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AUXIN REGULATION OF AXIAL GROWTH IN BRYOPHYTE SPOROPHYTES: ITS POTENTIAL SIGNIFICANCE FOR THE EVOLUTION OF EARLY LAND PLANTS[1]

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To identify developmental mechanisms that might have been involved in the evolution of axial sporophytes in early land plants, we examined the effects of auxin-regulatory compounds in the sporophytes of the hornwort *Phaeoceros personii*, the liverwort *Pellia epiphylla*, and the moss *Polytrichum ohioense*, members of the three divisions of extant bryophytes. The altered growth of isolated young sporophytes exposed to applied auxin (indole-3-acetic acid) or an auxin antagonist (*p*-chlorophenoxyisobutyric acid) suggests that endogenous auxin acts to regulate the rates of axial growth in all bryophyte divisions. Auxin in young hornwort sporophytes moved at very low fluxes, was insensitive to an auxin-transport inhibitor (N-[1-naphthyl]phthalamic acid), and exhibited a polarity ratio close to 1.0, implying that auxin moves by simple diffusion in these structures. Emerging liverwort sporophytes had somewhat higher auxin fluxes, which were sensitive to transport inhibitors but lacked any measurable polarity. Thus, auxin movement in liverwort sporophytes appears to result from a unique type of apolar facilitated diffusion. In young *Polytrichum* sporophytes, auxin movement was predominantly basipetal and occurred at high fluxes exceeding those measured in maize coleoptiles. In older *Polytrichum* sporophytes, acropetal auxin flux had increased beyond the level measured for basipetal flux. Insofar as acropetal and basipetal fluxes had different inhibitor sensitivities, these results suggested that moss sporophytes carry out bidirectional polar transport in different cellular pathways, which resembles the transport in certain angiosperm structures. Therefore, the three lineages of extant bryophytes appear to have evolved independent innovations for auxin regulation of axial growth, with similar mechanisms operating in moss sporophytes and vascular plants.

Key words: auxin; axial growth; bryophyte sporophytes; hornworts; liverworts; mosses; polar auxin transport.

Recent paleobotanical work and molecular sequence analyses have provided new insights into the evolution of early land plants (for reviews, see Graham, 1993; Kenrick and Crane, 1997; Niklas, 1997; Bateman et al., 1998; Graham et al., 2000). In particular, it is firmly established that ancient charophycean green algae gave rise to the early land plants (Graham, 1993; Karol et al., 2001). The fossil record from the Middle Ordovician shows the first microfossils, including obligate spore tetrads with sporopollenin-impregnated walls, imperforate cuticles, and narrow tubes, that can tentatively be attributed to land plants (Gray, 1985; Edwards and Wellman, 2001; Graham and Gray, 2001). The presence of obligate spore tetrads is consistent with the interpretation that the earliest land plants exhibited a bryophyte-grade of structural organization, at least with respect to spore morphology (Gray, 1985; Graham and Gray, 2001). Furthermore, available molecular sequence information supports the perspective that the three extant bryophyte lineages (hornworts, liverworts, and mosses) diverged earlier than the monophyletic lineage evolving into extant vascular plants (Qiu et al., 1998; Nickrent et al., 2000; Renzaglia et al., 2000; Karol et al., 2001). However, the macrofossil record for putative bryophytes is too fragmentary at present to confirm this perspective (Kenrick and Crane, 1997; Niklas, 1997; Bateman et al., 1998; Edwards, 2000; Goffinet, 2000; Kenrick, 2000).

Living charophycean algae have haplobiontic life cycles, with a dominant haploid gametophyte and a diploid phase solely consisting of the zygote that undergoes meiosis to produce four haploid zoospores (Graham and Wilcox, 2000); therefore, it is often proposed that the first land plants evolved a multicellular diploid embryo through the intercalation of mitotic divisions in the zygote prior to sporic meiosis (e.g., Graham, 1993; Hemsley, 1994; Graham and Wilcox, 2000). The origin of the embryo, and its subsequent elaboration into a complex axial sporophyte, was accomplished by several innovations critical to the widespread colonization of terrestrial environments: (1) jacketed sporangia capable of producing numerous meiospores and (2) erect axes that grow above the gametophyte and are well suited for aerial dispersal of those spores. In all extant nonseed plants except for a few genera of specialized aquatic liverworts, the sporophyte is elevated above the prostrate thallus, low-lying clump, or subterranean axis of the gametophyte. Moreover, complex sporophytes with erect axes containing vascular tissue have almost completely dominated fossil terrestrial flora ever since the early Devonian period ca. 400 million years ago (my BP) (Taylor and Taylor, 1993; Kenrick and Crane, 1997). Despite the obvious evolutionary significance of erect sporophytes, the botanical literature is silent about plausible physiological mechanisms that might have acted to generate the sporophytic axes of early land plants (Cooke et al., in press).

For several reasons, an examination of the axial sporophytes of extant bryophytes should reveal useful information for considering the evolutionary origins of these structures in the early land plants. First of all, because bryophytes represent the

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earliest divergent lineages of extant land plants, one bryophyte lineage may have retained the ancestral process that these plants used to generate axial sporophytes. Secondly, bryophyte embryos first develop as spherical or oblong structures, almost all of which will eventually undergo polarized growth to form the tripartite axes characteristic of mature bryophyte sporophytes (Smith, 1955; Bold et al., 1987; Crum, 2001). Bryophyte embryogenesis can thus be said to consist of an initial stage of spherical growth and a subsequent stage of axis elongation, which parallels the most plausible scenario for the evolution of early land plant sporophytes (Graham, 1993; Hemsley, 1994; Niklas, 1997). Finally, each bryophyte division utilizes unique morphological processes for generating its axes (Doyle, 1970; Crum, 2001), which raises the possibility that further investigation may disclose the common origin of the sporophytic axes of one bryophyte lineage and the vascular plants. Of particular interest is the opportunity to use developmental evidence in order to evaluate alternative hypotheses concerning the phylogenetic relationships among bryophyte lineages and other related lineages (Kenrick and Crane, 1997; Bateman et al., 1998; Qiu et al., 1998; Goffinet, 2000; Nickrent et al., 2000; Renzaglia et al., 2000; Karol et al., 2001; Delwiche et al., in press).

