et al*. (1997). (*b*) *Phaeoceros laevis*. Adapted from Carothers & Duckett (1980) and Renzaglia & Duckett (1989). (*c*) *Blasia pusilla*. Adapted from Renzaglia & Duckett (1987). (*d*) *Aulacomnium palustre*. Data derived from Bernhard & Renzaglia (1985). Drawing by H. Dee Gates.

its bearing on plant phylogenetics will be reviewed elsewhere (Renzaglia & Garbary 2000). Here we consider general features of the mature spermatozoid, especially of biflagellated cells, and evaluate the selective processes that have acted to bring about these phenotypes.

Biflagellated sperm cells are produced by charophycean algae (except Zygnematales), bryophytes and most lycophytes (figures 6 and 7), while all other tracheophytes with motile sperm produce multiflagellated cells (figures 8 and 9). The mature biflagellated cell in bryophytes is a helical cylinder, with an anteriorly positioned locomotory apparatus and four organelles: an anterior mitochondrion, a compacted central nucleus and a posterior plastid with an associated mitochondrion (figure 6*b–d*). In addition to flagella and basal bodies, the locomotory apparatus consists of a lamellar strip and a narrow band of microtubules (the so-called spline), which extend around the cell providing a framework for positioning of organelles. The lamellar strip is composed of centrin, a calcium-binding contractile protein, and as such functions as an MTOC for the spline microtubules and in positioning the flagella (Vaughn *et al*. 1993). At cellular

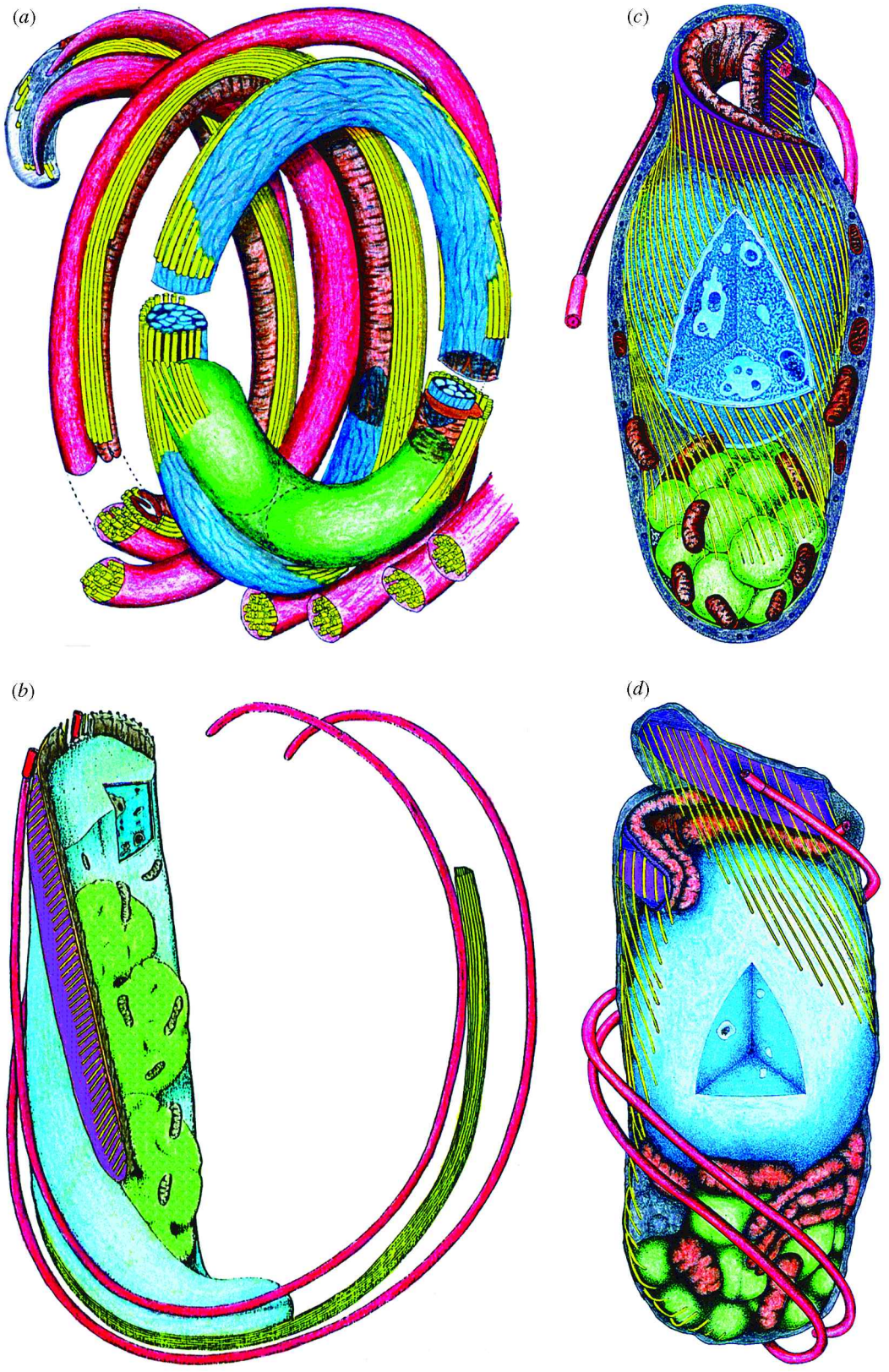


Figure 7. Reconstructions of mature biflagellated spermatozoids of lycophytes. Colour coding: red (pink) = flagella and basal bodies; blue = nucleus; brown = mitochondria; yellow = spline microtubules; purple = lamellar strip; green = plastid; grey = extraneous cytoplasm. (*a*) *Selaginella kraussiana*. Adapted from Renzaglia *et al*. (1998). (*b*) *Lycopodium obscurum*. Adapted from Maden *et al*. (1996). (*c*) *Palhinhaea cernua*. Adapted from Robbins & Carothers (1978). (*d*) *Lycopodiella lateralis*. Adapted from Maden *et al*. (1997).

maturity, the lamellar strip regresses to a dense rim along the leading edge of the cell (figure 6*b–d*).

Among the algal outgroups to land plants, only Charales possess coiled spermatozoids (figure 6*a*). In male gametes of *Chara* and *Nitella*, the same basic organization as in bryophytes is exhibited; the primary difference is in the number of organelles. Approximately 30 mitochondria are aligned at the cell anterior and up to six starch-filled

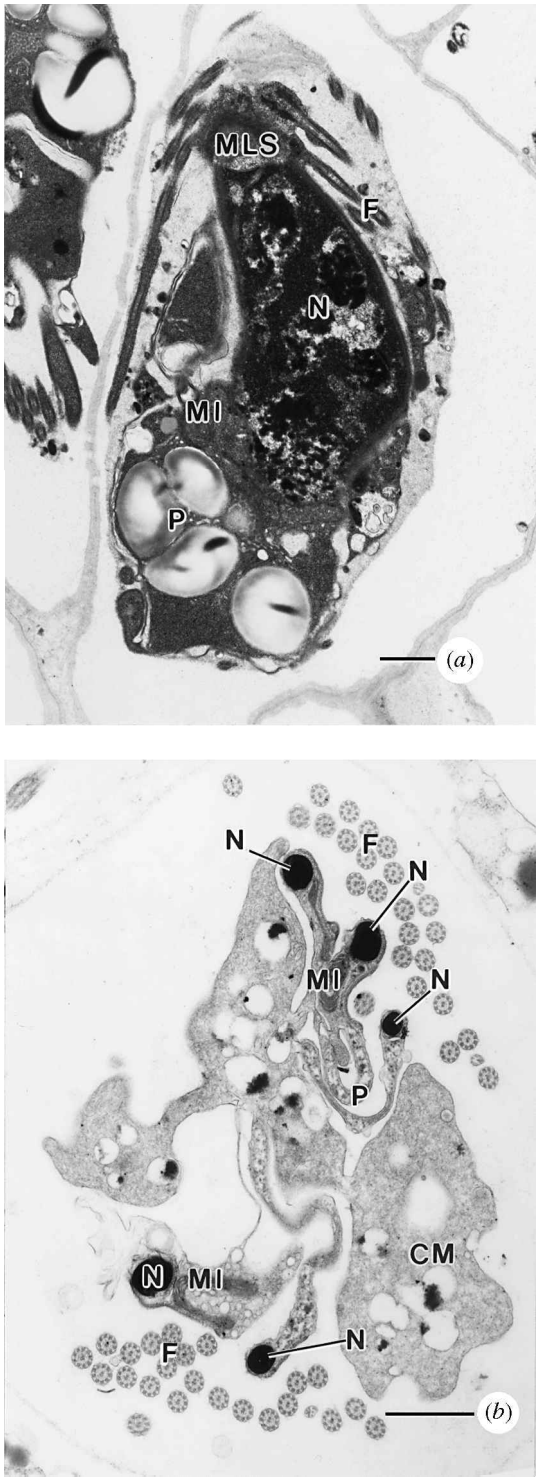


Figure 8. Transmission electron micrographs of multiflagellated male gametes of lycophytes. (*a*) *Phylloglossum drummondii*. This spermatozoid contains approximately 20 flagella (F) aligned in a coil over the multilayered structure (MLS) at the cell anterior. The nucleus (N) is broad, slightly coiled and contains uncondensed inclusions. Numerous mitochondria (MI) and a plastid (P) with large starch grains are positioned at the rear of the cell. Bar = 1.0 μm. (*b*) *Isoëtes bolanderi*. Approximately 20 flagella (F) are distributed along the length of this narrow and coiled spermatozoid. The nucleus (N) is a compacted narrow cylinder and it overlaps with two mitochondria (MI). An inconspicuous, posterior plastid (P) lacks starch. Upon motility, the large central mass of cytoplasm (CM) is shed. Bar = 1.0 μm. Unpublished micrograph by Gayleen Cochran.

plastids are associated with scattered mitochondria at the cell terminus. Gametes of *Chara* never possess a lamellar strip but those of *Nitella* and *Coleochaete* possess this component of the locomotory apparatus. In contrast to bryophytes, in which the plates of the lamellar strip are oriented at 45° to the longitudinal axis of spline microtubules, in algal cells this angle is consistently 90° (Graham 1993).

Mature spermatozoids of hornworts, mosses and liverworts are immediately distinguished from each other by major architectural differences. Hornwort sperm cells are extremely small (*ca*. 3.0 μm in diameter) and bilaterally symmetrical (figure 6*b*). The two basal bodies insert the parallel flagella into the cell anterior at approximately the same level. Unlike spermatozoids of all other archegoniates, which are sinistrally coiled, hornwort gametes exhibit a dextral coil. In contrast, the bilateral spermatozoids of liverworts and mosses are asymmetrical (figure 6*c*,*d*). In both groups of bryophytes, the two basal bodies are markedly different in length, structure and insertion into the cell. As a consequence, the flagella are staggered in their emergence from the cell. When viewed from the cell anterior, the basal body that is positioned further forwards is situated on the right and the more posterior basal body is on the left side of the cell. The anterior basal body in both mosses and liverworts contains dorsal microtubule triplets that extend in front of the basal body proper. Similarly, in both groups, the posterior basal body exhibits a unique yet consistent structure with ventral microtubule triplet extensions accounting for most of its length. The striking commonalities in construction of this complex locomotory apparatus, including basal bodies and flagella, strongly support monophyly of liverworts and mosses (Renzaglia & Duckett 1991; Garbary *et al*. 1993). The primary differences in moss compared with liverwort sperm cells are the occurrence of a stray microtubule and the position of the plastid and associated posterior mitochondrion along the inner nuclear surface (figure 6*d*) and not at the cell terminus as in hepatics (figure 6*c*).

Lycophyte spermatozoids are structurally diverse, probably more so than in any other land plant group (figures 7 and 8) (Maden *et al*. 1996, 1997; Renzaglia *et al*. 1998, 1999). Within Lycopodiaceae, gametes may be biflagellated and coiled (figure 7*b*), multiflagellated and coiled (figure 8*a*) or biflagellated with a more ovoid outline (figure 7*c*,*d*). The lamellar strip persists in the mature gamete and the angle between lamellar strip and spline ranges from 45° to 90° (Maden *et al*. 1996, 1997). Compared with bryophyte spermatozoids, those of lycopsids contain more cytoplasm, including numerous small mitochondria at the cell posterior. Plastid number varies from one to five. Plastids are either positioned on the inner nuclear surface as in the more coiled gametes (figure 7*b*) or aggregated at the cell posterior in ovoid cells (figure 7*c*,*d*). Apparent evolutionary trends within the family include an increase in cell length and diameter, and a decrease in coiling (Maden *et al*. 1997). Flagella are slightly staggered in the smaller coiled sperm cells of *Huperzia* and *Lycopodium* and more widely separated in the more specialized ovoid cells of *Palhinhaea* and *Lycopodiella*. These ideas are contrary to those of Robbins & Carothers (1978), who speculated that an ovoid cell with widely spaced flagella is the primitive condition in archegoniates.

