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Current Opinion in Plant Biology

Early evolution of the vascular plant body plan — the missing mechanisms

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The complex body plan of modern vascular plants evolved by modification of simple systems of branching axes which originated from the determinate vegetative axis of a bryophyte-grade ancestor. Understanding body plan evolution and homologies has implications for land plant phylogeny and requires resolution of the specific developmental changes and their evolutionary sequence. The branched sporophyte may have evolved from a sterilized bryophyte sporangium, but prolongation of embryonic vegetative growth is a more parsimonious explanation. Research in the bryophyte model system *Physcomitrella* points to mechanisms regulating sporophyte meristem maintenance, indeterminacy, branching and the transition to reproductive development. These results can form the basis for hypotheses to identify and refine the nature and sequence of changes in development that occurred during the evolution of the indeterminate branched sporophyte from an unbranched bryophyte-grade sporophyte.

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Polysporangiophyte origins — the interplay of phylogeny and development

The origin of vascular plants is arguably the most important evolutionary event in the development of the terrestrial biosphere. In modern terrestrial biomes, vascular plants are the dominant source of primary productivity, providing the foundation for virtually all terrestrial ecosystems. Current understanding of phylogeny indicates that vascular plants (tracheophytes) form a monophyletic group [1]. All extant tracheophytes are polysporangiophytes, i.e. plants with branched sporophytes, but the polysporangiophytes also include extinct lineages that did not produce vascular tissue (i.e. xylem with tracheids as conducting elements and phloem) [2]. Early polysporangiophytes had simple sporophytes consisting of undifferentiated, dichotomously branching axes [1,3]. Nevertheless, the evolution of the branched sporophyte paved the way to indeterminate modular growth which, in concert with the evolution of highly specialized conductive tissues (xylem and phloem), led to nutritional independence of the diploid phase. Combined with subsequent evolution of specialized vegetative organs (stems, leaves, roots) these led to the plant sporophyte-sustained ecosystems that are ubiquitous on land today.

Currently, there is intense interest in the origins of polysporangiophytes and vascular plants, which has generated several recent reviews of the topic [4,5•,6••,7••,8•]. Polysporangiophytes, along with the three bryophyte lineages — liverworts, hornworts and mosses — form the embryophyte clade [1,8•,9•]. Although there is wide agreement that polysporangiophytes (including vascular plants) evolved from bryophytic grade embryophytes [1,9•] somewhere between the mid-Ordovician and the mid-Silurian, 450–430 Ma ago [10–13], several aspects of this process remain obscure or are in dispute: the immediate ancestor or sister group of polysporangiophytes; the specific changes that initiated polysporangiophyte origins; the homologies of ancestral polysporangiophyte vegetative organs; the exact evolutionary sequence of changes leading to modern plant structure; and of fundamental importance, the genetic regulatory mechanisms that underlie the changes.

Attempts to answer these questions using a phylogenetic approach — that is, resolve phylogeny and use it to infer steps and mechanisms of evolutionary change, and homologies — have thus far not borne fruit. Despite a plethora of studies employing molecular markers, the phylogenetic relationships between the basal embryophytes lineages (liverworts, hornworts, mosses, and polysporangiophytes) remain largely unresolved [14,15]. Because of the depth of phylogenetic divergences associated with the early stages of embryophyte and polysporangiophyte evolution, as well as the taxon-sampling limitations inherent to molecular phylogenetics [16,17], the phylogenetic approach may never bring full resolution to the basal nodes of embryophyte phylogeny. However, an

evolutionary-developmental (evo-devo) approach that first, recognizes what structural changes result from the functional changes of specific genes, second, records the sequence in which comparable structural changes have occurred in the fossil record, and third, screens the genomes of living plants with similar structural features for the presence of orthologues, can provide a framework for formulating testable hypotheses of plant body plan evolution. Such an approach may be better suited for understanding the developmental changes that led to the evolution of polysporangiophytes and, thus, illuminate the homologies of the polysporangiophyte sporophyte.