Because the embryos of all land plants, including angiosperms, have similar spherical and axial stages (Bold et al., 1987; Gifford and Foster, 1989; Cooke et al., in press), it is appropriate to utilize current knowledge about auxin regulation of axis elongation during angiosperm embryogenesis as the starting point for designing working hypotheses about the same process in young bryophyte sporophytes. In angiosperms, the hormone auxin (indole-3-acetic acid) regulates both phases of embryo development through several mechanisms such as alternative pathways for auxin biosynthesis, homeostatic control over auxin levels, and auxin concentration gradients (Cooke et al., in press; Ljung et al., 2002; Ribnicky et al., 2002). For example, a pronounced surge in free auxin levels appears to mediate the rapid cell proliferation during the initial stage of carrot zygotic embryogenesis (Ribnicky et al., 2002). Specific inhibitors of polar auxin transport are reported to block or alter subsequent polarized growth in the developing embryos of many angiosperms (Schiavone and Cooke, 1987; Liu et al., 1993; Fischer et al., 1997; Hadfi et al., 1998). Recent molecular investigations have substantiated the interpretation from physiological experiments that auxin acts as the key regulator of axis elongation during angiosperm embryogenesis (Souter and Lindsey, 2000; Hamann, 2001). For example, in *Arabidopsis*, *gnom* mutant embryos develop into enlarged spherical structures unable to initiate a polarized growth axis. The molecular basis of the *gnom* phenotype appears to be that the embryos fail to localize the auxin efflux carrier PIN1 in the proper position for carrying out polar auxin transport (Steinmann et al., 1999).

Unfortunately, few researchers have investigated auxin biosynthesis, movement, or action in bryophyte sporophytes (for review, see Cooke et al., 2002). In liverworts, auxin-treated setae elongate at more than twice the rates observed in control setae (Schnepf et al., 1979; Thomas, 1980). In addition, an auxin antagonist markedly reduced elongation rates of *Pellia* setae, which suggests very strongly that seta elongation is principally regulated by endogenous auxin under normal conditions. Nevertheless, agar-block studies involving long-term equilibration resulted in similar levels of auxin accumulation in both donor and receiver blocks, regardless of seta orientation (Thomas, 1980). These results hinted at the possibility that axial auxin movement is not polarized in *Pellia* setae; however, lateral auxin movement did appear to mediate phototropic curvature of these setae (Ellis and Thomas, 1985). The only observations available on the auxin responses of moss sporophytes come from the work of French and Paolillo (1975a), who observed that high levels of exogenous auxin could slightly increase the elongation of intact *Funaria* sporophytes growing attached to the gametophytes and could partially compensate for the inhibitory effect of apical decapitation under the same growth conditions. There is no current evidence of polar auxin transport being involved in axial growth of bryophyte sporophytes.

By contrast, in many structures of vascular plant sporophytes, auxin movement often occurs by polar transport in which auxin moves in a specific, generally basipetal, direction over a short distance through transporting cells (Goldsmith, 1977; Lomax et al., 1995). In the chemiosmotic model, the electrochemical H^+ gradient across the plasma membrane is the ultimate driving force for polar transport (Raven, 1974; Rubery and Sheldrake, 1974; Goldsmith, 1977). Apoplastic indole-3-acetic acid (IAA) in the cell wall (pH 5) is thought to cross the plasma membrane passively as protonated indole-3-acetic-acid (IAAH) (pKa 4.7) or via the IAA-influx carrier AUX1 acting as a proton symporter (Bennett et al., 1996) at the apical ends of transporting cells (Swarup et al., 2001). Indole-3-acetic acid in the cytosol (pH 7) is transported back into the apoplast via IAA-efflux carriers encoded by the PIN genes in *Arabidopsis* (Muller et al., 1998; Steinmann et al., 1999). Therefore, the polarity of auxin transport is usually attributed to the asymmetric localization of auxin carriers at opposite ends of the transporting cells (Jacobs and Gilbert, 1983; Estelle, 1998; Palme and Galweiler, 1999; Swarup et al., 2000).

Both the influx and efflux carriers are sensitive to several inhibitors. The compounds N-(1-naphthyl)phthalamic acid (NPA) and 2,3,5-triiodobenzoic acid (TIBA) have traditionally been used to inhibit the efflux component of the polar auxin transport mechanism (Thomson et al., 1973). The NPA acts as a phytotropin to inhibit both lateral and axial auxin transport, but the inhibitory effects of TIBA are restricted to axial transport (Lomax et al., 1995). The influx carrier is sensitive to (1-napthoxy)acetic acid (NOA) (Imhoff et al., 2000; Parry et al., 2001). Although these inhibitors were originally thought to act directly on the auxin carriers (Thomson et al., 1973; Lomax et al., 1995), more recent work has suggested that the efflux-carrier inhibitors may instead interfere with the membrane-trafficking system responsible for inserting the carriers into the plasma membrane (Geldner et al., 2001). Nevertheless, these inhibitors at low concentrations have their more pronounced effects on those developmental processes that depend on auxin transport.

The overall objective of this paper is to characterize the role that auxin plays in the axial elongation of the sporophytes of common plants representing the three divisions of extant bryophytes. Our aim is to use the information gathered to aid our consideration of the evolutionary origins of these structures in the early land plants. Intact sporophytes of the hornwort *Phaeoceros pearsonii* (Howe) Prosk., the liverwort *Pellia epiphylla* (L.) Corda, and the moss *Polytrichum ohioense* Renauld & Cardot were exposed to exogenous auxin as well as to an auxin antagonist to evaluate whether auxin acts to regulate axial elongation in these sporophytes. Conventional agar-block tech-

niques for measuring polar transport of radiolabelled auxin were modified to accommodate the small cross-sectional areas of bryophyte sporophytes. Those techniques were then applied to measure auxin transport in axial sections in the absence or presence of inhibitors that affect various transport steps. These experiments demonstrated that auxin plays distinctive roles in the regulation of axial growth of the sporophytes from different bryophyte divisions. This knowledge has significant implications for our considerations about the origins of axial sporophytes in the early land plants.

MATERIALS AND METHODS

Plant material—*Phaeoceros pearsonii* gametophytes bearing young sporophytes were collected on Thurber Road in Santa Cruz, California, USA, by Drs. Daniel Norris and William Doyle. *Pellia epiphylla* gametophytes with preemergent sporophytes were collected on the campus of the University of Pittsburgh at Bradford in Bradford, Pennsylvania, USA, with the assistance of Drs. Francis Mulcahy and Mary Puterbaugh. *Polytrichum ohioense* gametophytes with dormant sporophytes were collected on the campus of the University of Maryland in College Park, Maryland, USA. All three species were grown on soil from the original habitat at room temperature under ca. 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of fluorescent light until the young sporophytes reached the desired length of 1 cm. Older moss sporophytes 2–3 cm in length were also used in certain experiments. Coleoptiles of *Zea mays* cv. Jubilee (R H Shumways, Graniteville, South Carolina, USA) were grown in the dark for 4 d after which they received 8–10 h of red light at 2.46 $\mu mol \cdot m^{-2} \cdot s^{-1}$ before being returned to darkness until used. The coleoptiles were cut just above the soil line and then scored to remove the primary leaf from the center of the coleoptile so that only coleoptile tissues were used.