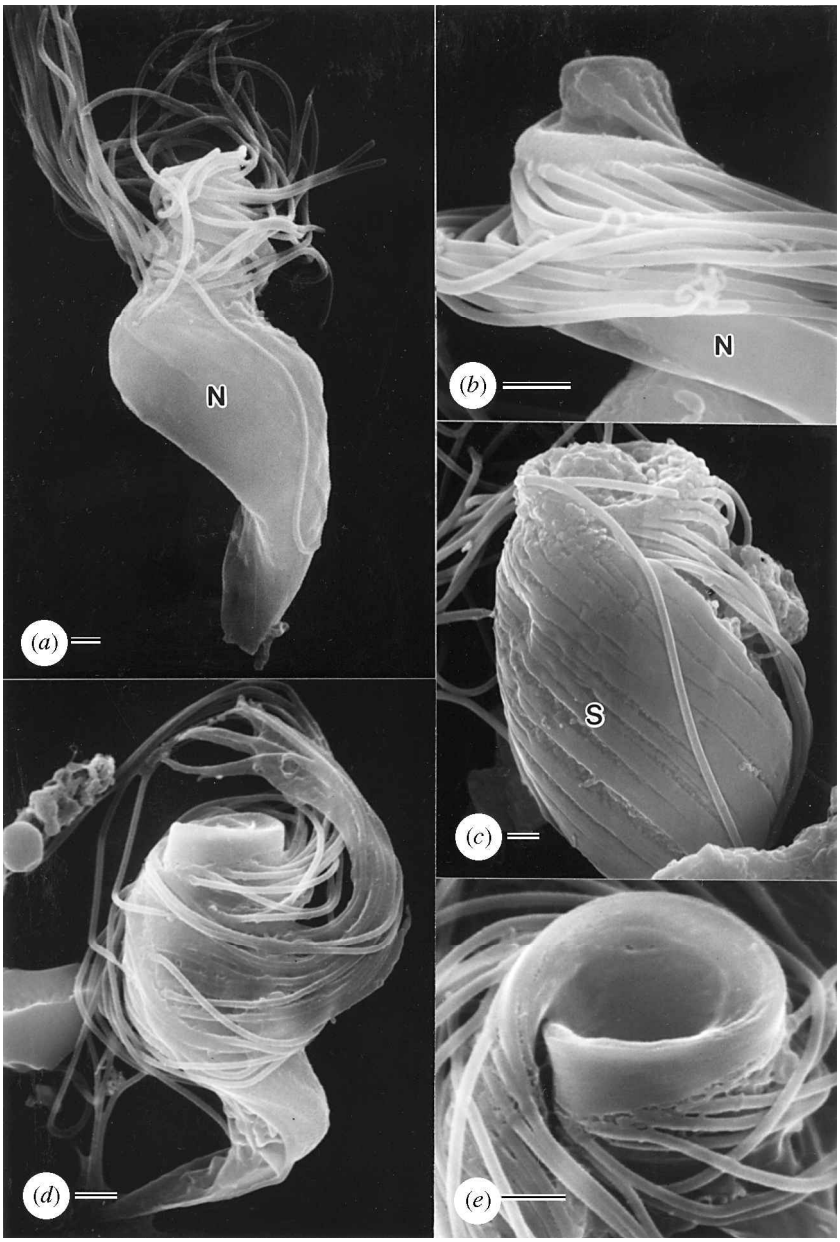


Figure 9. Scanning electron micrographs of multiflagellated sperm cells of pteridophytes. Bars = 1.0 μm. (*a*) *Equisetum arvense* whole spermatozoid with approximately 54 flagella arranged in coils around the cell anterior. The nucleus (N) is broad in the middle and tapers on both ends. A spline of up to 300 microtubules overlies the nucleus and provides a framework for the coils. Abundant organelles extend along the inner nuclear region (not visible). (*b*) Higher magnification of anterior of *Equisetum arvense* spermatozoid showing coils along which flagella are inserted into the cell and narrow anterior of nucleus (N). (*c*) Spermatozoids of *Psilotum nudum* are broad and coiled with approximately 36 flagella inserted along the anterior coil. The spline (S), consisting of up to 200 microtubules, associates in small bands that are visible over the broad nucleus. Unpublished micrograph by Thomas Johnson. (*d*) The sperm cell of *Angiopteris evecta* resembles that of *Equisetum* and *Psilotum* in that it is coiled with numerous flagella inserted along the anterior coils, and organelles line the inner nuclear region. Like sperm cells of leptosporangiate ferns, this cell is more flattened and ribbon-shaped than that of *Equisetum* and *Psilotum*. (*e*) Higher magnification of anterior of *Angiopteris evecta* spermatozoid showing ribbon-shaped anterior coils along which the flagella are inserted into the cell.

The only homosporous lycophyte with multiflagellated sperm (*ca*. 20 flagella) is *Phylloglossum* (figure 8*a*): this condition in a sole member of the Lycopodiaceae most certainly is an autapomorphy (Renzaglia & Whittier 1993; Renzaglia & Maden 2000).

The similarity in mature gamete structure between *Selaginella* and the bryophytes is intriguing from an evolutionary perspective. Like bryophytes, *Selaginella* produces small, coiled, biflagellated sperm cells with four organelles arranged similarly along a microtubular spline (figure 7*a*). No lamellar strip exists in the mature cell. However, unlike bryophyte spermatozoids, those of *Selaginella* possess exceptionally long anterior mitochondria that occupy more of the cell length than the nucleus. Comprehensive developmental data on *Selaginella kraussiana* reveal further deviations from bryophyte spermatogenesis as well as substantial similarities with Lycopodiaceae and other pteridophytes (Renzaglia *et al*. 1999). These studies reinforce the notion that developmental data are crucial in the determination of structural homology.

Although sperm cells of *Isoëtes* have not been thoroughly examined ultrastructurally, preliminary observations reveal that these cells are coiled, highly streamlined and possess approximately 20 flagella (figure 8*b*). Unique among land plant gametes is the lack of a starch-filled plastid in the mature cell. Commonalities with the *Selaginella* spermatozoid and marked structural deviations from those of homosporous lycopsids suggest this cell is derived from within the heterosporous lycopsid lineage and bears no direct evolutionary relationship with the multiflagellate spermatozoid of *Phylloglossum*.

The remaining pteridophytes (*Equisetum*, *Psilotum* and ferns) produce large (up to 18 μm in diameter) coiled, multiflagellated sperm cells with abundant organelles (figure 9). Similarities among all of these cells are numerous and point to a common origin. Most notable is the cell anterior that bears a coiled locomotory apparatus with over 34 flagella (figure 9*b*,*c*,*e*). A broad, microtubular band outlines the cell and the nucleus extends along the mid-spline region to the cell tip (figure 9*a*–*d*). Details of

spermatozoid structure in these plants will be presented elsewhere (Renzaglia & Garbary 2000).

All spermatozoids of land plants exhibit coiling, either external in cell architecture or internal in arrangement of organelles. Coiling is the direct result of cellular elongation associated with extension of the single band of spline microtubules within the constraints of a spherical cell, i.e. compaction and elongation necessarily parallel the circular boundaries of the cell. The complicated layout of land plant sperm cells most certainly provides a hydrodynamically sound architecture. Cellular elongation or streamlining reduces excess baggage and, perhaps even more importantly, is instrumental in movement of the spermatozoid through the narrow tube of the archegonial neck. The convergence in structure of archegonia and the egg apparatus of Charales may explain similarities in sperm cell shape between Charales and basal land plants and may account for the marked differences between these cells and those of Coleochaetales.

In bryophytes and perhaps some lycophytes, sperm cells have remained relatively small and simple. The low haploid DNA contents of these plants are correlated with sperm size and are reflected in small cell size (Renzaglia *et al*. 1995). Among bryophytes, hornworts have the smallest genome size and the smallest sperm cell. The symmetric placement of flagella at the anterior is an effective organization to move an extremely small cell through water. With greater DNA content and increase in cell size, it would follow that asymmetry and staggering of flagellar insertion may be necessary to facilitate movement. Data from sperm cell structure suggest that hornworts diverged from other bryophytes before flagellar staggering evolved and that mosses and liverworts acquired staggering from a common ancestor with larger spermatozoids than those of the hornwort–embryophyte progenitor. If cells are symmetrical, as in hornworts, the direction of coiling affords no selective advantage and is free to change without consequence to swimming performance. Once asymmetry was established, as in all other land plants, the different constructions of the right and left cellular halves may have had 'locked in' genetic determination of coiling direction. Based on these ideas, the prototypic spermatozoid of archegoniates was probably a minute biflagellated cell with minimal sinistral coiling. Observations of remarkably well-preserved sperm cells in Devonian gametophytes strengthen the speculation that primitive land plants produced small coiled gametes (Remy *et al*. 1993; Duncan *et al*. 1997).

## 6. SPOROPHYTES

Liverworts, hornworts and mosses are readily distinguished from polysporangiate tracheophytes by their unbranched, monosporangiate sporophytes that are nutritionally dependent on the persistent, photosynthetic gametophyte. Given the likely origin of the sporophyte through a delay in meiosis (i.e. antithetic theory), the lack of elaboration of the sporophyte in bryophytes is viewed as a plesiomorphy among embryophytes. Indeed, contemporary evidence, both molecular and morphological, supports the basal position of bryophytes in land plant phylogeny (Graham 1993). However, re-evaluation of sporophyte structure suggests that the interpretation of bryophyte sporophytes as less complex than those of pteridophytes is an oversimplification. In pteridophytes, in addition to facilitating spore production and dispersal, the sporophyte is responsible for vegetative growth, including maximum light harvesting and mechanical support. In bryophytes, where the gametophyte is the vegetative phase, the sole function of the sporophyte is to produce and disperse spores. In effect, each bryophyte sporophyte is a solitary sporangium, with or without a stalk, that is designed to make and release spores and as such has undergone virtually no selection for vegetative growth. It is not surprising then that bryophyte sporophytes, although they are unbranched and lack leaves, produce sporangia that are the most complicated of any produced by land plants. Lines of specialization have led to elaborate spore production and dispersal mechanisms, such as the peristome of true mosses, cellular elaters of liverworts and the highly complicated sporangium of hornworts that continues to elongate throughout the growing season. No parallels of such intricate sporangial complexity are found in any tracheophyte, for indeed these roles have been taken over by the vegetative sporophytic tissues, not the sporangium itself.

This new look at sporophyte complexity in bryophytes may be extended further in an evaluation of reproductive potential in sporophytes of embryophytes. Moss, liverwort and hornwort sporophytes are limited in their capacity for spore production because individually they bear only a single sporangium. However, when the totality of sporophytes that are potentially produced by a single gametophyte is considered, a very different interpretation emerges. During their lifetime and even within a single growing season, the typical sexual bryophyte gametophyte generates a multitude of sporophytes. In gametophytes of 'promiscuous' bryophytes, it is conceivable that the total number of spores produced by the 'population' of sporophytes on a single gametophyte may exceed that of a single pteridophyte sporophyte during a growing season. Moreover, unlike the multiple sporangia of a pteridophyte sporophyte (generated by a single gametophyte), the numerous sporophytes produced by a solitary bryophyte gametophyte are potentially products of independent fertilizations. Given outcrossing, each sporophyte is genetically unique. In bryophytes, evolution has maximized both sexual reproduction and the consequential production of genetically diverse sporangia, while in tracheophytes, vegetative growth is responsible for increased development of genetically similar sporangia.

Among tracheophytes, each gametophyte of heterosporous forms has the potential to produce only a solitary sporophyte. Basal homosporous tracheophytes, in contrast, often have the capacity to produce several sporophytes and longer-lived subterranean gametophytes may yield multiple sporophytes (Bierhorst 1971; A. R. Maden, unpublished data). Subterranean gametophytes are difficult to find in nature and therefore they have been insufficiently examined. As a result, the extent of sporophyte production by a single gametophyte in basal pteridophytes remains unknown. Nevertheless, it can be concluded that the ability to generate multiple sporophytes significantly increases reproductive potential in land plants with persistent gametophytes.