Hypotheses for the origin of polysporangiophytes

Currently, there are three competing hypotheses for the origin of polysporangiophytes (including vascular plants). Two of these hypotheses are explicit [6••,7••,18••] and a third is implicit from traditional paleobotanical inference and systematic analyses (e.g. [1,3,9•]). All three assert that polysporangiophytes evolved from bryophyte-grade plants and that the ancestral polysporangiophyte had a branching sporophyte which bore terminal sporangia and lacked vascular tissue. However, the three hypotheses differ in the evolutionary mechanisms they predict and the homologies they propose for the axes of the ancestral polysporangiophyte.

Traditional (implicit) hypothesis — branching of a bryophyte-grade sporophyte

The idea that the vascular plant sporophyte originated by increase in length and branching of a bryophyte-grade sporophyte has a long history [19]. This hypothesis, formulated by Campbell [20] and implicit in Bower's [21] discussions of ''the origin of the polysporangiate state'', is reflected in more recent applications, such as models of growth [22] or the coding of morphological characters for phylogenetic analyses (e.g. [1]). However, this 'traditional' hypothesis groups together ideas that are rather diverse and lack specificity. Whereas most of these ideas imply modification of a moss-like sporophyte (e.g. [1,9•,22]), some involve modification of a hornwort sporophyte (e.g. [19]). None of the authors proposes a complete, internally consistent suite of specific changes in development, nor explicit ways to test the hypotheses. Further, specific homologies are not proposed for the vegetative body of the ancestral polysporangiophyte sporophyte. Nevertheless, the implicit ideas denote an upward outlook for morphological evolution (i.e. evolutionary view that interprets evolution/homologies of descendants from characters of ancestors [21]) and in most cases (except for the hornwort-to-polysporangiophyte hypothesis) have the bryophyte seta as the starting point.

Interpolation hypothesis

A second hypothesis (the interpolation hypothesis [18••]) proposes that the polysporangiophyte sporophyte evolved from a bryophytic sporophyte by intercalation of a novel vegetative axial organ between the foot and the sporangium. The hypothesis, as formulated, has a set of correlates that are mutually inconsistent. On the one hand, the hypothesis proposes that intercalation of the vegetative organ occurs early in embryogeny, before sporangial differentiation. On the other hand, the bryophyte sporophyte is seen as a footed sporangium (termed a sporogonium). The seta, when present, is interpreted as an extension and integral part of the sporangium, which implies that the bryophyte embryo expresses only two types of developmental programs — vegetative in the foot and reproductive in the sporangium. Given these constraints (and because the foot has no role in this hypothesis), intercalation of a vegetative organ *before sporangial differentiation* is not possible, except through repression of the reproductive developmental program in the sporangial primordium. Therefore, the vegetative axial organ proposed by this hypothesis is not novel; it is a sterilized sporangial primordium (implicit in Fig. 3 of [18••]). The intercalation hypothesis is, thus, essentially a sterilization hypothesis (see below).

The interpolation hypothesis only implies sporangial sterilization and does not propose specific changes in development with genetic/regulatory origins, thus it is not testable. By proposing that the vegetative organ of the polysporangiophyte sporophyte is a (leafless) stem, this hypothesis suggests homology of the early polysporangiophyte sporophyte with a modern tracheophyte stem. This view corresponds to a downward outlook on evolution (i.e. interpreting the structures of extinct plants by reference to living plants [21,23•]) that goes against the large body of evidence generated by paleobotanical studies over the last two centuries, which demonstrates that the sporophytic axes of early polysporangiophytes differed dramatically from the stems of modern plants in morphology (e.g. absence of leaves and phytomeres), anatomy (e.g. absence of tracheids as water conducting cells, stelar organization, sterome), and branching (apical isotomous) (e.g. [1,3]).