Growth response assays—Stock solutions (10^{-2} mol/L) of *p*-chlorophenoxyisobutyric acid (PCIB) (Aldrich Chemical Company, Milwaukee, Wisconsin, USA) or indole-3-acetic acid (IAA) (Sigma, St. Louis, Missouri, USA) were prepared in 95% ethanol and then were added to 0.7% cool molten phytoagar (Sigma) to obtain final concentrations of 10^{-5} mol/L in 100 × 15-mm polystyrene petri dishes. An equivalent amount of 95% ethanol was added to the control plates. Intact sporophytes of *Phaeoceros pearsonii*, *Pellia epiphylla*, and *Polytrichum ohioense* were carefully dissected from surrounding gametophytic tissue under a dissecting microscope. *Polytrichum* sporophytes were completely removed from the gametophyte, while the sporophyte of *Pellia epiphylla* and *Phaeoceros pearsonii* were dissected with minimal gametophytic tissue remaining near the foot that could not be removed because of the delicate nature of the structures. Then 25–30 sporophytes for each treatment were placed horizontally on the agar surface of the petri plates, their original lengths were measured with the ocular micrometer of a dissecting microscope at 75× magnification. The dishes were placed in the constant darkness except for brief intervals needed to measure their lengths under the dissecting microscope every 24 h. The data from each treatment of each species were presented as the mean net growth of the sporophytes at each 24-h interval ± the standard error among replicate sporophytes.

Auxin transport assays—The experiments designed to measure auxin transport in bryophyte sporophytes were carried out using conventional agar-block methods (McCready and Jacobs, 1963; Mitchell and Livingston, 1968), modified to accommodate the small cross-sectional areas of bryophyte sporophytes. All donor blocks contained 10^{-6} mol/L 5-[^{3}H]-IAA (specific activity of 25 Ci/mmol, American Radiolabeled Chemicals, St. Louis, Missouri, USA), and receiver blocks were composed either of water agar or 10^{-5} mol/L N-(1-naphthyl)phthalamic acid (NPA) (Pfaltz and Bauer, Stamford, Connecticut, USA). NPA stock (10^{-3} mol/L) in 95% ethanol was added to molten Bactoagar (Difco Laboratories, Detroit, Michigan, USA) and allowed to cool in a 3-mm-diameter glass tube to create receiver blocks with a final concentration of 10^{-5} mol/L NPA. Sporophyte sections 5 mm in length were cut with a miniature scalpel (Roboz Surgical Instruments, Rockville, Maryland, USA) from the midregion of the setae of *Polytrichum ohioense* and *Pellia epiphylla*. Sections (5 mm) were similarly cut from the immature capsule located just above the intercalary meristem of *Phaeoceros pearsonii*. These sections were placed in a horizontal orientation between the 3-mm-diameter cylindrical donor and receiver blocks of 1.8% Bactoagar mounted on microscope slides under a glass chamber designed to maintain high humidity at room temperature. A physical gap separated the lanolin-mounted slides to ensure that no capillary movement of water occurred between the agar blocks. Single time point experiments for characterizing the transport polarity and inhibitor effects in each species utilized 5–15 sporophyte sections, which were placed in the transport chamber for 3 h. Time course experiments for measuring the accumulated amount of basipetal transport in each species used five different sections for each time point taken every hour for 5 h. In either case, the receiver block from each section was placed in 5 mL of Biosafe II scintillation fluid overnight and then counted for 5 min in an LKB Wallac 1219 Rack Beta liquid scintillation counter (95% counting efficiency, LKB Instruments, Gaithersburg, Maryland, USA). Identical methods were used for measuring auxin transport in 5-mm sections obtained 1.0 cm below the apical region of *Zea mays* coleoptiles.

Additional experiments were run to evaluate auxin transport in the setae of older sporophytes (2–3 cm in length) of *Polytrichum ohioense*. Experiments were carried out in the same manner as the assay described earlier with the variation that 10^{-5} mol/L NPA or 10^{-5} mol/L NOA (Aldrich Chemical Company) was added to both the donor and the receiver blocks. The NOA was dissolved in 95% ethanol at a stock concentration of 10^{-2} mol/L. The control donor and receiver blocks had 1% (v/v) 95% ethanol added to simulate the amount of added ethanol in the inhibitor solutions of the other trials. The transport assay was run on 10 sporophytes for each treatment for 3 h. Identical methods were used for measuring auxin transport in *Zea mays* coleoptiles obtained as described earlier.

Data analysis for transport experiments—All counts per minute (cpm) from the liquid scintillation counter were divided by the counting efficiency to yield the corresponding disintegrations per minute (dpm), which were converted into curies (2.2×10^6 dpm = 1 Ci) and then into moles by dividing by the specific activity of [^{3}H]-IAA (25 Ci/mmol) reported by the manufacturer. Time course data for basipetal transport were directly plotted as amount (fmol) vs. time (h). The slope of each best-fitted line represented the intensity (amount per unit time) of basipetal transport. Basipetal transport velocity (distance per unit time), which corresponded to the transport rate of the first molecules to reach the receiver block, was calculated as the length of the section divided by the x-intercept of the line. Transport flux (amount per unit area per unit time) was calculated by dividing the transport intensity by the cross-sectional area of each section, as determined from measuring the dimensions of the seta, capsule, and coleoptile section with an ocular micrometer in a dissecting microscope. Cross-sectional areas for solid moss sections were calculated by the formula πr^2, where r is the radius, and for hollow liverwort and hornwort sections by the formula $\pi(r_o^2 - r_i^2)$, where r_o is the outer radius and r_i is the inner radius. Cross-sectional areas for hollow elliptical maize coleoptiles were calculated by the formula $\pi(R_a R_b - r^2)$, where R_a is the minor outer radius of the ellipse, R_b is the major outer radius of the ellipse, and r is the radius of the hollow inner circle. Transport polarity was calculated as the ratio of basipetal transport intensity to acropetal transport. Percentage inhibition was calculated as the ratio of transport intensity in inhibited structures vs. the transport intensity in control structures times 100%. The data are presented as the mean ± the standard error among replicate sections.

RESULTS

General sporophyte morphology—In general, almost all sporophytes of the three lineages of extant bryophytes mature as tripartite linear structures, but the developmental processes responsible for generating these sporophytes are strikingly different in the three lineages (Smith, 1955; Bold et al., 1987; Renzaglia et al., 2000; Crum, 2001). The mature sporophyte of the representative liverwort *Pellia epiphylla* develops an