Because selection for individual sporophytes of bryophytes has acted to enhance the manufacture and dispersal of spores, it is not surprising that a number of diverse mechanisms for elevating the sporangium have evolved. To expose spores to sufficient wind currents, sporangia must be raised above the gametophyte surface. Some of these strategies for sporangial elevation involve extension of gametophytic tissue (archegoniophore in Marchantiales and pseudopodium in *Sphagnum* and Andreaeales), while most involve growth of sporophytic tissue. In those with gametophytic elevation of sporangia, the seta remains short and the sporangium relatively simple, e.g. no conducting tissue is present in *Sphagnum* or Andreaeales. Elaboration of sporophytic tissue entails either the production of a seta (true mosses, *Takakia*, leafy and simple thalloid hepatics) or the development of a basal meristem (hornworts).

In hepatics, seta extension is rapid and involves the extension of turgid cells. Mechanical strength is provided by hydrostatic pressure against the cell wall from within the large central vacuole. Elongation of the archegoniophore and development of protective structures around the developing sporophyte also entail rapid wall expandability and extension of ephemeral organs, an innovation that is common in a variety of hepatic tissues. The strategy in these plants is for 'instantaneous' and effective dispersal of spores. This process is facilitated by elaters, specialized elongated sterile cells with spiralled wall thickenings. With an ephemeral, elongated, mature sporophyte it follows that there would be no selection for elaboration of conducting tissue, photosynthetic regions and stomata in liverworts. Such tissues and cells are adaptations beneficial to sporophytes, such as those of mosses and hornworts where sporophyte extension is gradual and brought about by cell division followed by gradual elongation. Mechanical strength and movement of materials, especially upwards to the region where spores differentiate, are crucial to the success of sporophytes that persist through the growing season and disperse spores over an extended period. Hence, selective pressures in at least some of these lineages have favoured the retention of sporophytic innovations related to increased strength (cells with thickened walls), photosynthetic function (stomata and assimilative regions) and enhanced transport of nutrients (conducting tissue). Sporophytes of hepatics require a comparable period of time to mature but this process occurs within the confines of protective gametophytic tissues which provide a continuous supply of water and nutrients.

Not insignificant in effecting spore dispersal is the fact that many bryophytes grow on vertical substrates. In such situations, the entire plant is elevated above ground level and is exposed to wind currents that facilitate maximum spore dispersal. Sporophytes of epiphytic mosses, for example, are often less elongated than those of ground dwelling taxa. Thus, location of habitat must be factored into the equation for successful spore dissemination.

Different strategies of sporophyte elevation are complemented by divergent morphogenetic designs in embryo and sporophyte growth and differentiation. Salient features of sporophyte development in bryophyte lineages are considered below. The hornwort zygote is dissected first by a vertical wall, not a transverse partition as in mosses and liverworts. The endothecium in hornworts and *Sphagnum* forms a columella only and is not responsible for producing sporogenous tissue as in other bryophytes. As in gametangial ontogeny, apical cell involvement is echoed in the formative phase of embryogenesis in mosses. Later in development, before differentiation of the capsule, an intercalary meristem typically forms and brings about further elongation of the sporophyte (Roth 1969). In both mosses and hornworts, photosynthetic capability in the sporophyte is partially responsible for continued growth. An assimilative layer extends the length of the hornwort sporophyte. In mosses, the photosynthetic region extends along the entire capsule and includes internal specialization of aerenchyma. In contrast, the hepatic sporophyte is contained within protective gametophytic cells until spores have matured. Thus, gametophytic nurturing occurs through the life of the hepatic sporophyte.

Taken as a whole, the fundamental differences in development, structure, and spore dispersal strategies suggest divergence of the three groups of bryophytes prior to specialization of mechanisms for elongation and continued growth. With this scenario, the prototypic bryophyte sporophyte would be a small mass of cells with a fertile internal region, as predicted by Graham (1993), Mishler & Churchill (1984) and Hemsley (1994). Because a placenta, with specialized cells at the sporophyte/gametophyte interface, is ubiquitous in embryophytes (Ligrone *et al.* 1993), this specialized absorptive region was probably in place in the early stages of sporophyte diversification. The occurrence of transfer cells in gametophyte tissue surrounding the zygote of *Coleochaete* supports this supposition. Thus, certain Charophyceae were preadapted for embryo evolution (Graham 1993). As an unelongated mass of cells, the ancestral sporophyte would not have acquired structures necessary for sustained growth, e.g. localized meristems, photosynthetic zones, conducting tissue and stomata. In particular, stomata, assimilative regions and conducting tissues are adaptive specializations that enable persistence of an elongated sporophyte through the growing season. Therefore the homology of such structures among basal land plants requires evaluation.

Stomata occur in *Sphagnum* and the true moss lineage (but not basal taxa such as *Takakia* and Andreaeales) and in two of the five widely recognized genera of hornworts. It should be noted that *Megaceros*, the hornwort genus with the largest sporophytes, does not possess stomata. In many mosses, instead of distinct guard cells, stomate ontogeny uniquely produces a binucleate cell with a central pore formed by incomplete septum formation during cytokinesis (Sack & Paollilo 1985). Separation of adjacent cells along the middle lamella is commonplace in hornwort gametophytes. It would follow then that the genetic potential for producing two adjacent cells that separate from each other, i.e. stomata, is inherent in these plants. Since this innovation is restricted in occurrence to only one lineage of anthocerotes (*Phaeoceros–Anthoceros*), stomata probably evolved independently in mosses and hornworts. This interpretation is reasonable given the simplicity of stomatal design and the apparent lack of diurnal cycles in bryophyte stomata (Paton & Pearce 1957). Certainly, much more complicated structures and processes have evolved multiple times in embryophytes;

secondary tissues and heterospory are two excellent examples (Bateman *et al*. 1998; Niklas 1997). Bateman & DiMichele (1994) concluded that heterospory evolved a minimum of 11 times during embryophyte history.

Among bryophytes, conducting tissues are relatively widespread in gametophytes but restricted in distribution within sporophytes to mosses, a feature that suggests affinities with tracheophytes. However, because of developmental and ultrastructural divergences, contemporary investigations have called into question the homology of moss and tracheophyte conducting cells and have resulted in the rejection of this presumption (see Ligrone *et al*., this issue and references therein). Most features of sieve cells, the food-transporting cells of pteridophytes, which are shared with leptoids, the food-conducting cells of mosses, are found exclusively in the Polytrichales, a relatively derived moss taxon. The fundamental cytological design, involving an endoplasmic microtubule system, is shared by mosses and liverworts and is absent in tracheophytes. Similarly, the concept of homology between hydroids, water-conducting cells of mosses, and tracheids, water-conducting cells of tracheophytes, has been severely challenged. Basal moss clades, except *Takakia*, are devoid of conducting cells and those of *Takakia* more closely resemble water-conducting cells of the liverwort *Haplomitrium* than they do those of the true moss lineage. Clearly, conducting-cell homology among bryophytes and tracheophytes requires further evaluation and can no longer be regarded as a working precept.

The primitive method of sporangial dehiscence is the formation of a single suture. Among archegoniates this condition is present in all basal clades. The process may involve a specialized line of cells as in *Haplomitrium* (Bartholomew-Began 1991) or it may occur between seemingly undifferentiated cells as in *Takakia* and *Huperzia* (Renzaglia *et al*. 1997; A. R. Maden, unpublished data). Hornwort sporophytes typically dehisce along two sutures on either side of the sporangium, while capsules of more advanced liverworts split into four valves and those of mosses produce an operculum with an underlying elaborate peristome.

Spores and sporogenesis are fundamentally conserved in embryophytes and provide a wealth of phylogenetically informative characters (Brown & Lemmon 1988, 1990*b*; Garbary & Renzaglia 1998). For example, monoplastidic meiosis is an ancestral condition that characterizes Coleochaetales, hornworts, mosses and primarily basal taxa of liverworts, lycophytes and ferns (Renzaglia *et al*. 1994; Brown & Lemmon 1997). Associated with monoplastidy in archegoniates, but not in green algae, is a unique quadripolar microtubule system (QMS) that is organized at the plastids and predicts the polarity of the two meiotic divisions (Brown & Lemon 1997). The occurrence of a QMS in both monoplastidic and more-derived polyplastidic meiocytes of liverworts and the putative loss of this microtubule assemblage in tracheophytes provides a well-defined cytomorphogenetic transformation series in land plants (Brown & Lemmon 1997).

The spore wall (sporoderm) of embryophytes shows continuity in structure, typically with a pecto-cellulosic inner layer (intine) and an outer, sculptured layer (exine), which is impregnated with sporopollenin (Blackmore & Barnes 1987). As a protective coating around the single cell contained within, the sporoderm was a necessary adaptation for survival and dispersal of airborne spores. Intine and exine are derived from the cytoplasm and are deposited in a centripetal fashion. Tripartite lamellae are major components of the exine in most mosses, liverworts and tracheophytes, but apparently are lacking in hornworts and Andreaeales (Brown & Lemmon 1988; Renzaglia & Vaughn 2000). A primexine is laid down during meiotic cytokinesis in liverworts and hornworts and this layer provides a template within which wall layers are successively deposited. Premeiotic patterning of wall sculpturing is restricted to certain liverworts (*Haplomitrium*, *Pallavicinia* and *Nowellia*) (Brown *et al*. 1986); with a more comprehensive survey, this feature may be informative in defining clades within hepatics. Wall-sculpturing patterns involving deposition of a perine from extrasporal tissue are shared by mosses and pteridophytes (Blackmore & Barnes 1987). A true perine is lacking in hornworts and liverworts, where outer exine layers are responsible for wall ornamentation. In hornworts, a dense layer is deposited late in development (Renzaglia & Vaughn 2000). This 'pseudoperine' is differentiated from true perine because it is derived from the spore mother cell wall and not the tapetum.

Elaters consisting of entire cells are restricted to liverworts and hornworts. These cellular entities are the product of controlled mitotic divisions and thus the ratio of spores to elaters often is fixed within a taxon. In general, the ratio of spores to elaters is greater than four to one in hepatics; mitotic divisions occur in sporogenous cells after the elater mother cell is delimited. In comparison, elaters of hornworts are laid down in tiers that alternate with spore-generating layers. Typically four spores are produced per elater, but in some instances mitotic divisions in the elater mother cell decrease the spore-to-elater ratio (Schuster 1984*c*). The lack of specialized wall thickenings in most taxa and the tendency for adjacent elaters to adhere to one another has led to the concept that the 'pseudoelater' of hornworts is multicellular. The curious occurrence of elaters with spiralled wall thickenings in *Megaceros* and *Dendroceros* suggests homology of these cells with morphologically similar elaters of hepatics. However, because of the variability in elater structure in hornworts and the existence of spiralled elaters in presumably derived taxa (*Megaceros* and *Dendroceros*), the resemblance of elaters of certain hornworts and liverworts is most realistically interpreted as a convergence. Further developmental studies are required to test this hypothesis.