Sterilization hypothesis

Another recent hypothesis [6••,7••] also proposes that the polysporangiophyte sporophyte evolved by sterilization of a sporangial axis. Bolstered by a recent upsurge in support for a hornwort-polysporangiophyte clade in molecular phylogenies (e.g. [24,25]), this sterilization hypothesis explains the evolution of the polysporangiophyte sporophyte from a hornwort-type sporophyte. A key premise of the hypothesis is that, like in hornworts, the embryo of the polysporangiophyte precursor had an upper tier which expressed sporangial identity from its initiation and which developed a basal meristem with indeterminate growth that expressed the sporangial developmental program. Evolution of the polysporangiophyte sporophyte, thus, involved several changes, including (i) a

switch to vegetative growth by repression of the reproductive developmental program in the area of the embryo pre-determined to produce sporangial tissue (sporangium primordium and basal meristem); (ii) a shift of the expression domain for the indeterminate growth program from an intercalary area (the basal meristem between the sporangium primordium and the foot primordium) into a superficial, apical location; and (iii) an inversion in the polarity of cell divisions in the meristem leading to basiscopic positioning of their differentiating derivatives and a basipetal pattern of overall tissue maturation.

The resulting vegetative axial organ is considered to be a leafless shoot and the process responsible for the shifts in the timing and position of different developmental programs is homeotic mutation [6••]. Specific regulatory changes responsible for the hypothesized developmental transitions are not proposed in this hypothesis. Nevertheless, the sterilization hypothesis does provide a starting point (the hornwort sporophyte), as well as a coherent sequence of steps, all of which represent statements that are testable within an evo-devo framework. As currently formulated, the homology of the early polysporangiophyte sporophyte associated with this hypothesis is equivocal: it is either a combined bryophyte sporangium and seta [7••]; a part of the moss sporophyte, not necessarily the seta [7••]; or homology of the polysporangiophyte sporophyte apical meristem with the basal meristem of mosses and hornworts [6••]. Further, the postulate that the vegetative sporophyte of early polysporangiophytes was a shoot [6••] implies homology with the tracheophyte stem and represents an additional example of the downward outlook on evolution, as discussed above, under the intercalation hypothesis.

Fundamentally, the interpolation and sterilization hypotheses both propose that the divergence between polysporangiophytes and their bryophyte sister group (whichever that may be) marks a transition from an embryo that expresses a reproductive developmental program from the earliest stages of embryogeny (in the bryophyte sister group), to an embryo that expresses a vegetative developmental program in the early stages of embryogeny as a result of sporangial sterilization (Figure 1). Coupled with information from the fossil record, recent developments in molecular developmental biology now provide additional clues that point to an alternative hypothesis for the origin and homologies of the vascular plant sporophyte, henceforth referred to as the apical growth hypothesis.

An alternative — the apical growth hypothesis

The key premise of this hypothesis is that in early developmental stages the bryophyte embryo follows a vegetative developmental program which produces a vegetative axis, and the sporangium is produced by a subsequent switch to a reproductive developmental program in the apical portion of that axis. Prolonged apical growth, via an apical meristem, and branching are the primary differences between the polysporangiophyte sporophyte and that of bryophyte-grade plants. The apical growth hypothesis proposes that the change from the bryophytic sporophyte morphology to the polysporangiophyte sporophyte resulted from a heterochronic change — in timing of the transition to reproductive growth. More specifically, polysporangiophytes originated as a result of changes in the embryogeny and early sporophyte development of a bryophyte-grade plant by prolongation and amplification of vegetative growth in the embryonic axis and the advent of branching, both arising from an apical cell; and by a corresponding delay in the transition to the reproductive growth program producing the sporangium (Figure 1).