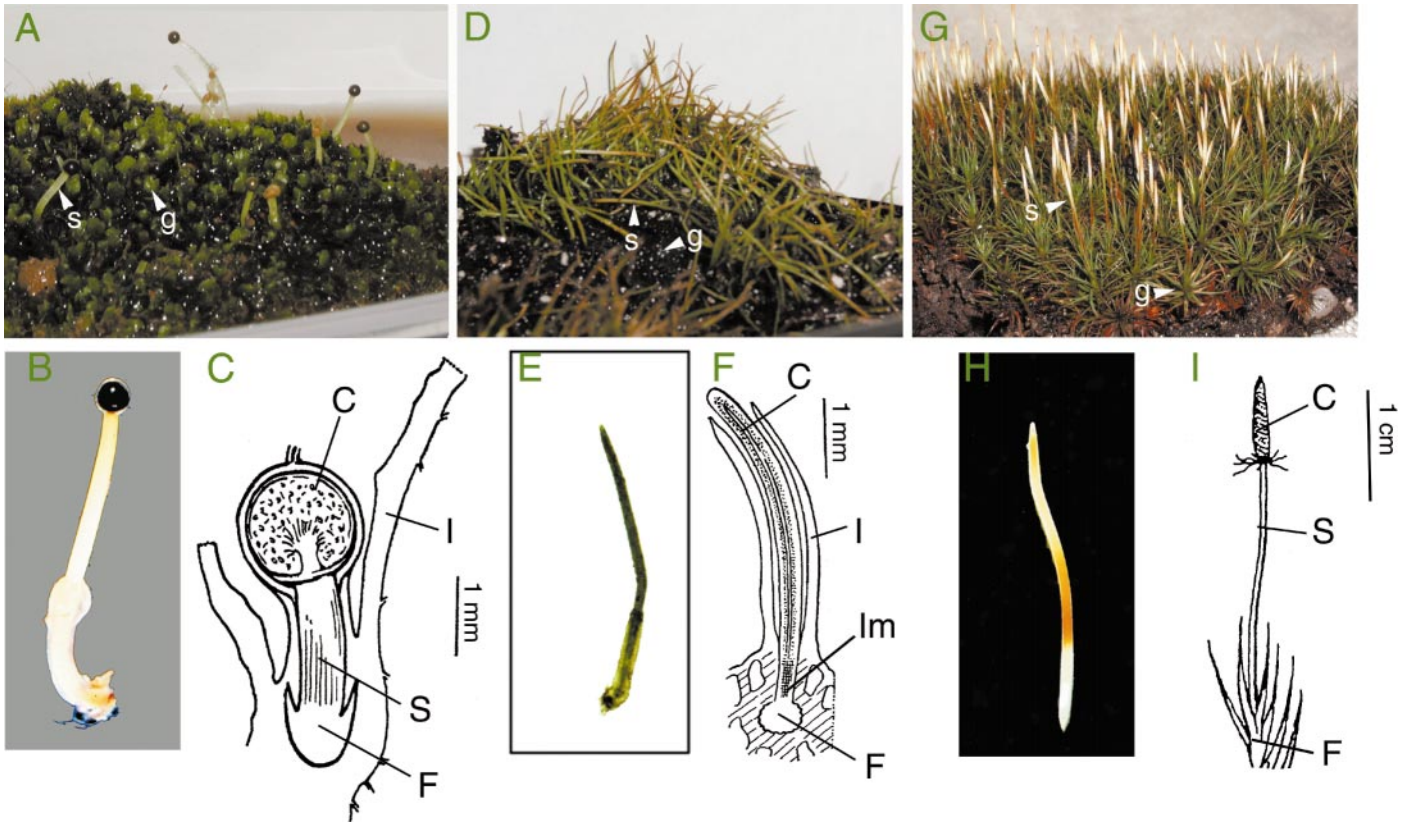

Fig. 1. A–C. The sporophytes of *Pellia epiphylla*. A. Gametophytes bearing sporophytes. B. Detached sporophyte. C. Drawing of longitudinal section of sporophyte. Reprinted with permission from Watson (1971). D–F. The sporophytes of *Phaeoceros pearsonii*. D. Gametophytes bearing sporophytes. E. Detached sporophyte. F. Drawing of longitudinal section of sporophyte. Reprinted with permission from Watson (1971). G–I. The sporophytes of *Polytrichum ohioense*. G. Gametophytes bearing sporophytes. H. Detached sporophyte. I. Drawing of longitudinal section of sporophyte. Reprinted with permission from Bold (1973). Abbreviations: C, capsule; S, seta; F, foot; Im, intercalary meristem; I, involucre; s, sporophyte; g, gametophyte.

apical capsule, a basal foot, and an intermediate seta (Fig. 1A-C). The liverwort embryo differentiates into three tiers destined to develop into those three structures in the mature sporophyte. Subsequently, the unexpanded sporophyte remains protected by the enlarged calyptra and the adjacent involucre while the capsule undergoes precocious differentiation to produce mature spores and narrow elators (Fig. 1B). The unexpanded seta is uniformly composed of unspecialized cells lacking any differentiation (Thomas, 1980). When the environmental conditions are conducive to spore dispersal, the seta cells start rapidly absorbing water resulting in diffuse cell elongation in the absence of compensatory cell division or substantial wall biosynthesis (Thomas and Doyle, 1976). This simple turgor-driven mechanism mediates seta elongation at a rate approaching 1 mm/h (Watson, 1971). Therefore, it appears that the sole purpose of the ephemeral liverwort seta is to suddenly elevate the mature capsule above the gametophyte to effect the almost simultaneous dispersal of its spores.

The mature sporophyte of a typical hornwort like *Phaeoceros pearsonii* consists of a linear sporangium and a basal foot with an intervening intercalary meristem that divides to generate new sporangial cells throughout sporophytic growth (Fig. 1D-F). The hornwort embryo also exhibits a basal tier destined to become the foot and an apical tier representing the future tip of the capsule (Campbell, 1918; Smith, 1955; Crum, 2001). The intermediate tier develops into a narrow band of dividing cells called an intercalary meristem that undergoes unifacial divisions on its capsule side, with the result that the new cells compose almost the entire capsule (Fig. 1F). The persistent activity of the intercalary meristem generates an indeterminate capsule, in which spore development is a sequential process with new sporogenous cells originating near the meristem and mature spores being released from distal regions over several months. Thus, the axis of the hornwort sporophyte is largely composed of the linear capsule itself (Fig. 1F), in marked contrast to the elongated setae of the other bryophytes.

The mature sporophyte of the mosses, such as *Polytrichum ohioense*, can also be divided into a foot, seta, and capsule (Fig. 1G–I). The young *Polytrichum* sporophyte grows as a bipolar structure with transient apical cells at opposite poles. The activity of these apical cells and their derivatives results in the javelin-shaped structure illustrated in Fig. 1H (Smith, 1955; Lal and Bhandari, 1968; Bold et al., 1987; Crum, 2001). Subsequently, an intercalary meristem arises near the base of the future capsule, and the unifacial activity of this meristem is responsible for generating all the remaining seta cells (French and Paolillo, 1975b). Because seta elongation in the mosses depends in part on repeated cell divisions, it is a gradual process, as opposed to the rapid growth of liverwort setae by simple cell elongation. The *Polytrichum* seta has considerable cellular differentiation with an outer epidermis, an underlying cortex, and a central strand composed of water-conducting cells called hydroids, sugar-conductive cells called