## 7. PHYLOGENY INTERPRETED FROM MALE GAMETOGENESIS

A comprehensive data set on spermatogenesis was developed to address relationships among pteridophytes and bryophytes (Garbary *et al*. 1993). The original data set consisted of 90 characters. Accumulation of additional developmental and experimental data from other organisms has resulted in a deeper understanding of character homology, and the original 90-character data set has now been reduced to 72 more informative characters. Thus, data from male gametogenesis may provide the single best set of morphological characters in terms of homology. Recent efforts to increase taxon sampling and to complete data on critical pteridophyte and bryophyte genera have

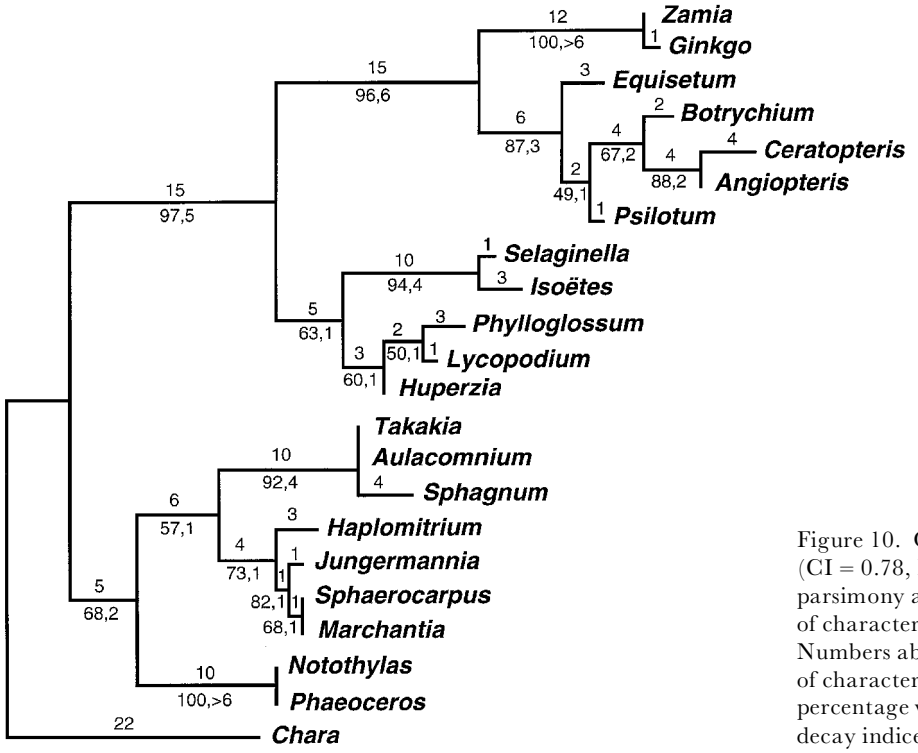


Figure 10. One of three trees of length 164 (CI = 0.78, RI = 0.88) obtained from a maximum parsimony analysis (implemented with PAUP* 4.0) of characters derived from spermatogenesis. Numbers above each branch represent the number of characters supporting the branch. Bootstrap percentage values (100 replications) followed by decay indices are given below the branch.

enabled greater resolution of relationships, especially within lycophytes. Of special importance were the architectural and developmental data for *Selaginella kraussiana* (Renzaglia *et al*. 1998) and *S. australiensis* (Renzaglia *et al.* 1999); previous data were derived mainly from the mature sperm structure (Robert 1974). The 1993 analysis resulted in a highly intuitive cladogram with bryophytes monophyletic and with mosses and liverworts forming a single clade. The only apparent anomaly was the placement of *Selaginella* on the bryophyte rather than the tracheophyte clade. Subsequent cladistic analyses of this sperm data set resolve the anomalous position of *Selaginella* (Maden *et al*. 1997; this paper), and place it with other lycophytes.

The current maximum parsimony (MP) analysis of 21 archegoniates using 72 characters produces three equally parsimonious trees that have the same basic topology (figure 10) as those published previously (except for the more appropriate position here of *Selaginella* with the lycophytes), in which there is a dichotomy between bryophytes and tracheophytes. The monophyly of bryophytes receives weak bootstrap (BS) support (68% BS, decay index of 2), whereas the tracheophyte clade is strongly supported (97% BS, decay index of 5). Within bryophytes, all three groups are monophyletic and the moss and hornwort clades are strongly supported. There is only weak support for the moss plus liverwort clade (BS 57%, decay index of 1); however, three fundamental characters support this clade, i.e. dimorphic basal body structure, the presence of a spline aperture and the location of the stellate pattern in the flagellum.

Lycophytes form a clade sister to a monophyletic assemblage containing ferns, *Equisetum*, *Psilotum* and seed plants. The clade of heterosporous taxa (*Isoëtes* and *Selaginella*) is strongly supported (BS 94%, decay index of 4), whereas its sister group, containing the remaining homosporous lycophytes, receives weaker support (BS 60%, decay index of 1). This analysis of lycophytes is congruent with those based on *rbcL* (Wikström & Kenrick 1997; Korall *et al*. 1999), except for the placement of *Phylloglossum* in a clade with *Lycopodium*, not *Huperzia*. However, relationships among these three homosporous taxa are poorly resolved based on spermatogenesis. Because there is enormous variation in male gamete structure within lycophytes, it would be useful to expand the data set to include all generic segregates of lycophytes (Øllgaard 1987) and to further sample subgenera within *Selaginella*.

The placement of *Equisetum* in the fern lineage is controversial (Kenrick & Crane 1997*a*,*b*); however, the first unequivocal evidence that supported the hypothesis that horsetails are reduced ferns came from male gametogenesis characters (Bierhorst 1971). This hypothesis later found support in an analysis combining *rbcL* and morphological characters (Pryer *et al*. 1995) as well as an analysis of mtSSU rDNA sequences (Duff & Nickrent 1999). A sister relationship between *Psilotum* and eusporangiate ferns is supported by a number of molecular studies (Manhart 1995; Pryer *et al*. 1995; Pahnke *et al*. 1996; Malek *et al*. 1996; Wolf 1997; Wolf *et al*. 1998; Hedderson *et al*. 1998).

Manual adjustments on tree topology were conducted using MacClade (Maddison & Maddison 1992) to study the effect on tree length (TL). Only two other hypotheses were reasonably consistent with these data. Tree length was only two steps longer (TL = 166) when figure 10 was constrained to match the results from the overall morphological data set (figure 11) and the molecular data set (figure 12), both of which resolve hornworts as sister to all other embryophytes. The embryophyte clade minus hornworts is supported by a character associated with the staggering of the basal bodies, a fundamental feature of motile cell organization (Renzaglia & Duckett 1991; see discussion in § 5). An additional single step (i.e. to TL = 167) was required to make mosses sister to tracheophytes while retaining hornworts as the basal lineage. The moss/tracheophyte clade is supported by two characters: the first

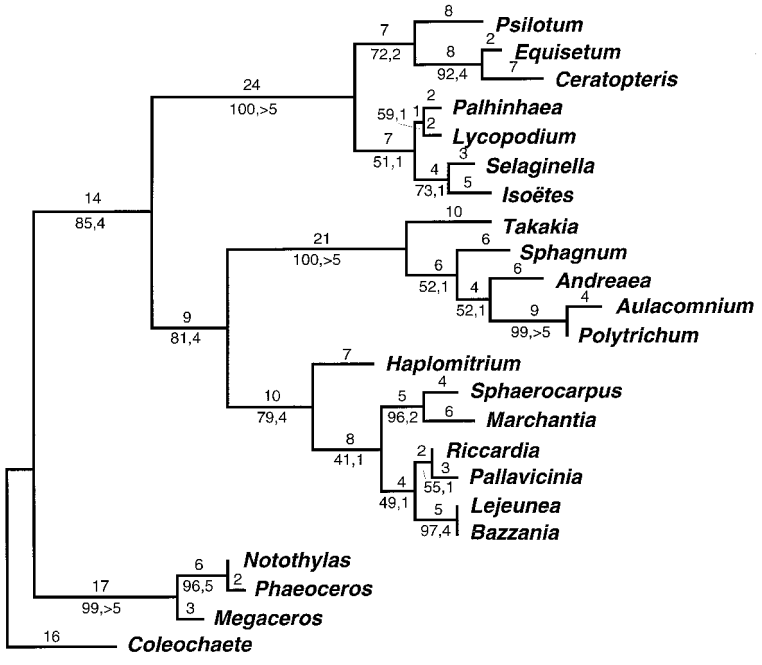


Figure 11. The single most parsimonious tree of length 264 (CI = 0.70, RI = 0.84) obtained from a maximum parsimony analysis (implemented with PAUP* 4.0) of 125 developmental and morphological characters. Numbers above each branch represent the number of characters supporting the branch. Bootstrap percentage values (100 replications) followed by decay indices are given below the branch.

involves spline growth (in association with nuclear shaping) and the second involves the direction of nuclear shaping. Other tree topologies had even less support, and when the liverworts were placed as the outgroup to the remaining land plants (i.e. the Mishler & Churchill (1984) hypothesis), the cladogram was five steps longer than the most parsimonious tree (TL = 169). For this arrangement, there were no characters that supported the clade consisting of all embryophytes minus liverworts.

The lack of congruence between the tree topology based on spermatogenesis (figure 10) and that based on overall morphology (figure 11) may be due to inherent problems in the spermatogenesis data set. First, because of convergences in cell architecture between spermatozoids of Charales and archegoniates, *Chara* may not be an ideal choice for an outgroup. However, spermatogenesis in *Coleochaete*, another potential charophyte outgroup, is insufficiently known, hence many characters cannot be coded for this genus. Moreover, based on the limited data available, spermatozoids of *Coleochaete* lack the presumed ancestral features of archegoniate sperm cells. For example, no coiling has been described in coleochaetalean spermatozoids. One could argue that the selective pressures for motile cells of land plants are decidedly different from those confronting aquatic algae and thus evolution of these cells in archegoniates was rapid and directional. Moreover, multicellular gametangia and the problems related to negotiating passage through an archegonial neck provided additional selective forces. As noted above, similarities in architecture of charalean and bryophyte spermatozoids may reflect similar structure of the oogonium with its crown cells and the archegonium with its neck cells and the common constraints of movement through a narrow channel.

Given the results of our successive MP analyses of the spermatogenesis data set, we regard the occurrence of bryophyte monophyly as an artefact. However, we are convinced that more detailed analyses of sperm cell ultrastructure in a wide range of charophycean algae will provide abundant data for a robust reanalysis and will enable refined evaluation of preadaptations in these cells that led to the architecture of sperm cells of basal embryophytes.

Our MP analyses have dealt only with land plants with flagellated sperm. The comparison of flagellated and non-flagellated sperm by Southworth & Cresti (1997) suggests that analogous features may be present in non-flagellated seed plants, and that there are some remnants of structural homology between the two architectures. However, there may be too few characters on which to attempt a phylogenetic analysis across the transition between these sperm types. The absence of comparable studies of spermatogenesis in gymnosperms and basal angiosperms is also an impediment to a critical analysis of non-flagellated sperm evolution.

## 8. PHYLOGENY INTERPRETED FROM MORPHOLOGY AND DEVELOPMENT

A comprehensive data set incorporating morphological, developmental and ultrastructural features was compiled to approach questions relating to the position of the three bryophyte groups within the global phylogeny of plants (Garbary & Renzaglia 1998). Specific characters to resolve relationships among groups of tracheophytes generally were not included. Of the 125 characters, 62 were coded for the gametophyte generation and 63 were coded for the sporophyte generation. To curtail overweighting of characters related to spermatogenesis, only 11 informative features of male gametogenesis were included. To help ensure character homology, features of gametophytes were considered independent of sporophytes.

The analysis presented here of 22 ingroup taxa represents a slightly revised character and organism matrix relative to Garbary & Renzaglia (1998). A single most parsimonious tree results from MP analysis of the morphological data (figure 11). This tree is highly resolved as shown by the fact that 13 of the 20 total ingroup clades

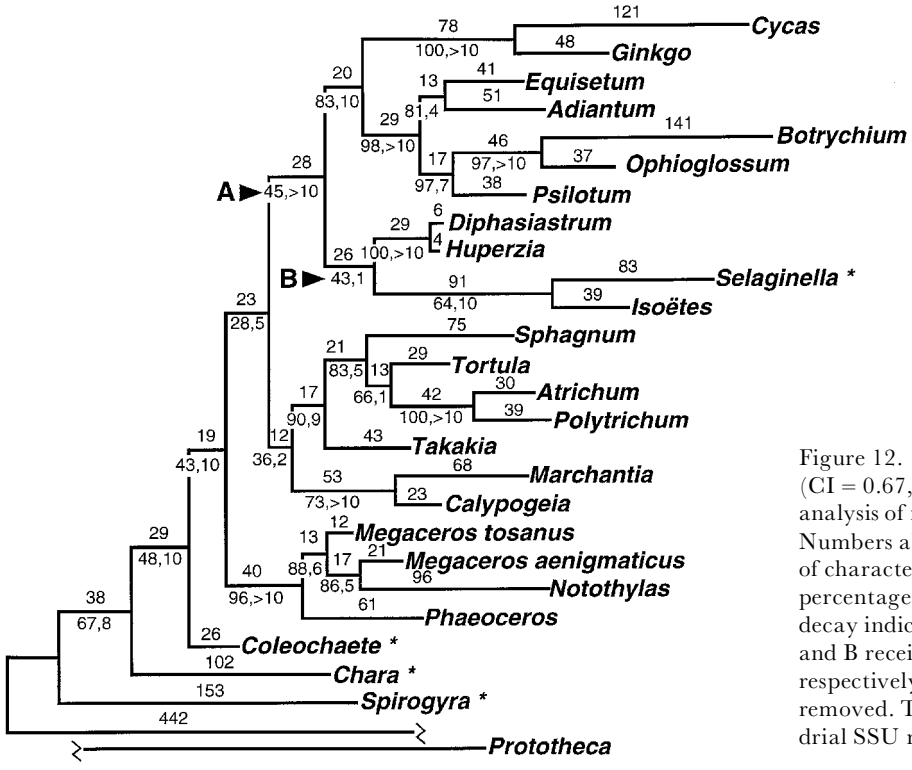


Figure 12. The single MP tree of length 2543 (CI = 0.67, RI = 0.53) resulting from a combined analysis of nuclear and mitochondrial SSU rDNA. Numbers above each branch represent the number of characters supporting the branch. Bootstrap percentage values (100 replications) followed by decay indices are given below the branch. Nodes A and B received BS support of 55% and 61%, respectively, when the outgroup *Prototheca* was removed. Taxa with an asterisk (*) lack mitochondrial SSU rDNA sequences.

receive BS support of 70% or greater. Strong BS support exists for the basal position of hornworts, the monophyly of hornworts, mosses and liverworts, the moss/liverwort clade, tracheophytes and ferns. Among these primary clades, only the separation of lycophytes from other tracheophytes received weak support (51% BS, decay index of 1). The remaining weakly supported nodes exist mainly within the moss and liverwort clades. Despite different taxon sampling, this phylogram is generally congruent with the analysis of spermatogenous characters (figure 10) with the following three exceptions: (i) the position of hornworts as sister to the remaining land plants; (ii) the relative positions of *Psilotum* and *Equisetum*; and (iii) the resolution of relationships within the moss clade. The two primary features of this cladogram are the position of hornworts basal to all other land plants and the position of mosses and liverworts as monophyletic and sister to tracheophytes.