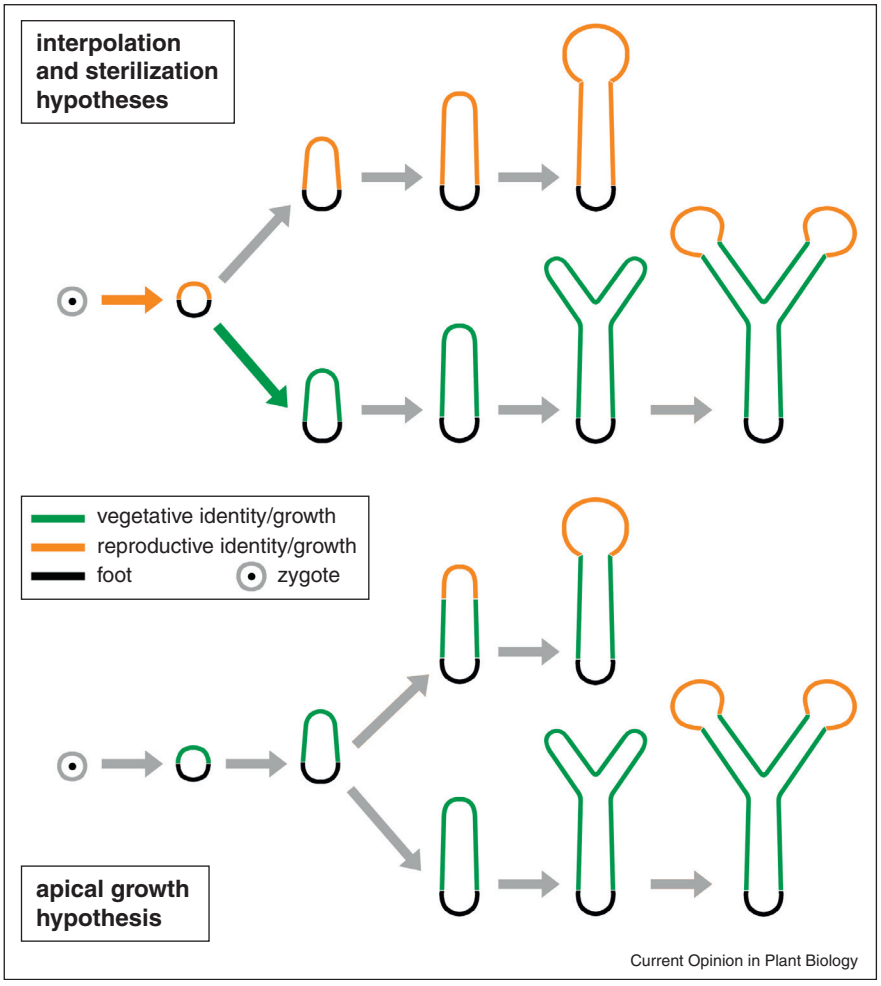
The apical growth hypothesis explicitly predicts the origins and homologies of the vascular plant sporophyte within a developmental context and is the most parsimonious hypothesis in terms of changes required for the evolution of the ancestral polysporangiophyte body plan. This hypothesis is congruent with the morphology of sporophytes known for the most ancient polysporangiophytes (e.g. *Cooksonia* [26]) and incorporates recent developments from molecular biology.

Parsimony and novelty

In terms of life cycle, the fundamental developmental change that marks the transition from charophycean algae to embryophytes is the delay in onset of a reproductive program (meiotic division — sporogenesis) and intercalation of vegetative growth (mitotic divisions producing a multicellular diploid individual — the sporophyte; Bower's [27] intercalation hypothesis). The premise of the apical growth hypothesis is a natural extension of that developmental change in evolutionary time. Modification of a stem-group bryophyte embryo with early vegetative growth to prolong apical meristematic activity before the change to reproductive growth (i.e. sporogenesis) is a far more parsimonious explanation for the evolutionary origin of the polysporangiophyte body plan [28•] than that proposed by sterilization hypotheses which involve a reversal from early vegetative growth (in the ancestral embryophyte) to expression of a reproductive developmental program at the beginning of embryogeny (in the stem-group bryophyte), followed by an additional reversal to early vegetative growth in the ancestral polysporangiophyte (Figure 2).

The evolutionary novelty in the context of the apical growth hypothesis is prolonged embryonic growth from an apical cell. A transient apical cell responsible for development of the embryonic longitudinal axis is present in mosses and at least some liverworts (e.g. [29]) but is not known in hornworts. The quasi-ubiquitous presence of apical cells and apical meristems across major