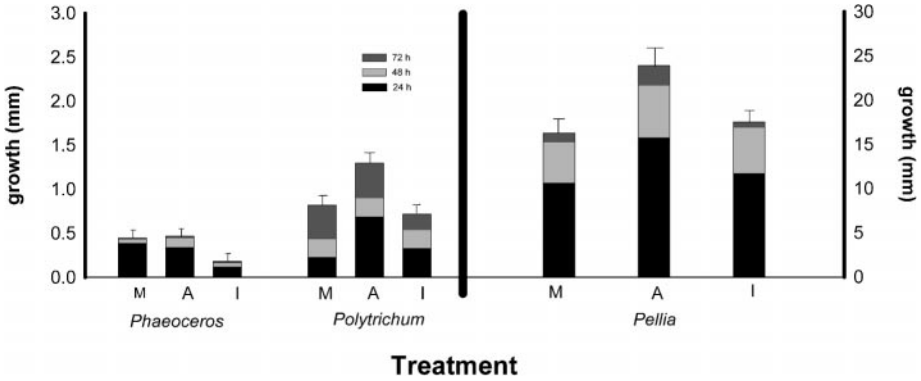

Fig. 2. In vitro growth of isolated sporophytes of *Phaeoceros pearsonii*, *Pellia epiphylla*, and *Polytrichum ohioense* measured every 24 h for 72 h in response to control medium (M), indole-3-acetic acid (A), or *p*-chlorophenoxyisobutyric acid (I). Data are presented as means + 1 SE among 25–30 replicate sporophytes.

leptoids, and supportive stereids (Hébant, 1977). Although spore maturation is a simultaneous process within the late-maturing capsule, the spores are gradually disseminated in most mosses due to the activity of the peristome.

Auxin effects on axial elongation—Young sporophytes from *Phaeoceros pearsonii*, *Pellia epiphyll*, and *Polytrichum ohioensis* were isolated from their respective gametophytes and exposed to an auxin (IAA) or an auxin antagonist (PCIB) at a concentration of 10^{-5} mol/L to determine if auxin acts to regulate axis elongation in bryophyte sporophytes. The elongation responses of 25 sporophytes exposed to each treatment were measured every 24 for 72 h (Fig. 2).

Immature sporophytes of *Phaeoceros pearsonii* ranged in length from 4 to 14 mm at the start of the experiment. Control sporophytes exhibited a mean increase of 0.39 mm in the first day and 0.06 mm over the next 2 d for a total mean increase of 0.45 mm. The growth response of these hornwort sporophytes was almost identical to that of the IAA treatment, with a mean increase of 0.34 mm in the first 24 h and a total mean increase of 0.47 mm for the entire experiment. However, hornwort sporophytes subjected to the PCIB treatment grew only 0.18 mm over 3 d, which represented a 60% reduction in total growth relative to the control sporophytes.

The initial lengths of immature setae of *Pellia epiphylla* ranged from 8 to 24 mm at the beginning of the experiment. Liverwort sporophytes in the controls elongated an average of 16.29 mm over 72 h, while the IAA-treated sporophytes grew 25.90 mm over the same interval, which means that IAA promoted the elongation of liverwort setae by 58%. The liverwort sporophytes grown in the PCIB treatment had a mean total increase of 17.53 mm, which was not significantly different from the control response.

The young sporophytes of *Polytrichum ohioense*, which had initial lengths of 12 to 21 mm, grew rather consistently within each treatment during the entire experiment. Moss sporophytes displayed a total mean increase of 0.82 mm in the control treatment vs. 1.30 mm and 0.72 mm in the IAA and PCIB treatments, respectively. Thus, IAA caused an increase in total elongation approaching 60% in these sporophytes.

In conclusion, overall growth rates of young *Pellia* and *Polytrichum* sporophytes significantly increased in response to exogenous IAA, but they did not respond to the anti-auxin treatment. By contrast, young *Phaeoceros* sporophytes reacted with the opposite sensitivity to these experimental treatments. The experiment thus suggested that endogenous IAA acts to regulate axis elongation in all three bryophytes.

Time course of basipetal auxin movement—Axial sections from the sporophytes of *Phaeoceros pearsonii*, *Pellia epiphylla*, and *Polytrichum ohioense* and from the coleoptiles sections of *Zea mays* were placed into a conventional agar-block apparatus with ^{3}H-IAA in the donor blocks to determine the time course of basipetal movement. The amount of ^{3}H-IAA was measured in the receiver blocks every hour for 5 h to construct the curves depicted in Fig. 3 and characterized in Table 1.

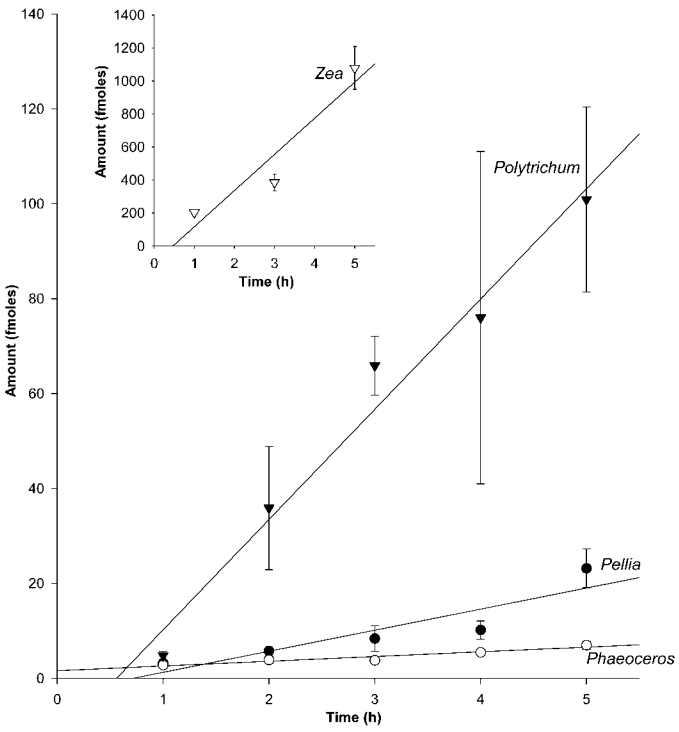

Fig. 3. Basipetal auxin movement in isolated sporophytes of *Phaeoceros pearsonii* (open circles), *Pellia epiphylla* (closed circles), and *Polytrichum ohioense* (closed triangles) and in isolated coleoptiles of *Zea mays* (open triangles) in an agar-block apparatus over 5 h. Data are presented as means ± 1 SE among five to 15 replicate structures for every collection of each species at each hour.

TABLE 1. Key parameters characterizing basipetal auxin transport in three bryophyte sporophytes and in maize coleoptile. The equations correspond to the best-fit lines calculated on the basis of the amount of radioactivity in the receiver blocks at each hour starting at 1 h, as depicted in Fig. 3.