The characters supporting the individual clades are largely discussed by Garbary & Renzaglia (1998) and will not be repeated here. The primary branching within land plants (i.e. embryophytes minus hornworts) is supported by four synapomorphies: (i) the change from a dorsiventral to an axial gametophyte; (ii) the presence of flagellar staggering; (iii) the presence of flavonoids; and (iv) the occurrence of grana end membranes. The positions of *Megaceros* and *Takakia* are of interest as these taxa lack stomata and they are the apparent basal clades of both hornworts and mosses, respectively. This provides strong support for our contention that stomata may have evolved several times within land plants. A similar tree topology is supported by our molecular analysis (below) with respect to the position of *Takakia* but not the relationships within hornworts.

Manipulations of the cladogram in figure 11 using MacClade do not provide even weak support for any other phylogenetic hypotheses. Thus, forcing bryophyte monophyly (i.e. the spermatogenesis hypothesis) produces a cladogram three steps longer (TL = 267); making mosses sister to tracheophytes adds four steps (TL = 268); reproducing the Mishler & Churchill (1984) hypothesis (i.e. liverworts sister to remaining embryophytes) was seven steps longer (TL = 271). Thus the total morphology tree supports the primary features of the tree from spermatogenesis. Based on the nature of the characters supporting the primary clades we consider the cladogram in figure 11 to be an accurate representation of relationships at the base of the land plant clade. It is also congruent with previously published trees based on molecular data (see § 9).

## 9. PHYLOGENY INTERPRETED FROM NUCLEAR AND MITOCHONDRIAL SSU rDNA

To test hypotheses based on morphology and to evaluate morphological character evolution, we conducted analyses of 22 land plants using a data set combining sequences from nuclear and mitochondrial SSU rDNA. This MP analysis includes previously published sequences in addition to new sequences including *Psilotum*, *Megaceros aenigmaticus*, *Polytrichum* and *Cycas* for mitochondrial SSU rDNA and *Megaceros tosanus* for nuclear SSU rDNA (see Duff & Nickrent (1999) for details on methods). Mitochondrial SSU rDNA sequences were not available for *Spirogyra*, *Chara*, *Coleochaete* and *Selaginella*, so these taxa were scored as 'missing' in the data matrix. The single tree resulting from a heuristic search of 3647 characters (562 informative) shows the same basic topology as the total morphology tree (figure 12). The tree is well-resolved with 14 of the total 21 ingroup nodes receiving BS support of 70% or more. Strong BS support exists for the monophyly of hornworts, mosses, liverworts and ferns, but

unlike the morphological tree (figure 11), only weak support is obtained for the basal position of hornworts among land plants (BS 43%), a moss–liverwort clade (BS 36%) and tracheophytes (BS 45%). It is of interest that BS support for the tracheophyte clade (A in figure 12) increases to 55% and the lycophyte clade (B in figure 12) increases to 61% when the distant outgroup *Prototheca* is removed from the analysis. Support for these and other relationships, including the basal position of hornworts, is greatly strengthened by the addition of mitochondrial SSU rDNA sequences of algal outgroups that are more closely related to embryophytes than *Prototheca* (e.g. *Chara*) (C. L. Parkinson and J. D. Palmer, unpublished data). This tree topology is also congruent with the maximum likelihood tree derived from the mitochondrial SSU rDNA data alone (Duff & Nickrent 1999). Additional analyses utilizing more genes and greater taxon sampling point to even stronger support for both the basal position of the hornworts and the sister relationship of liverworts and mosses (D. L. Nickrent, R. J. Duff, C. L. Parkinson and J. D. Palmer, unpublished data).

Despite poor bootstrap support for some critical basal clades in the present analysis, rearrangements of major branches in figure 12 to match other proposed topologies result in even weaker support. For example, a cladogram congruent with the Mishler & Churchill (1984) hypothesis is six steps longer than the MP tree, and a monophyletic bryophyte topology is eight steps longer. It has become apparent that the phylogenetic signal present in nuclear SSU rDNA is low, especially for deeper nodes on the land plant tree (Soltis *et al.* 1999); thus this partition contributes a significant amount of homoplasy to a combined data set.

## 10. CONGRUENCE OF MORPHOLOGICAL AND MOLECULAR PHYLOGENIES

The three separate data sets are largely congruent and point to a single topology for the relationships of the major groups of extant embryophytes. The key features of this phylogenetic hypothesis are as follows: (i) that hornworts, liverworts, mosses, lycophytes and the remaining pteridophytes and seed plants are each monophyletic; (ii) that hornworts are sister to the remaining land plants; (iii) that mosses plus liverworts are monophyletic; and (iv) that this moss/liverwort clade is sister to another comprising tracheophytes. Of these features it is the basal position of the hornworts and the sister group relationship between mosses and liverworts that seem to be most debated.

Because of their universal occurrence, SSU rDNA genes have been utilized extensively in phylogenetic studies of the living world, and specifically nuclear SSU rDNA sequences are informative in reconstructing plant, algal and protist phylogenies (Soltis & Soltis 1998; Soltis *et al.* 1999). Our primary conclusions are congruent with nuSSU rDNA phylogenies (e.g. Hedderson *et al.* 1998; Soltis *et al.* 1999), although it should be noted that some analyses of this gene have given radically different results (e.g. Bopp & Capesius 1996; Capesius & Bopp 1997). Previous molecular analyses have also supported the basic tree topologies shown in figures 11 and 12. Malek *et al.* (1996) derived a sister group relationship between mosses and liverworts by using sequences of the mitochondrial *cox3* gene. Steinhauser *et al.* (1999) and Beckert *et al.* (2000) arrived at the same conclusion based on the joint possession of a group I intron and a phylogenetic analysis of mitochondrial *nad5* sequences. A recent study employing five genes from the chloroplast genome (but with limited taxon sampling) also derived the same basic tree topology (Nishiyama & Kato 1999). Although a number of molecular analyses and biochemical characters have produced conclusions that are not consistent with our tree (reviewed by Qiu & Palmer 1999), these were often compromised by small numbers of taxa, short genes (or incomplete sequences), saturated third positions in protein-coding genes, rate heterogeneity and problematic analyses (e.g. Van de Peer *et al.* 1990; Waters *et al.* 1992; Manhart 1994; Mishler *et al.* 1994; Bopp & Capesius 1996; Lewis *et al.* 1997). Thus, we do not consider them compelling refutations of our primary conclusions. The distribution of mitochondrial introns discussed by Qiu *et al.* (1998) supports liverworts as the basalmost land plant lineage, and is thus inconsistent with our conclusion. It should be noted that their data set has a high level of homoplasy as several lineages higher in the cladogram also possess the same character state as liverworts. Thus, the absence of these three introns can be explained as arising from a loss in the common ancestor to liverworts. Given our limited understanding of mitochondrial genome evolution in plants, we suggest that phylogenetic relationships based on mitochondrial introns be inferred with caution.

## 11. INFERENCES ABOUT LAND PLANT EVOLUTION

It is premature to enumerate detailed speculations on morphological transformation series in bryophytes. A number of hypotheses have been laid out in the above discussion and these will require rigorous testing as robust molecular and total evidence analyses on more taxa become available. In this section we make limited inferences about character evolution based on the consensus topology outlined in §10.

Monosporangiate sporophytes and continuity in gametophyte features such as apical organization and gametangial development support bryophytes as basal land plant lineages. Thalloid gametophytes occur in hornworts and as such are best viewed as plesiomorphic among land plants. Early patterns in sporeling development suggest that an axial, erect habit is ancestral to the prostrate growth form of many mature liverwort and pteridophyte gametophytes (Whittier & Braggins 1992; Bartholomew-Began 1991; Bartholomew & Crandall-Stotler 1986). Leafy buds that originate from moss protonemata are also upright. Thus, the moss/liverwort progenitor was probably a branched, upright, leafless axis. Lateral appendages, 'leaves' and the procumbent habit apparently evolved a number of independent times in gametophytes of mosses and liverworts. Parallel acquisition of 'leaves' in such taxa as *Takakia*, true mosses, *Haplomitrium*, *Phyllothallia*, *Fossombronia* and Jungermanniales is supported by divergent developmental strategies as well as our phylogenetic analyses.

Sporophyte reduction has occurred in all three bryophyte lineages and in our analyses is best illustrated by the hornwort *Notothylas*. Both the molecular and morphological trees support the hypotheses that the highly

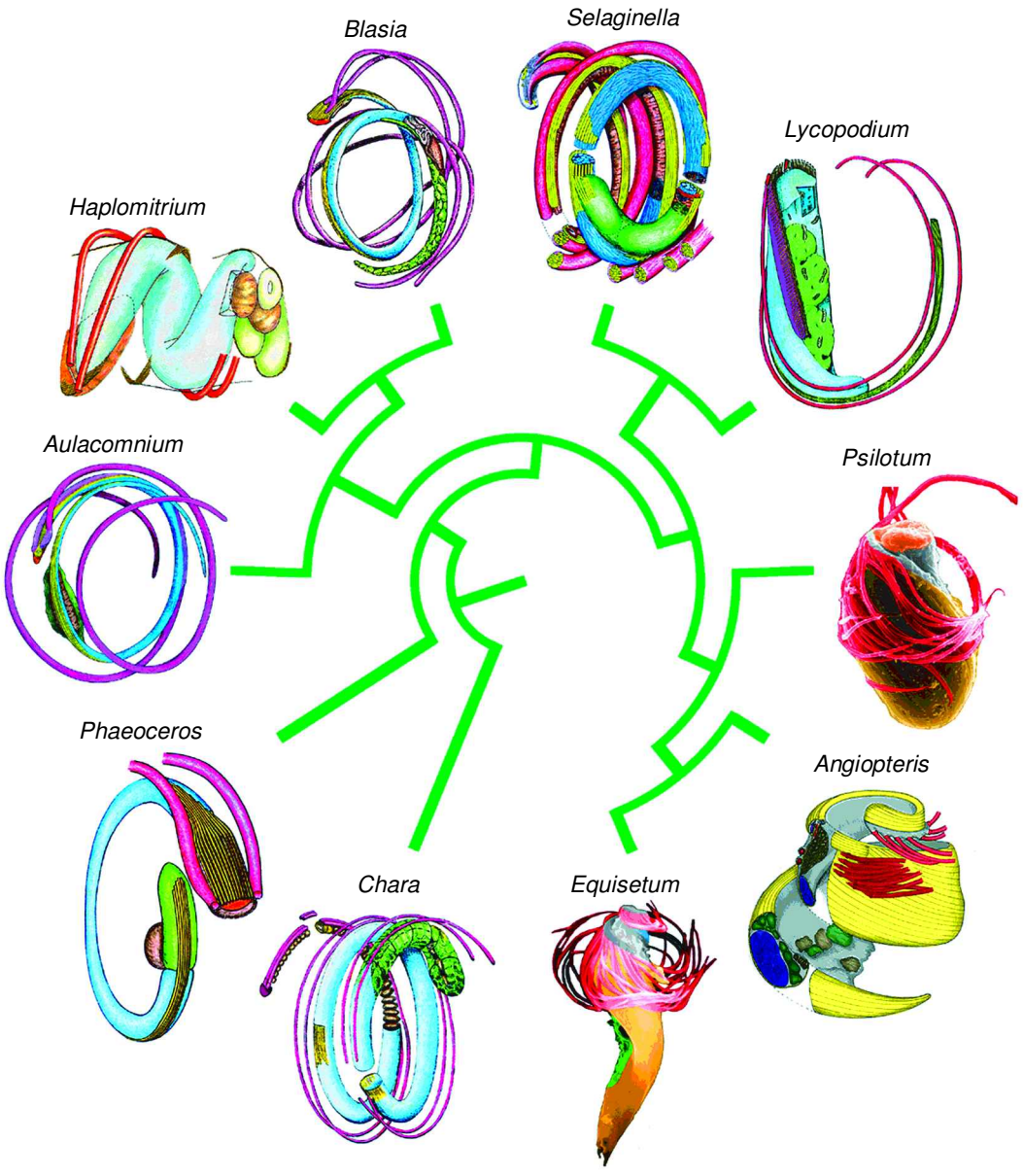


Figure 13. Sperm cell architecture mapped onto a cladogram based on total morphology and nuclear plus mitochondrial SSU rDNA sequences. See § 11 for explanation of the figure. Colour coding: red = flagella and basal bodies; blue = nucleus; brown = mitochondria; yellow = spline microtubules; orange or purple = lamellar strip; green = plastid; grey = extraneous cytoplasm.