Figure 1



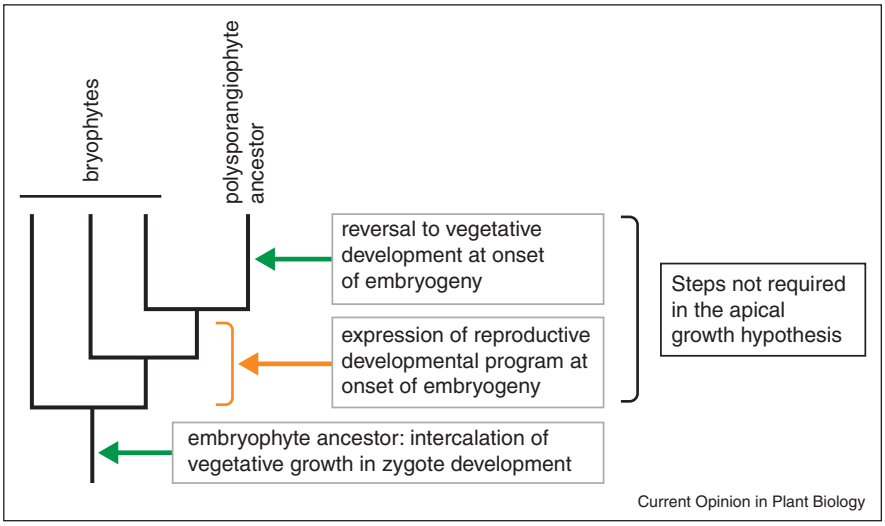
Comparison of developmental trajectories (zygote to mature sporophyte) in the interpolation and sterilization hypotheses (top panel) and the apical growth hypothesis (bottom panel); in each panel the lower developmental trajectory represents the polysporangiophyte ancestor and the upper trajectory represents the bryophyte-grade sister group. Whereas the interpolation and sterilization hypotheses propose evolution of the polysporangiophyte sporophyte from a bryophyte-grade sporophyte in which the reproductive developmental program was expressed from the earliest stages of embryogeny, the apical growth hypothesis proposes that the polysporangiophyte sporophyte evolved from a bryophyte ancestor with early embryogeny characterized by vegetative growth.

lineages and ploidy levels of life cycle phases (e.g. [30–32,33•]) suggests that an intimate association between longitudinal polarity and apical meristems is a fundamental property of growth in photosynthetic multicellular eukaryotes. Although the embryos of all extant embryophytes have well-defined longitudinal polarity, it is unclear whether embryonic growth from an apical cell was established very early in embryophyte evolution. If that were the case, then that potential has been explored to a modest extent by liverworts, to a more considerable extent by mosses (where it is replaced by growth from a seta meristem in later developmental stages), and was lost in hornworts (and replaced by growth from a basal meristem). Irrespective of when the embryonic vegetative axis derived from an apical cell arose during embryophyte evolution, the apical growth hypothesis predicts that longitudinal polarity was present in the polysporangiophyte precursor.

Developmental mechanisms

Several lines of evidence indicate that the sporophytes of bryophyte-grade embryophytes have the potential for indeterminate growth and branching, like the polysporangiophyte precursor. Rare, yet taxonomically diverse examples of teratological branched (dichotomously and even trichotomously) bryophyte sporophytes are reported in the literature [21,34,35•,36]. This evidence comes mostly from mosses which, although not necessarily closest to polysporangiophytes phylogenetically, may be most similar morphologically to the polysporangiophyte ancestor and closest to the latter in terms of developmental potential. Interestingly, Leitgeb [34] illustrates

Figure 2



The sequence of character changes in embryophyte phylogeny implied in the interpolation and sterilization hypotheses. This sequence lowers parsimony as compared to the apical growth hypothesis, by including two additional changes.

two examples of branched embryos in liverworts; the basal position of the lineage among embryophytes, along with occurrences of apical cells in liverwort embryo development [29], suggest that the genetic tool kit for apical growth and branching of the sporophyte may have arisen very early in embryophyte evolution.

Recent gene silencing and physiological studies in *Physcomitrella patens* provide clues to potential mechanisms of sporophyte branching. Sporophytes with multiple sporangia have been shown to develop as a result of first, disruption of the *TEL* genomic locus (also involved in shoot development in the *P. patens* gametophyte and angiosperm sporophytes [37]); second, disruption of the *LFY* locus (that controls the first zygotic cell division in *P. patens*, functions in the vegetative to reproductive transition in angiosperms, and is expressed in gymnosperm reproductive meristems [38]); and third, auxin transport inhibitors [39]. Perhaps the most spectacular evidence comes from apogamous *P. patens* sporophytes produced on gametophytic protonemata by deletion of polycomb repressive complex 2 (PRC2) genes (*CLF* [40••] and *FIE* [41]). Although haploid, these structures express *MKN* (class 1 KNOX) genes which are typically expressed in the meristematic areas of wild type *P. patens* sporophytes [42••]. The apogamous sporophytes produced by Okano *et al.* [40••] display prolonged apical growth (from apical cells that express *MKN* genes) and branching. Together, these demonstrate that the moss genome harbors the potential for indeterminate growth and branching of the sporophyte.