Organism	Structure	Best-fit line	Velocity (mm/h)	Mean cross-sectional area (mm^2)	Flux ($fmol \cdot mm^{-2} \cdot s^{-1}$)
Phaeoceros pearsonii	Immature capsule	$y = 1.0x + 1.6$	n/d[a]	0.17	1.6×10^{-3}
Pellia epiphylla	Young seta	$y = 4.4x - 3.2$	6.9	0.21	5.9×10^{-3}
Polytrichum ohioense	Young seta	$y = 23.2x - 13.0$	8.9	0.14	4.6×10^{-2}
Zea mays	Coleoptile	$y = 219.3x - 101.8$	11.0	1.99	3.1×10^{-2}

[a] not determined.

Time course data from immature sporophytes of *Phaeoceros pearsonii* were represented by the line $y = 1.0x + 1.6$ (see Table 1, Fig. 3) whose slope corresponded to 1.0 fmol auxin moved per hour. The velocity (distance per unit time) of auxin transport could not be resolved for *Phaeoceros* sporophytes because the limited amount of auxin transported resulted in a best-fit line that did not cross the *x*-axis. Hornwort sporophytes had a mean cross-sectional area of 0.17 mm^2, which meant that their basipetal flux of auxin movement was equal to 1.6×10^{-3} $fmol \cdot mm^{-2} \cdot s^{-1}$. Instead of using the conventional term of auxin *amounts* to compare auxin movement among the different structures, auxin *fluxes* were utilized here to normalize the profound size differences among these structures. Auxin movement in young setae of *Pellia epiphylla* was characterized by the line $y = 4.4x - 3.2$. Extrapolating this line to the x-axis resulted in the calculated auxin movement velocity of 6.9 mm/h. These setae had an average cross-sectional area of 0.21 mm^2, with a calculated flux of 5.9×10^{-3} $fmol \cdot mm^{-2} \cdot s^{-1}$. Thus, the auxin flux in *Pellia* setae was over 3.5-fold higher than the flux in *Phaeoceros* sporophytes. Time course data for the young setae of *Polytrichum ohioense* were best represented by the line $y = 23.2x - 13.0$, which led to a predicted velocity for auxin movement of 8.9 mm/h. Moss setae displayed a mean cross-sectional area of 0.14 mm^2, which was somewhat less than that of the other two bryophyte sporophytes. However, their basipetal flux of 4.6×10^{-2} $fmol \cdot mm^{-2} \cdot s^{-1}$ was almost 30 times higher than the flux in *Phaeoceros* sporophytes and almost eight times higher than the flux in *Pellia* sporophytes.

Even though the maize coleoptile is a hollow structure, it offers a much greater cross-sectional area for auxin movement (1.99 mm^2) than do the bryophyte sporophytes. The line $y = 219.3x - 101.8$ best represented the time course data for maize coleoptiles, which had a transport velocity of 11 mm/h. Their slope of 219.3 fmol auxin transported per hour is approximately 10-fold higher than the slope of the movement in moss sporophytes. However, the auxin flux in maize coleoptiles was 3.1×10^{-2} $fmol \cdot mm^{-2} \cdot s^{-1}$, which is 67% of the flux recorded in *Polytrichum* sporophytes. Thus, the moss sporophyte appears to have evolved a mechanism for moving auxin that is comparable to those acting in flowering plant structures.

Polarity and inhibitor sensitivity of auxin movement—Polar auxin transport in flowering plants is typically sensitive to certain inhibitors (see Introduction). Thus, to compare this process in bryophyte sporophytes vs. maize coleoptiles, auxin movement was characterized in both acropetal and basipetal directions in the presence or absence of these inhibitors.

Control sections from immature capsules of hornwort sporophytes transported 5.9 ± 0.7 fmol (326 ± 39 dpm) ^{3}H-IAA in the basipetal direction and 6.1 ± 0.3 fmol (337 ± 14 dpm) in the acropetal direction during 3-h experiments, which resulted in a basipetal to acropetal (B/A) polarity ratio of 1.0 (Table 2). The absence of evident auxin polar transport, along with the very low levels of auxin movement measured in Fig. 2, suggests that auxin movement may result from simple diffusion in hornwort sporophytes. The addition of the inhibitor NPA to the receiver block had virtually no effect, which is consistent with an apparent lack of an auxin transport apparatus (Table 2). Thus, this experiment suggested that auxin

TABLE 2. Transport polarity and NPA sensitivity of auxin transport in three bryophyte sporophytes and a maize coleoptile placed in the agar-block apparatus for 3 h. Data are presented as the mean ± the standard error among 5–15 replicated structures. Abbreviations: dpm, disintegrations per minute; B/A, basipetal to acropetal; NPA, N-(1-naphthyl)phthalamic acid.

Organism	Structure	Treatment	Basipetal transport		Acropetal transport		Polarity B/A
			dpm	fmol	dpm	fmol	
Phaeoceros pearsonii	Immature capsule	Control	326 ± 39	5.9 ± 0.7	337 ± 14	6.1 ± 0.3	1.0
		10^{-5} mol/L NPA	365 ± 29	6.6 ± 0.5	320 ± 29	5.8 ± 0.5	—
		Percent inhibition	0		6		—
Pellia epiphylla	Young seta	Control	461 ± 97	8.4 ± 1.8	416 ± 49	7.6 ± 0.9	1.1
		10^{-5} mol/L NPA	205 ± 16	3.7 ± 0.3	277 ± 45	5.0 ± 0.8	—
		Percent inhibition	56		33		—
Polytrichum ohioense	Young seta	Control	3793 ± 444	69.0 ± 8.1	409 ± 36	7.4 ± 0.7	9.3
		10^{-5} mol/L NPA	3165 ± 556	57.5 ± 10.1	352 ± 16	6.4 ± 0.3	—
		Percent inhibition	17		14		—
Zea mays	Coleoptile	Control	233 972 ± 5088	4254 ± 92.5	347 ± 52	6.3 ± 0.9	674
		10^{-5} mol/L NPA	2433 ± 388	44.2 ± 7.1	339 ± 52	6.2 ± 1.0	—
		Percent inhibition	99		2		—

efflux carriers are absent from hornwort sporophytes and/or they are almost completely insensitive to transport inhibitors.

Seta sections from *Pellia epiphylla* sporophytes were similarly measured to have a B/A ratio of 1.1 (Table 2), suggesting that auxin movement also lacks polarity in these sporophytes. However, 10^{-5} mol/L NPA in the receiver blocks reduced basipetal movement from 8.4 ± 1.8 fmol to 3.7 ± 0.3 fmol auxin (56% inhibition) and acropetal movement from 7.6 ± 0.9 fmol to 5.0 ± 0.8 fmol (33% inhibition). This sensitivity to NPA suggests that auxin efflux carriers are present in liverwort sporophytes. The somewhat higher NPA-mediated inhibition of basipetal transport implies that efflux carriers may be somewhat more likely to reside near the basal ends of seta cells.