simplified sporophyte of *Notothylas* is derived from the more complicated sporophytic structure that typifies the group. This concept was promoted by traditional morphologists (Schuster 1984*c*, 1992; Proskauer 1960; Lang 1907; Campbell 1895; Bartlett 1928) but has been called into question by more contemporary neontologists (Mishler & Churchill 1985; Graham 1993). *Notothylas* occurs in disturbed sites that presumably select for rapid generation time and reduced complexity of both life cycle phases. In general, the rate of genomic evolution, and consequently phenotypic change, is greater in small organisms that grow and reproduce rapidly, a phenomenon that in turn is often correlated with low genome size (Niklas 1997; John & Miklos 1988; Renzaglia *et al.* 1995). Thus, the interpretation of small ephemeral taxa such as *Notothylas* among anthocerotes and *Riccia* among hepatics as derived is consistent with life history phenomena and the seasonal instability experienced by these plants.

Homology of stomata is assumed in many morphological phylogenies (Kenrick & Crane 1997*a*,*b*; Mishler & Churchill 1984, 1985). Developmental, physiological and morphological differences among stomata of hornworts, mosses and tracheophytes as well as the occurrence of these structures in specialized and restricted mosses and hornworts suggest that reappraisal of stomate homology is warranted. Interpretation of independent acquisition of stomata in three lineages is equally parsimonious with the view that stomata evolved once in embryophytes and were lost in liverworts, three hornwort genera and basal mosses. The speculation that bryophyte groups diverged prior to elongation and elaboration of sporophytes supports the contention that stomata are homoplastic in these plants; there would be no selective forces favouring

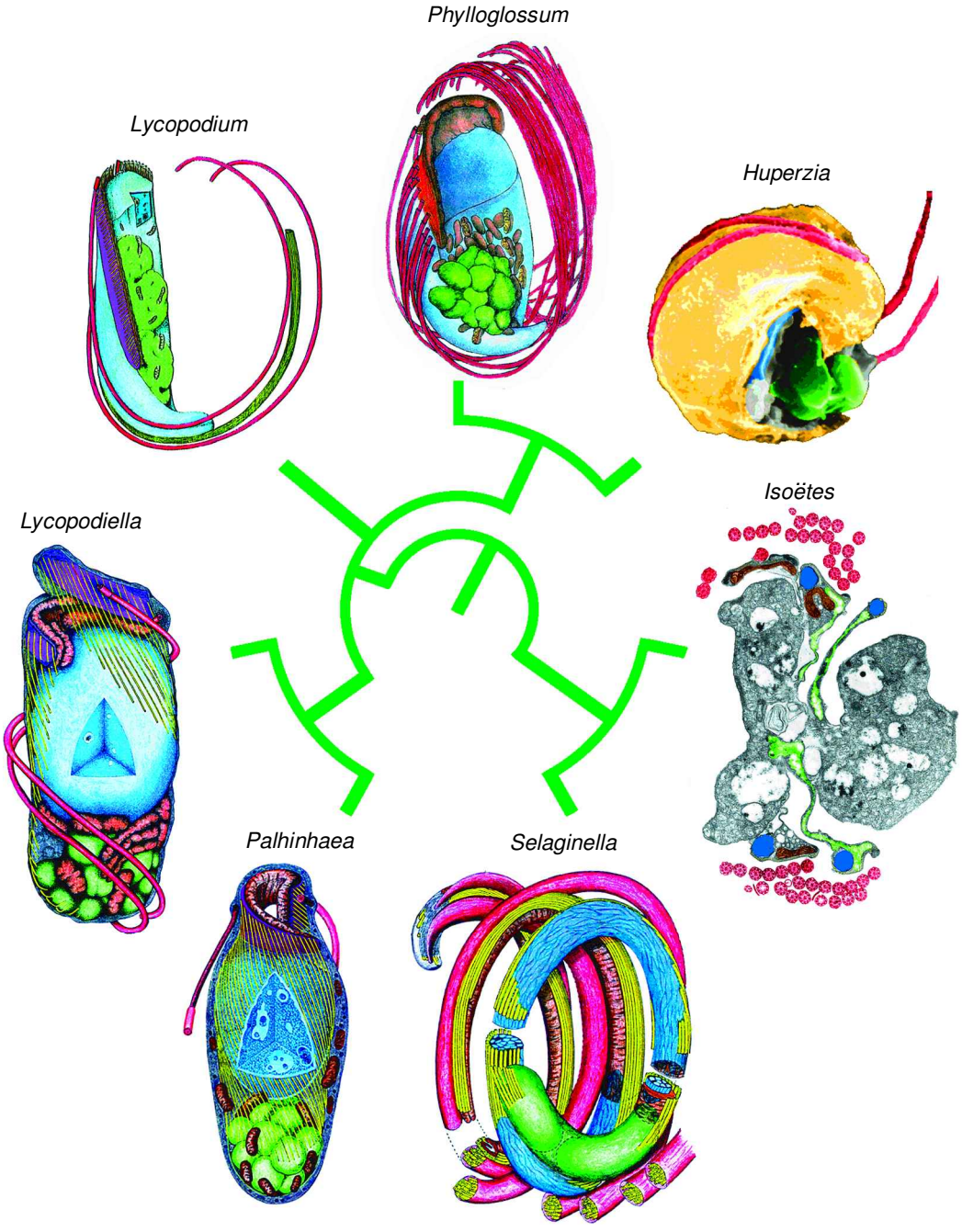


Figure 14. Lycophyte sperm cell architecture mapped onto a cladogram derived from spermatogenesis and *rbcL* sequences (Wikström & Kenrick 1997). Multiflagellated spermatozoids have evolved two independent times in lycophytes, once in the heterosporous *Isoëtes* and once in the homosporous *Phylloglossum*. Within Lycopodiaceae, biflagellated sperm cells of *Lycopodium* and *Huperzia* are minute and coiled while those of *Lycopodiella* and *Palhinhaea* are more ovoid.

stomate evolution in the unexpanded, ephemeral ancestral sporophyte. Modern comprehensive comparative studies are required to further test stomate homology among bryophytes and basal pteridophytes.

Ultrastructural data on food-conducting cells support a moss–liverwort clade and provide refutation of the precept that leptoids in mosses are homologous to sieve cells of tracheophytes. Because water-transporting cells probably evolved at least three times in liverworts and twice in mosses (Ligrone *et al.*, this issue), care must be exercised in interpreting homology of these cells among taxa.

Scrutiny of sperm cell structure and evaluation of evolutionary trends based on molecular and morphological trees reveals examples of parallelisms, divergences and convergences among archegoniates (figures 13 and 14). The plesiomorphic architecture of plant spermatozoids is a slightly coiled biflagellated cell. Presumably, increased compaction and coiling occurred independently in several lineages, including the Charales, mosses, liverworts, heterosporous lycophytes and ferns (figure 13). Hornwort sperm cells remained small and diverged from other bryophytes before flagellar staggering evolved. An

autapomorphy of hornworts is the reverse in directionality of coiling, which, as noted above, was probably inconsequential to the hydrodynamics of this minute cell. With resolution of a moss–liverwort clade, the detailed similarities in locomotory apparatus microstructure between these two groups are interpreted as homologous. Flagellar staggering involving dimorphic basal bodies and an aperture in the spline were features of the prototypic moss–liverwort sperm cell that further specialized after historical separation of the two lineages (Renzaglia *et al.* 1995).

Multiflagellated sperm cells evolved within three separate lineages of pteridophytes: in *Phylloglossum*, a homosporous lycophyte (Renzaglia & Maden 2000), in *Isoëtes*, a heterosporous lycophyte and in the *Equisetum–Psilotum*–fern–seed plant lineage (figures 13 and 14). Additional specializations within the lycophytes include decreased coiling and increased flagellar stagger in taxa with subterranean gametophytes, and extreme streamlining in heterosporous taxa. Commonalities in sperm cell development and organization provide strong support for a fern–*Equisetum–Psilotum* clade, an assemblage that has been proposed by other contemporary analyses (Kenrick & Crane 1997*a*,*b*; Duff & Nickrent 1999).

## 12. CONCLUSIONS AND PROSPECTS

Morphological and molecular data have now converged to provide resolution of the problem of bryophyte phylogeny in the context of the evolution of basal land plants. Phylogenetic analyses of total evidence, two morphological and one molecular data set, provide support for hornworts as the basalmost clade of extant terrestrial organisms. Furthermore, there is reasonable support for the recognition of mosses and liverworts as sister groups. Although these conclusions are contrary to some current interpretations, we feel that they provide the best explanations of character homology and adaptive radiation within the three bryophyte groups and between these clades and tracheophytes.

The ongoing difficulties in resolving precise relationships among basal land plants are in part due to their rapid radiation in a virtually uninhabited ancient landscape and in part to the limitations of data sets. Given the limited number of surviving lineages in basal embryophytes, the huge gaps between charophycean algae and land plants, and between bryophytes and lycophytes (among extant organisms), morphological homology may remain difficult to resolve. Conflicting data may reflect the almost simultaneous radiation of the bryophyte lineages and considerable convergence based on common evolutionary potential. Resolution of these problems requires clear phylogenetic hypotheses and the willingness to analyse homology based on developmental and ultrastructural features and not on superficial functional similarities of mature structures or organs. Thus we argue that the homologies in stomatal and transport cell characters that were previously used to support the ancestral liverwort hypothesis are untenable.

Molecular data are rapidly accumulating, and we are currently analysing sequences of multiple genes from all three plant genomes (i.e. nucleus, mitochondrion and chloroplast) sampled from all appropriate extant lineages. From these data, and from combined analyses with morphological data, a more resolved phylogeny should emerge that will probably mirror our general conclusions. Such congruence will allow the phylogenetic debate to proceed beyond that of branching patterns so that plant biologists can investigate equally interesting questions in evolutionary biology, including rates and patterns of evolutionary change. With robust phylogenies and the accumulation of new morphogenetic and ultrastructural data on key taxa, we will be in a position to make more accurate and precise inferences about structural homologies in basal land plants.

This work would not have been possible without technical and conceptual contributions of present and previous students, collaborators and colleagues, who have enabled the development of our ideas. In particular we note the involvement of J. G. Duckett, D. P. Whittier, K. C. Vaughn, A. R. Maden, R. Ligrone, S. J. Schmitt, H. D. Gates, K. D. McFarland, D. K. Smith, H. A. Crum and L. E. Graham. This work was supported by grants to K.S.R., D.L.N. and D.J.G. from the National Science Foundation, USA (DEB-9207626, MCB-9808752 and DEB-9527735), and the Natural Sciences and Engineering Research Council, Canada. We thank J. B. Bozzola at the Micro-Imaging and Analysis Center, SIUC, and Thomas Johnson for technical assistance in preparing the manuscript.

## REFERENCES

Bartholomew, S. E. & Crandall-Stotler, B. J. 1986 The sporeling ontogeny of *Monoclea gottschei* subsp. *elongata*. *Bryologist* **97**, 244–252.

Bartholomew-Began, S. E. 1991 A morphogenetic re-evaluation of *Haplomitrium* Nees (Hepatophyta). *Bryophytorum Bibliotheca*, Band 41. Berlin: J. Cramer.

Bartlett, E. M. 1928 The comparative study of the development of the sporophyte in the Anthocerotaceae, with special reference to the genus *Anthoceros*. *Ann. Bot.* **42**, 409–430.