Interestingly, expression of CLF in the apogamous sporophytes of the *CLF* deletion lines leads to arrest of meristematic activity and development of sporangium-like organs. Completing the picture, in wild-type *P. patens* CLF expression is detected throughout the developing sporophyte starting at the time the apical cell stops dividing [40••]. These suggest that CLF acts in the developmental program for transition to sporophyte reproductive development, repressing vegetative apical growth and inducing sporangium development.

Maintenance of meristematic activity and indeterminate growth at the sporophyte axis tip of the ancestral polysporangiophyte was very likely under control of class 1 KNOX genes. *KNOX1* genes, required for maintenance of meristematic activity in seed plants [43], have been established before the divergence of mosses [44] and function in *Physcomitrella* sporophyte development [42••,45]. Sakakibara *et al.* [42••] showed that they are expressed in the meristematic areas of the moss embryo (apical and seta meristem) while these are active, probably regulating the frequency of cell division and cell growth, but their signal ceases with onset of sporangium development. These suggest conserved KNOX1 functions between mosses and angiosperms which, along with comparable loss-of-function phenotypes between the two lineages, suggest that *KNOX1* genes acquired sporophyte meristem maintenance function at least as early as the last common ancestor of mosses and polysporangiophytes [46•].

PIN-mediated polar auxin transport (PAT) is a key signaling mechanism in the patterning and maintenance of indeterminate vegetative growth throughout tracheophytes. The hormone auxin is a master regulator of embryo and postembryonic development in vascular

plants, and it has been hypothesized that genetic changes in auxin action were instrumental in the evolution of body plans in early embryophytes [47•]. While in liverwort and hornwort sporophytes auxin movement is apolar, the moss seta hosts significant basipetal PAT in early developmental stages, which regulates its elongation [47•,48]. As in seed plants, basipetal PAT regulates endogenous auxin distribution during establishment of longitudinal polarity in *P. patens* embryogeny [39]. The molecular mechanism for moss sporophyte PAT is not known. While *PIN* orthologs are present in the *P. patens* genome [49], these are not functionally related to the PIN1 auxin efflux carrier [50] and it has been hypothesized that PIN-mediated PAT only became a key signaling mechanism during the evolution of vascular plants [51•].

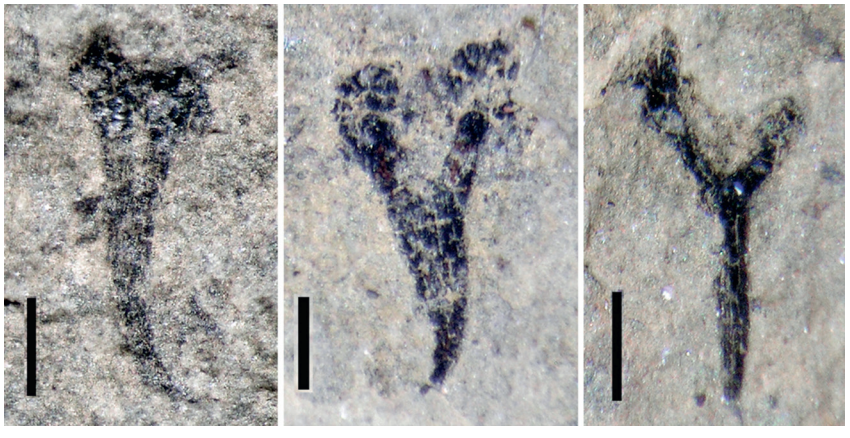
The role of PAT in shoot patterning is conserved across vascular plants [52•]. Together with the evidence for basipetal PAT in mosses, these indicate that an auxin-dependent mechanism for vegetative growth generating longitudinally polarized axial sporophytes was in place in the last common ancestor of mosses and polysporangiophytes [47•]. In this context, the sporophytes with multiple sporangia produced by disruption of auxin transport in *P. patens* [39] may indicate that temporal modulation of auxin homeostasis was required for branching in the ancestral polysporangiophyte sporophyte, especially if it is proven that in the *P. patens* experiments branching occurs before onset of the reproductive developmental program.