The B/A ratio of 9.3 of the control setae of *Polytrichum ohioense* sporophytes confirmed basipetal auxin transport (Table 2). However, the addition of NPA in the receiver blocks effected only slight, but equivalent inhibitions of basipetal transport (17%) and acropetal transport (14%). These results could be attributed to preferential but not exclusive distribution of auxin efflux carriers to basal locations, even though the carriers and/or their intracellular transport system were apparently insensitive to NPA. Because of the structural complexity of the *Polytrichum* seta, a second possibility was that basipetal and acropetal transport might occur in the peripheral cortex and central vascular strand, respectively, within the *Polytrichum* seta.

By contrast, maize coleoptiles exhibited a B/A ratio of 674, which means that auxin transport is almost exclusively basipetal in these structures. NPA caused a 99% inhibition of basipetal transport, but it did not affect the negligible amount of acropetal transport.

The possibility that *Polytrichum* setae might have two opposing pathways for auxin transport was evaluated by examining the transport capabilities of older moss sporophytes, which would presumably contain more mature vascular tissue. Transport assays were performed in the agar-block apparatus as before in the presence or absence of the inhibitors of the auxin influx carrier (NOA) and the auxin efflux carrier (NPA). In these experiments, 10^{-5} mol/L NPA or 10^{-5} mol/L NOA were incorporated into both the donor and receiver blocks to ensure effective inhibition.

In the control experiments, older moss sporophytes transported 54.6 ± 8.6 fmol of auxin in the basipetal direction over 3 h (Table 3), which is roughly similar to the basipetal transport in younger moss sporophytes (Table 2). However, acropetal auxin transport in older sporophytes was 61.7 ± 14.7 fmol (Table 3), which is almost nine times higher than the acropetal transport in young sporophytes (Table 2). Auxin transport in older sporophytes was strongly affected by NPA: basipetal transport was reduced to 31.9 ± 4.5 fmol (41.4% inhibition) while acropetal transport was decreased to 24.8 ± 4.5 fmol auxin (59.9% inhibition). NOA application resulted in only 4% inhibition of basipetal transport but 64.7% inhibition of acropetal transport. The enhanced levels of acropetal transport and its pronounced sensitivity to NOA suggest that the bidirectional auxin transport occurs in *Polytrichum* sporophytes via two different pathways. On the other hand, the control coleoptile sections of *Zea mays* transported 12 266.6 ± 717.8 fmol auxin in the basipetal direction. NPA greatly decreased this transport to 54.3 ± 6.9 fmol auxin (99.6% inhibition). Acropetal transport in coleoptile sections was less affected by NPA because 47.3 ± 2.5 fmol auxin was reduced to 39.8 ± 4.0 fmol (15.7% inhibition). With the addition of NOA to the re-

TABLE 3. Transport polarity and inhibitor sensitivity of auxin transport in older moss sporophytes and maize coleoptiles placed in the agar-block apparatus for 3 h. Data are presented as the mean ± the standard error among 10 replicate structures. Abbreviations: dpm, disintegrations per minute; NPA, N-(1-naphthyl)phthalamic acid; NOA, (1-napthoxy)acetic acid.

Organism	Structure	Treatment	Basipetal transport			Acropetal transport		
			dpm	fmol	Percent inhibition	dpm	fmol	Percent inhibition
Polytrichum ohioense	Older seta	Control	3001 ± 474	54.6 ± 8.6	—	3394 ± 810	61.7 ± 14.7	—
		10^{-5} mol/L NPA	1757 ± 250	31.9 ± 4.5	41.4	1362 ± 247	24.8 ± 4.5	59.9
		10^{-5} mol/L NOA	2881 ± 506	52.4 ± 9.2	4.0	1199 ± 150	21.8 ± 2.7	64.7
Zea mays	Coleoptile	Control	674 662 ± 39 480	12 266.6 ± 717.8	—	2599 ± 140	47.3 ± 2.5	—
		10^{-5} mol/L NPA	2986 ± 378	54.3 ± 6.9	99.6	2190 ± 221	39.8 ± 4.0	15.7
		10^{-5} mol/L NOA	31 952 ± 8054	5809.6 ± 146.4	52.6	1703 ± 139	31.0 ± 2.5	34.5

ceiver blocks, both basipetal and acropetal auxin transport decreased substantially in maize coleoptiles by 52.6% and 34.5%, respectively.

DISCUSSION

Auxin regulation of axis elongation—The sporophytes of three different bryophytes in this paper exhibit distinctive processes of axial elongation, which correlate with the profound differences in their auxin responses. The linear sporophytes of the hornwort *Phaeoceros pearsonii* do not develop specialized setae for axis elongation, but instead they have a persistent intercalary meristem that generates an elongated capsule. Although the auxin-antagonist experiment suggests that endogenous auxin regulates the activity of this meristem, this auxin appears to move by simple diffusion in the apoplast of the elongating capsule without any detectable polarity or carrier activity. The sporophytes of the liverwort *Pellia epiphylla* and of the moss *Polytrichum ohioense* have both evolved intervening setae that are specialized for axis elongation. Liverwort setae are uniformly composed of parenchymatous cells, which elongate by means of diffuse growth (Thomas, 1980). Our results confirm that this elongation process is sensitive to auxin levels, as was reported in earlier work (Schnepf et al., 1979; Thomas, 1980). Even though auxin does not exhibit polar movement within *Pellia* setae, the experiments with transport inhibitors reveals that its movement does involve the activity of transmembrane auxin carriers in seta cells. Thus, this type of auxin movement can be classified as apolar facilitated diffusion.

By contrast, young moss setae develop a subapical meristem beneath the apical region destined to become the capsule (Wenderoth, 1931; French and Paolillo, 1975b). In addition, the older setae of many mosses, including *Polytrichum ohioense*, develop a central strand of vascular tissue (Hébant, 1977). Therefore, the cell types and meristematic activity in *Polytrichum* setae are somewhat similar to those features in vascular plant axes. This paper has presented considerable evidence that auxin acts to regulate the elongation of *Polytrichum* setae. In young setae, exogenous auxin mediates a 50% increase in axis elongation, and it undergoes polar transport in the basipetal direction at a flux (amount per cross-sectional area per unit time) greater than the flux measured in maize coleoptiles. In older setae, auxin is transported at high rates in both directions. In light of the differing inhibitor sensitivities of acropetal and basipetal transport, they may occur in separate cellular pathways within moss setae. Because acropetal transport becomes more pronounced near the time of vascular tissue differentiation in the setae, it seems reasonable to speculate that acropetal transport occurs in the vascular tissue to supply the auxin that is presumably required for delayed process of capsule differentiation. Lastly, polar auxin transport in moss setae bears some remarkable similarities to this process in vascular plant axes. Bidirectional polarized transport is also observed in certain vascular plant organs; for instance, auxin transport in angiosperm roots is basipetal in the peripheral cortex, but it is acropetal in the central stele (Rashotte et al., 2000; Swarup et al., 2001). This bidirectional auxin movement appears to play an essential role in pattern formation and cellular differentiation in roots (Sabatini et al., 1999; Friml et al., 2002; Grebe et al., 2002).