Bateman, R. M. & DiMichele, W. A. 1994 Heterospory: the most iterative key innovation in the history of the plant kingdom. *Biol. Rev.* **69**, 345–417.

Bateman, R. M., Crane, P. R., DiMichele, W. A., Kenrick, P. R. & Rowe, N. P. 1998 Early evolution of land plants: phylogeny, physiology and ecology of the primary terrestrial radiation. *A. Rev. Ecol. Syst.* **29**, 263–292.

Beckert, S., Steinhauser, S., Muhle, H. & Knoop, V. 2000 A molecular phylogeny of bryophytes based on nucleotide sequences of the mitochondrial sequences of the mitochondrial *nad5* gene. *Pl. Syst. Evol.* **218**, 179–192.

Bell, P. R. 1994 *Green plants: their origin and diversity*. Portland, OR: Dioscorides Press.

Bernhard, D. L. & Renzaglia, K. S. 1995 Spermiogenesis in the moss *Aulacomnium palustre*. *Bryologist* **98**, 52–70.

Bierhorst, D. W. 1971 *Morphology of vascular plants*. New York: MacMillan.

Blackmore, S. & Barnes, S. H. 1987 Embryophyte spore walls: origin, development, and homologies. *Cladistics* **3**, 185–195.

Bold, H. C., Alexopoulos, C. J. & Delevoryas, T. 1987 *Morphology of plants and fungi*, 5th edn. New York: MacMillan.

Bopp, M. & Capesius, I. 1996 New aspects of bryophyte taxonomy provided by molecular approach. *Bot. Acta* **109**, 1–5.

Bremer, K., Humphries, C. J., Mishler, B. D. & Churchill, S. P. 1987 On cladistic relationships in green plants. *Taxon* **36**, 339–349.

Brown, R. C. & Lemmon, B. E. 1988 Sporogenesis in bryophytes. *Adv. Bryol.* **3**, 159–223.

Brown, R. C. & Lemmon, B. E. 1990*a* Monoplastidic cell division in lower land plants. *Am. J. Bot.* **77**, 559–571.

Brown, R. C. & Lemmon, B. E. 1990*b* Sporogenesis in bryophytes. In *Microspores: evolution and ontogeny* (ed. S. Blackmore & R. V. Knox), pp. 55–94. London: Academic Press.

Brown, R. C. & Lemmon, B. E. 1993 Diversity of cell division in simple land plants holds clues to evolution of the mitotic and cytokinetic apparatus in higher plants. *Mem. Torrey Bot. Club* **25**, 45–62.

Brown, R. C. & Lemmon, B. E. 1997 The quadripolar microtubule system in lower land plants. *J. Plant Res.* **110**, 93–106.

Brown, R. C., Lemmon, B. E. & Renzaglia, K. S. 1986 Sporocytic control of spore wall patterns in liverworts. *Am. J. Bot.* **73**, 593–596.

Campbell, D. H. 1895 *The structure and development of mosses and ferns (Archegoniatae)*. New York: Macmillan.

Capesius, I. & Bopp, M. 1997 New classification of liverworts based on molecular and morphological data. *Pl. Syst. Evol.* **207**, 87–97.

Carothers, Z. B. & Duckett, J. G. 1980 The bryophyte spermatozoid: a source of new phylogenetic information. *Bull. Torrey Bot. Club* **107**, 281–297.

Crandall-Stotler, B. J. 1980 Morphogenetic designs and a theory of bryophyte origins and divergence. *BioScience* **30**, 580–585.

Crandall-Stotler, B. J. 1984 Musci, hepatics and anthocerotes—an essay on analogues. In *New manual of bryology*, vol. 2 (ed. R. M. Schuster), pp. 1093–1129. Nichinan, Japan: Hattori Botanical Laboratory.

Crandall-Stotler, B. J. 1986 Morphogenesis, developmental anatomy and bryophyte phylogenetics; contraindications of monophyly. *J. Bryol.* **14**, 1–23.

Crum, H. A. & Anderson, L. E. 1981 *Mosses of eastern North America*, vols. I–II. New York: Columbia University Press.

Duckett, J. G. 1970 Sexual behaviour of the genus *Equisetum*, subgenus *Equisetum*. *Bot. J. Linn. Soc.* **63**, 327–352.

Duckett, J. G. & Duckett, A. R. 1980 Reproductive biology and population dynamics of wild gametophytes of *Equisetum*. *Bot. J. Linn. Soc.* **79**, 205–210.

Duckett, J. G. & Ligrone, R. 1995 The formation of catenate foliar gemmae and the origin of oil bodies in the liverwort *Odotoschisma denudatum* (Mart.) Dum. (Jungermanniales): a light and electron microscope study. *Ann. Bot.* **76**, 405–419.

Duckett, J. G. & Renzaglia, K. S. 1988 Cell and molecular biology of bryophytes: ultimate limits to the resolution of phylogenetic problems. *Bot. J. Linn. Soc.* **98**, 225–246.

Duckett, J. G., Renzaglia, K. S. & Pell, K. 1990 Desiccation causes the proliferation of multicellular hairs but not mucilage papillae in *Cryptothallus mirabilis*: a correlated light and electron microscope study. *Can. J. Bot.* **68**, 697–706.

Duckett, J. G., Renzaglia, K. S. & Pell, K. 1991 A light and electron microscope study of rhizoid–ascomycete associations and flagelliform axes in British hepatics with observations on the effects of the fungi on host morphology, and comparisons with mycorrhizae in the Ericaceae. *New Phytol.* **118**, 233–257.

Duff, J. R. & Nickrent, D. L. 1999 Phylogenetic relationships among land plants using mitochondrial small-subunit rDNA sequences. *Am. J. Bot.* **86**, 372–386.

Duncan, T. M., Renzaglia, K. S. & Garbary, D. J. 1997 Ultrastructure and phylogeny of the spermatozoid of *Chara vulgaris* (Charophyceae). *Pl. Syst. Evol.* **204**, 125–140.

Fulford, M. 1956 The young stages of the leafy Hepaticae: a resumé. *Phytomorphology* **6**, 199–235.

Garbary, D. J. & Renzaglia, K. S. 1998 Bryophyte phylogeny and the evolution of land plants: evidence from development and ultrastructure. In *Bryology for the twenty-first century* (ed. J. W. Bates, N. W. Ashton & J. G. Duckett), pp. 45–63. Leeds: The British Bryological Society and Maney Publishing.

Garbary, D. J., Renzaglia, K. S. & Duckett, J. G. 1993 The phylogeny of land plants: a cladistic analysis based on male gametogenesis. *Pl. Syst. Evol.* **188**, 237–269.

Gifford, E. M. & Foster, A. S. 1988 *Morphology and evolution of vascular plants*, 3rd edn. New York: Freeman.

Gould, S. J. 1989 *Wonderful life—the Burgess Shale and the nature of history*. New York: W. W. Norton & Co.

Graham, L. E. 1993 *Origin of land plants*. New York: John Wiley.

Gunning, B. E. & Steer, M. W. 1996 *Plant cell biology: structure and function*. Sudbury, MA: Jones and Bartlett Publishers.

Hanson, D. T., Swanson, S., Graham, L. E. & Sharkey, T. D. 1999 Evolutionary significance of isoprene emission from mosses. *Am. J. Bot.* **86**, 634–639.

Hébant, C. 1977 The conducting tissues of bryophytes. *Bryophytorum Bibliotheca*, vol. 10. Vaduz: Cramer.

Hedderson, T. A., Chapman, R. & Cox, C. J. 1998 Bryophytes and the origins and diversification of land plants: new evidence from molecules. In *Bryology for the twenty-first century* (ed. J. W. Bates, N. W. Ashton, & J. G. Duckett), pp. 65–77. Leeds: The British Bryological Society and Maney Publishing.

Hemsley, A. R. 1994 The origin of the land plant sporophyte: an interpolational scenario. *Biol. Rev.* **69**, 263–273.

John, B. & Miklos, G. 1988 *The eukaryote genome in development and evolution*. London: Allen and Unwin.

Kenrick, P. & Crane, P. R. 1997*a* *The origin and early diversification of land plants: a cladistic study*. Washington, DC: Smithsonian Institution Press.

Kenrick, P. & Crane, P. R. 1997*b* The origin and early evolution of plants on land. *Nature* **389**, 33.

Korall, P., Kenrick, P. & Therrien, J. P. 1999 Phylogeny of Selaginellaceae: evaluation of generic/subgeneric relationships based on *rbcL* gene sequences. *Int. J. Plant Sci.* **160**, 585–594.

Lang, W. H. 1907 On the sporogonium of *Notothylas*. *Ann. Bot.* **21**, 201–210.

Lewis, L. A., Mishler, B. D. & Vilgalys, R. 1997 Phylogenetic relationships of the liverworts (Hepaticae), a basal embryophyte lineage, inferred from nucleotide sequence data of the chloroplast gene *rbcL*. *Mol. Phyl. Evol.* **7**, 377–393.

Ligrone, R., Duckett, J. G. & Renzaglia, K. S. 1993 The gametophyte–sporophyte junction in land plants. *Adv. Bot. Res.* **19**, 231–317.

McCourt, R. M. 1995 Green algal phylogeny. *Trends Evol. Ecol.* **10**, 159–163.

Maddison, W. P. & Maddison, D. R. 1992 *MacClade: analysis of phylogeny and character evolution, 3.0*. Sunderland, MA: Sinauer Associates.

Maden, A. R., Renzaglia, K. S. & Whittier, D. P. 1996 Ultrastructure of the spermatozoid of *Lycopodiella obscurum* (Lycopodiaceae). *Am. J. Bot.* **83**, 419–429.

Maden, A. R., Whittier, D. P., Garbary, D. J. & Renzaglia, K. S. 1997 Ultrastructure of the spermatozoid of *Lycopodiella lateralis* (R.Br.) B. Øllgaard (Lycopodiaceae). *Can. J. Bot.* **75**, 1728–1738.

Malek, O., Lättig, K., Hiesel, R., Brennicke, A. & Knoop, V. 1996 RNA editing in bryophytes and a molecular phylogeny of land plants. *EMBO J.* **15**, 1403–1411.

Manhart, J. R. 1994 Phylogenetic analysis of green plant *rbcL* sequences. *Mol. Phyl. Evol.* **3**, 114–127.

Manhart, J. R. 1995 Chloroplast 16S rDNA sequences and phylogenetic relationships of fern allies and ferns. *Am. Fern J.* **85**, 182–192.

Mishler, B. D. & Churchill, S. P. 1984 A cladistic approach to the phylogeny of 'bryophytes'. *Brittonia* **36**, 406–424.

Mishler, B. D. & Churchill, S. P. 1985 Transition to a land flora: phylogenetic relationships of the green algae and bryophytes. *Cladistics* **1**, 305–328.

Mishler, B. D., Lewis, L. A., Buchheim, M. A., Renzaglia, K. S., Garbary, D. J., Delwiche, C. F., Zechman, F. W.,

Kantz, T. S. & Chapman, R. L. 1994 Phylogenetic relationships of the 'green algae' and 'bryophytes'. *Ann. Mo. Bot. Gdn* **8**, 451–483.

Niklas, K. J. 1997 *The evolutionary biology of plants*. University of Chicago Press.

Nishiyama, T. & Kato, M. 1999 Molecular phylogenetic analysis among bryophytes and tracheophytes based on combined data of plastid coded genes and the 18S rRNA gene. *Mol. Biol. Evol.* **16**, 1027–1036.

Øllgaard, B. 1987 A revised classification of the Lycopodiaceae. *Opera Botan.* **92**, 153–178.

Oliver, M. J. & Wood, A. J. 1997 Desiccation tolerance in mosses. In *Stress-inducible processes in higher eukaryotic cells* (ed. T. Koval), pp. 1–26. New York: Plenum Press.

Pahnke, J., Goremykin, V., Bobrova, V., Troitsky, A., Antonov, A. & Martin, W. 1996 Utility of rDNA internal transcribed spacer sequences from the inverted repeat of chloroplast DNA in pteridophyte molecular phylogenetics. In *Pteridology in perspective* (ed. J. M. Camus, M. Gibby & R. J. Johns). Kew: Royal Botanic Gardens.

Paton, J. A. & Pearce, J. V. 1957 The occurrence, structure and functions of the stomata of British bryophytes. *Trans. Br. Bryol. Soc.* **3**, 228–259.

Pirozynski, K. A. 1981 Interactions between fungi and plants through the ages. *Can. J. Bot.* **59**, 1824–1827.

Pirozynski, K. A. & Malloch, D. W. 1975 The origin of land plants: a matter of mycotropism. *Biosystems* **6**, 153–164.