Basic homologies across embryophytes

Considered within the context of Bower's upward outlook [21,23•], the apical growth hypothesis implies homology between the branching axis of the polysporangiophyte sporophyte and the vegetative embryonic axis of the bryophyte-grade ancestor. A vegetative axis is present early in liverwort and moss embryogeny and part of this axis contributes to the seta (while the rest produces the sporangium). However, whereas in liverworts the seta is produced by some generalized cell division followed by cell elongation, in mosses the mature seta is largely the product of a basal meristem established between the foot and the sporangium primordium [7••,18••]. Developmental peculiarities notwithstanding, to the extent that the seta of bryophytes is an expression of the vegetative embryonic axis, the branching vegetative axis of the ancestral polysporangiophyte sporophyte (i.e. *Cooksonia*-type [26]) is homologous to a bryophytic seta. The greatest difference between the two is that the former typically branches and the latter usually does not [53••].

This basic homology is directly reflected in the sporophytes of many early polysporangiophytes that span the Late Silurian and beginning of the Early Devonian [12,54] (Figure 3). With their simple, undifferentiated branching axes terminated in sporangia, these plants were little more than branched bryophyte-type setae [22,53••]. Their potential for indeterminate growth and branching nevertheless provided the developmental background for subsequent evolutionary innovations at the base of the Devonian explosion in tracheophyte diversification. Starting as early as the late Silurian, these innovations included specialized conducting tissues (xylem and phloem) associated with increase in size and independence from the maternal gametophyte, and diversification of molecular pathways controlling branching architecture. These allowed for broad exploration of functional morphospace throughout the Early Devonian, which paved the way for the rise of the modern sporophyte body plans.

Figure 3



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Early Devonian (ca. 410 million years old) cooksonioid polysporangiophyte sporophytes from the Beartooth Butte Formation (Wyoming). The simple, undifferentiated branching axes terminated in sporangia reflect basic homology with the bryophyte sporophyte axis. Note diminutive size; scale bars 0.5 mm. USNM-P (Devonian/Beartooth Butte/Park Co., WY/Plantae/Hueber Research Coll./B13).

All extant tracheophytes (except for cases of secondary reduction and, possibly, the Psilotales) have complex sporophytes differentiated into specialized vegetative organs — stems, leaves, and roots. While the evolutionary origins of leaves are seeing some resolution [55], the origins of stems and roots remain blurry. This is due in part to the widely held view that the sporophyte axis of early polysporangiophytes is homologous to a stem (e.g. [4,56]). In this context, accumulating evidence for shared molecular mechanisms of development between angiosperm stems and roots (summarized in [57]) has been used to suggest that roots evolved from stems (e.g. [57,58]). However, a growing body of evidence indicates that, in angiosperms, cells with root pericycle-like identity and their meristematic derivatives are the most developmentally plastic cell population. They represent the transitional stage for totipotent callus tissue formation from root, as well as from above-ground organ tissues, and are responsible for development of shoot apical meristems from callus tissue, lateral root primordia, or root apical meristems [59,60•]. This evidence for broad morphogenetic potential of root-specific cells challenges ideas that roots evolved from stems and suggests morphogenetic equivalence between roots and stems, at least in angiosperms.