In summary, among the three bryophyte lineages, hornwort sporophytes appear to exhibit the simplest structural features and hormonal regulation for generating an elongated axis. Almost all liverwort sporophytes develop elongated setae for elevating their capsules, but liverwort setae have growth mechanisms and auxin movements that are quite different from those operating in moss setae. Moss setae and vascular plant organs have similar structural features and hormonal regulation, which may be indicative of common developmental mechanisms operating in both types of plant axes.

Evolutionary implications—As is clear from emerging perspectives from the field of evolutionary developmental biology (Raff, 1996; Knoll and Carroll, 1999; Peterson and Davidson, 2000; Cronk, 2001), the regulatory mechanisms operating in embryos and young organisms are generally conserved within particular lineages over great evolutionary time scales. The evidence is consistent with the notion that auxin has played a critical role in the regulation of plant developmental processes ever since the origin of the land plant lineage. First of all, auxin serves as the principal hormone for regulating embryo development, at least in vascular plants (Cooke et al., in press). Bryophyte gametophytes tend to have auxin biosynthetic pathways, auxin movement characteristics, and auxin-mediated responses that are rather similar to those features in vascular plant sporophytes (Cooke et al., 2002). This leads to the plausible interpretation that the vascular plants are not likely to have evolved de novo mechanisms governing auxin regulation of developmental processes, but rather they modified preexisting mechanisms already operating in the early land plants. Lastly, given that the bryophytes seem to represent the earliest divergent lineages of land plants (Kenrick and Crane, 1997; Qiu et al., 1998; Nickrent et al., 2000; Renzaglia et al., 2000; Karol et al., 2001; Delwiche et al., in press), the present report concerning auxin effects on the axial growth of bryophyte sporophytes may provide significant insights into the early evolution of land plant sporophytes.

The unique structural events and hormonal regulation in young hornwort and liverwort sporophytes make it impossible to link the process of axial elongation in either group to the comparable process in vascular plants. By contrast, the remarkable structural and physiological similarities in the axial elongation of moss setae and vascular plant axes supports the plausible interpretation that mosses may be the sister group to vascular plants. In particular, it appears reasonable to speculate that this elongation mechanism, which is based on persistent apical or subapical meristems, early axis differentiation, and bidirectional polarized auxin transport, evolved in their common ancestor before the divergence into separate lineages.

From these considerations, the following scenario for the evolution of the sporophytic axes of early land plants may be plausible. Microfossil evidence from the Middle Ordovician Period has indicated that the earliest land plants were likely to have a bryophyte-grade of structural organization, at least with respect to spore morphology (Gray, 1985; Edwards and Wellman, 2001; Graham and Gray, 2001). These first plants gave rise to different lineages, including those that would ultimately evolve into the hornwort, liverwort, and moss-vascular plant lineages. No paleobotanical evidence exists to resolve the issue of whether these lineages diverged before or after they evolved the ability to generate axial sporophytes. Nevertheless, insofar as extant bryophytes possess very different mechanisms for elevating their sporangia, it seems reasonable to propose that the diversification of bryophyte lineages did precede the in-

dependent origins of axial sporophytes. Of the earliest lineages of land plants, only the putative moss–vascular plant lineage appears to have evolved an elongation mechanism preadapted for generating the large multiaxial sporophytes that have been the most prominent members of the terrestrial flora ever since 400 my BP.

Our enthusiasm for this scenario is dampened by the realization that the question of bryophyte evolution can be viewed as yet another ''abominable mystery'' plaguing plant evolutionary biology (Kenrick and Crane, 1997; Niklas, 1997; Bateman et al., 1998; Goffinet, 2000). Molecular phylogenetic studies have unequivocally established the three bryophyte lineages as being the earliest divergent lineages of extant plants, although the specific order of their divergence remains unresolved to date (Qiu et al., 1998; Goffinet, 2000; Nickrent et al., 2000; Karol et al., 2001; Delwiche et al., in press). The earliest mesofossils with possible bryophyte affinities have been identified as miniature branching axes in Lower Devonian rocks (Edwards et al., 1995; Edwards, 2000; Edwards and Axe, 2000). The meager macrofossil record for putative bryophytes, viz. thalloid organisms bearing monosporangiate axes, consists of a few compression fossils, such as *Sporogonites* (Lower Devonian), perhaps representing an early hornwort or thalloid moss, and *Pallaviciniites* (Upper Devonian), closely resembling certain modern liverworts (Taylor and Taylor, 1993; Goffinet, 2000). By contrast, the macrofossil record of the early land plants appears to emphasize the rapid diversification of numerous multiaxial protracheophytes and vascular plants starting in Upper Silurian and Lower Devonian strata (Taylor and Taylor, 1993; Kenrick and Crane, 1997; Bateman et al., 1998). The evidence available from well-preserved fossils in the Rhynie Chert (Lower Devonian) indicates that these plants are likely to have undergone isomorphic alternation of generations, as opposed to the heteromorphic life cycles of extant bryophytes (Kenrick and Crane, 1997). Therefore, the central dilemma in bryophyte evolution is how to resolve the apparent conflict between the early divergence of bryophyte lineages, as predicted by molecular analyses, vs. the late appearance of recognizable bryophytes in the fossil record. Kenrick and Crane (1997) proposed that stem-group bryophytes have gone unrecognized because they may lack the most distinctive characteristics of extant crown groups. It is therefore conceivable that the monosporangiate axes of extant bryophytes do not represent the ancestral condition, but instead these axes were evolutionarily derived from reduced polysporangiate structures. This perspective must necessarily confound any facile interpretation that the auxin regulation of axial elongation in extant bryophyte sporophytes reflects the developmental mechanism involved in the evolution of the sporophytic axes of the earliest bryophytes.

A second, related problem arises in our interpretation that the underlying mechanism of axial elongation arose in the common ancestor of the mosses and vascular plants, which implies that the development of the sporophytic axis is a homologous process in these two groups. According to the most recent phylogenetic analysis of the mosses (Newton et al., 2000), the earliest divergent moss order is the problematic Sphagnales, which elevate their short sporophytes via extended gametophores. This mechanism of capsule elevation in Sphagnales may represent the basal state in the mosses, or it may be a derived adaptation in response to their aquatic habit (L. E. Graham, University of Wisconsin, Wisconsin USA, personal communication). In the former case then, the appearance of polar auxin transport in the sporophytic axes of later-divergent Polytrichales would be attributable to independent recruitment as opposed to a single origin in the common ancestor of these two groups.

In conclusion, the observations made in this paper indicate that the three divisions of extant bryophytes utilize different developmental mechanisms for regulating axial elongation of their sporophytes. The auxin regulation of axial elongation in moss sporophytes is quite reminiscent of the same process in certain vascular plant organs, which supports intriguing interpretation that it originated in a common ancestor of the moss and vascular plant lineages. Of course, the prediction that these two lineages are indeed sister to each other remains to be validated by further phylogenetic analyses.

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