Proskauer, J. 1960 Studies on Anthocerotales. VI. On spiral thickenings in the columella and its bearing on phylogeny. *Phytomorphology* **10**, 1–19.

Pryer, K. M., Smith, A. R. & Skog, J. E. 1995 Phylogenetic relationships of extant ferns based on evidence from morphology and *rbcL* sequences. *Am. Fern J.* **85**, 205–282.

Qiu, Y. L. & Palmer, J. D. 1999 Phylogeny of early land plants: insights from genes and genomes. *Trends Pl. Sci.* **4**, 26–30.

Qiu, Y. L., Cho, Y., Cox, J. C. & Palmer, J. D. 1998 The gain of three mitochondrial introns identifies liverworts as the earliest land plants. *Nature* **394**, 671–674.

Remy, W., Gensel, P. G. & Hass, H. 1993 The gametophyte generation of some early Devonian land plants. *Int. J. Pl. Sci.* **54**, 35–58.

Renzaglia, K. S. 1978 A comparative morphology and developmental anatomy of the Anthocerotophyta. *J. Hattori Bot. Lab.* **44**, 31–90.

Renzaglia, K. S. 1982 A comparative developmental investigation of the gametophyte generation in the Metzgeriales (Hepatophyta). *Bryophytorum Bibliotheca*, vol. 24. Vaduz: J. Cramer.

Renzaglia, K. S. & Bartholomew, S. E. 1985 Sporeling development in *Fossombronia cristula* Aust. with special reference to apical organization and growth. *Bryologist* **88**, 337–343.

Renzaglia, K. S. & Duckett, J. G. 1987 Spermatogenesis of *Blasia pusilla*, from antheridial initial through mature spermatozoid. *Bryologist* **90**, 468–501.

Renzaglia, K. S. & Duckett, J. G. 1988 Different developmental processes underlie similar spermatozoid architecture in mosses, liverworts and hornworts. *J. Hattori Bot. Lab.* **64**, 219–236.

Renzaglia, K. S. & Duckett, J. G. 1989 Ultrastructural studies of spermatogenesis in the Anthocerotophyta. V. Nuclear *metamorphosis and the posterior mitochondrion of Notothylas orbicularis* and *Phaeoceros laevis*. *Protoplasma* **151**, 137–150.

Renzaglia, K. S. & Duckett, J. G. 1991 Towards an understanding of the difference between the blepharoplasts of mosses and liverworts, and comparisons with hornworts, biflagellate lycopods and charophytes: a numerical analysis. *New Phytol.* **117**, 187–208.

Renzaglia, K. S. & Garbary, D. J. 2000 Motile male gametes of land plants: diversity, development and evolution. *Crit. Rev. Pl. Sci.* (In the press.)

Renzaglia, K. S. & Maden, A. R. 2000 Microtubule organizing centers and the origin of centrioles during spermatogenesis in the pteridophyte *Phylloglossum*. *Micros. Res. Tech.* **49**. (In the press.)

Renzaglia, K. S. & Vaughn, K. C. 2000 Anatomy, development and classification of hornworts. In *The biology of bryophytes* (ed. J. Shaw & B. Goffinet), pp. 1–19. Cambridge University Press.

Renzaglia, K. S. & Whittier, D. P. 1993 Spermatozoids of *Phylloglossum drummondii* are multiflagellated. *Am. J. Bot.* **80**, 111. (Suppl.)

Renzaglia, K. S., Maden, A. R., Duckett, J. G. & Whittier, D. P. 1994 Monoplastidy in spermatogenesis of *Lycopodium obscurum*. *Can. J. Bot.* **72**, 1436–1444.

Renzaglia, K. S., Rasch, E. M. & Pike, L. M. 1995 Estimates of nuclear DNA content in bryophyte sperm cells: phylogenetic considerations. *Am. J. Bot.* **82**, 18–25.

Renzaglia, K. S., McFarland, K. D. & Smith, D. K. 1997 Anatomy and ultrastructure of the sporophyte of *Takakia ceratophylla* (Bryophyta). *Am. J. Bot.* **84**, 1337–1350.

Renzaglia, K. S., Dengate, S. B. & Bernhard, D. L. 1998 Architecture of the spermatozoid of *Selaginella australiensis*. *Am. Fern J.* **88**, 1–16.

Renzaglia, K. S., Bernhard, D. L. & Garbary, D. J. 1999 Developmental ultrastructure of the male gamete of *Selaginella*. *Int. J. Pl. Sci.* **160**, 14–28.

Robbins, R. R. & Carothers, Z. B. 1978 Spermatogenesis in *Lycopodium*: the mature spermatozoid. *Am. J. Bot.* **65**, 433–440.

Robert, D. 1974 Étude ultrastructurale de la spermiogenése, notamment de la différentiation de l'appareil nucléaire, chez le *Selaginella kraussiana* (Kunze) A. Br. *Annales des Sciences Naturalles, Botanique, Paris*, Série 12. **15**, 65–118.

Roth, D. 1969 Embryo und Embryotheca bei den Laubmoosen. Eine histogenetische und morphologische Untersuchung. *Biblioth. Bot.* **129**, 1–49. Pls. 1–9.

Roux, W. 1895 *Gesammelte Abhandlungen zur Entwicklungsmechanik der Organismen*. Leipzig: Engelmann.

Sack, F. D. & Paollilo, D. J. Jr. 1985 Incomplete cytokinesis in *Funaria stomata*. *Am. J. Bot.* **72**, 1325–1333.

Schofield, W. B. 1985 *Introduction to bryology*. New York: Macmillan.

Schuster, R. M. 1966 *The Hepaticae and Anthocerotae of North America, east of the hundredth meridian*, vol. 1. New York: Columbia University Press.

Schuster, R. M. 1979 The phylogeny of the Hepaticae. In *Bryophyte systematics* (ed. G. C. S. Clarke & J. G. Duckett), pp. 41–82. London: Academic Press.

Schuster, R. M. 1984*a* Comparative anatomy and morphology of Hepaticae. In *New manual of bryology*, vol. 2 (ed. R. M. Schuster), pp. 760–891. Nichinan, Japan: Hattori Botanical Laboratory.

Schuster, R. M. 1984*b* Evolution, phylogeny and classification of the Hepaticae. In *New manual of bryology*, vol. 2 (ed. R. M. Schuster), pp. 892–1070 Nichinan, Japan: Hattori Botanical Laboratory.

Schuster, R. M. 1984*c* Morphology, phylogeny and classification of the Anthocerotae. In *New manual of bryology*, vol. 2 (ed. R. M. Schuster), pp. 1071–1092. Nichinan, Japan: Hattori Botanical Laboratory.

Schuster, R. M. 1992 *The Hepaticae and Anthocerotae of North America*, vol. V. Chicago: Field Museum of Natural History.

Smith, D. K. & Davison, P. G. 1993 Antheridia and sporophytes in *Takakia ceratophylla* (Mitt.) Grolle: evidence for reclassification among the mosses. *J. Hattori Bot. Lab.* **73**, 263–271.

Smith, E. C. & Griffiths, H. 1986*a* The occurrence of the chloroplast pyrenoid is correlated with the activity of a $CO_2$-concentrating mechanism and carbon isotope discrimination in lichens and bryophytes. *Planta* **198**, 6–16.

Smith, E. C. & Griffiths, H. 1986*b* A pyrenoid-based carbon concentrating mechanism is present in terrestrial bryophytes of the class Anthocerotae. *Planta* **200**, 203–212.

Smith, G. M. 1955 *Cryptogamic botany. II. Bryophytes and pteridophytes*, 2nd edn. New York: McGraw-Hill.

Soltis, D. E. & Soltis, P. S. 1998 Choosing an approach and an appropriate gene for phylogenetic analysis. In *Molecular systematics of plants. II. DNA sequencing* (ed. D. E. Soltis, P. S. Soltis & J. J. Doyle), pp. 1–42. Boston, MA: Kluwer Academic Publishers.

Soltis, P. S., Soltis, D. E., Wolf, P. G., Nickrent, D. L., Chaw, S.-M. & Chapman R. L. 1999 The phylogeny of land plants inferred from 18S rDNA sequences: pushing the limits of rDNA signal? *Mol. Biol. Evol.* **16**, 1774–1784.

Southworth, D. & Cresti, M. 1997 Comparisons of flagellated and nonflagellated sperm in plants. *Am. J. Bot.* **84**, 1301–1311.

Steinhauser, S., Beckert, S., Capesius, I., Malek, O. & Knoop, V. 1999 Plant mitochondrial RNA editing. *J. Mol. Evol.* **48**, 303–312.

Stewart, W. N. & Rothwell, G. W. 1993 *Paleobotany and the evolution of plants*, 2nd edn. Cambridge University Press.

Taylor, T. N. & Taylor, E. L. 1993 *The biology and evolution of fossil plants*. Englewood Cliffs, NJ: Prentice-Hall.

van de Peer, Y., De Baere, R., Cauwenberghsm, J. & Wachter, R. 1990 Evolution of green plants and their relationships with other photosynthetic eukaryotes as deduced from 5S ribosomal RNA sequences. *Pl. Syst. Evol.* **170**, 85–96.

Vaughn, K. C. & Harper, J. D. 1998 Microtubule organizing centers and nucleating sites in land plants. *Int. Rev. Cytol.* **181**, 75–149.

Vaughn, K. C. & Renzaglia, K. S. 1998 Origin of bicentrioles in anthocerote spermatogenous cells. In *Bryology for the twenty-first century* (ed. J. W. Bates, N. W. Ashton & J. G. Duckett), pp. 189–203. Leeds: The British Bryological Society and Maney Publishing.

Vaughn, K. C., Campbell, E. O., Hasegawa, J., Owen, H. A. & Renzaglia, K. S. 1990 The pyrenoid is the site of ribulose 1–5-bisphosphate carboxylase/oxygenase accumulation in the hornwort (Bryophyta: Anthocerotae) chloroplast. *Protoplasma* **156**, 117–129.

Vaughn, K. C., Ligrone, R., Owen, H. A., Hasegawa, J., Campbell, E. O., Renzaglia, K. S. & Monge-Najera, J. 1992 The anthocerote chloroplast: a review. *New Phytol.* **120**, 169–190.

Vaughn, K. C., Sherman, T. D. & Renzaglia, K. S. 1993 A centrin homologue is a component of the multilayered structure in bryophytes and pteridophytes. *Protoplasma* **175**, 58–66.

Waters, D. A., Buchheim, M. A., Dewey, R. A. & Chapman, R. L. 1992 Preliminary inferences of the phylogeny of bryophytes from nuclear-encoded ribosomal RNA sequences. *Am. J. Bot.* **79**, 459–466.

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

Whittier, D. P. 1981 Gametophytes of *Lycopodium digitatum* (formerly *L. complanatum* var. *flabelliforme*) as grown in axenic culture. *Bot. Gaz.* **142**, 519–524.

Whittier, D. P. 1983 Gametophytes of *Ophioglossum engelmannii*. *Can. J. Bot.* **61**, 2369–2373.

Whittier, D. P. & Braggins, J. E. 1992 The young gametophyte of *Phylloglossum* (Lycopodiaceae). *Ann. Mo. Bot. Gdn* **79**, 730–736.

Whittier, D. P. & Peterson, R. L. 1984 Gametophytes of *Botrychium lunarioides* and their mucilage-coated rhizoids. *Can. J. Bot.* **62**, 2854–2860.

Whittier, D. P. & Thomas, R. D. 1993 Gametophytes and young sporophytes of *Botrychium jenmanii* in axenic culture. *Int. J. Pl. Sci.* **154**, 68–74.

Whittier, D. P. & Webster, T. R. 1986 Gametophytes of *Lycopodium lucidulum* from axenic culture. *Am. Fern J.* **76**, 48–55.

Wikström N. & Kenrick, P. 1997 Phylogeny of Lycopodiaceae (Lycopsida) and the relationships of *Phylloglossum drummondii* Kunze based on *rbcL* sequences. *Int. J. Pl. Sci.* **158**, 862–871.

Wolf, P. G. 1997 Evaluation of *atpB* nucleotide sequences for phylogenetic studies of ferns and other pteridophytes. *Am. J. Bot.* **84**, 1429–1440.

Wolf, P. G., Pryer, K. M., Smith, A. R. & Hasebe, M. 1998 Phylogenetic studies of extant pteridophytes. In *Molecular systematics of plants. II. DNA sequencing* (ed. D. E. Soltis, P. S. Soltis & J. J. Doyle), pp. 540–561. Boston, MA: Kluwer Academic Publishers.