As discussed earlier, the view that early polysporangiophytes had stems corresponds to a downward outlook on evolution [23•] that disregards the large body of paleobotanical evidence which demonstrates that the sporophytic axes of early polysporangiophytes differed dramatically from the stems of modern plants (e.g. [1,53••]). If one adopts the upward outlook for inferring homology, both stems and roots evolved from the ancestral sporophytic axes of early polysporangiophytes. The intersection of the developmental mechanisms shared by stems and roots can provide clues to the controls of development in the early polysporangiophyte axis (the 'ancestral meristem' of Steeves & Sussex [61]). These include WUSCHEL, its paralog WOX5, and CLAVATA genes, components of the pathways controlling homeostasis of stem cells in the apical meristems of both shoots and roots in *A. thaliana* [57], whose homologs have been identified in *P. patens* [62–64], although their functions are unknown.

The ancestral polysporangiophyte

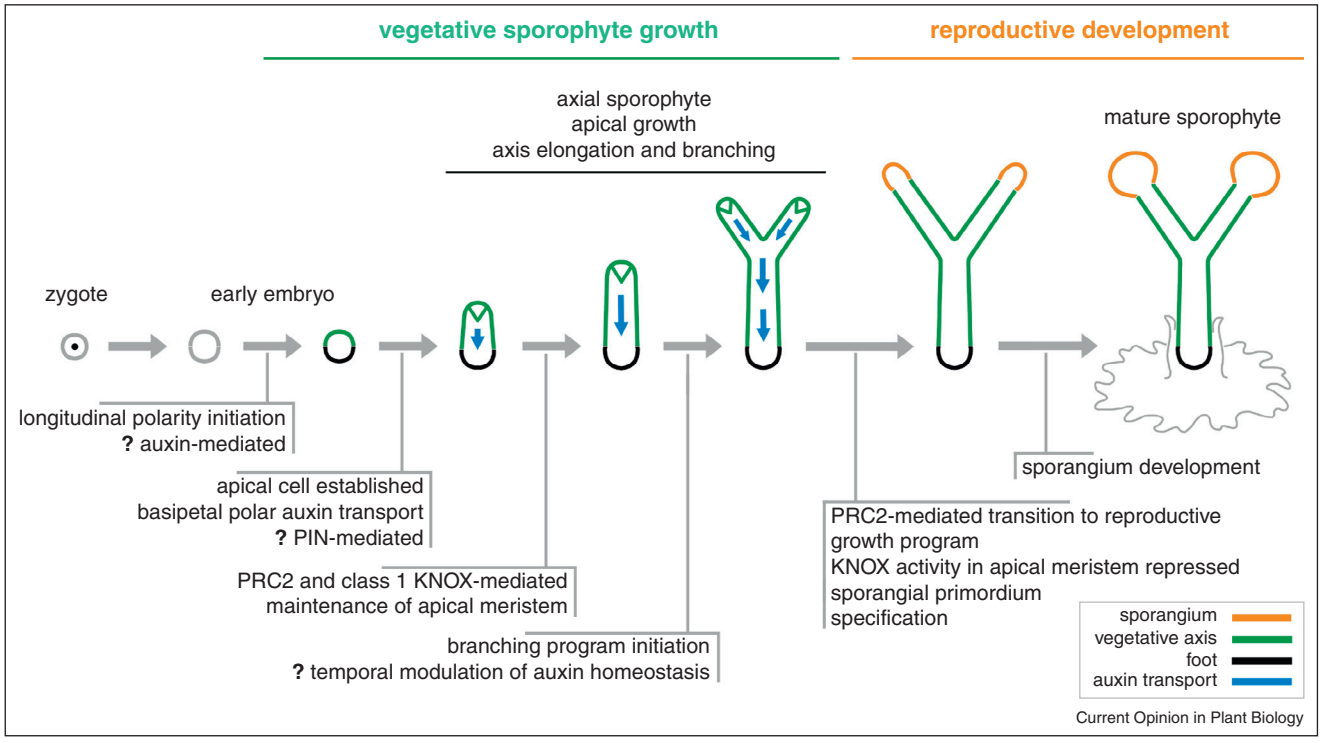
Paleobotanical data, in combination with information on developmental mechanisms, help paint a more detailed picture of the earliest polysporangiophytes. The fossil record shows that the earliest polysporangiophytes had vegetative sporophytes that were unlike those of modern vascular plants. Too small to be physiologically independent (Figure 3), these sporophytes remained physically attached to the gametophytes [53••] which were most likely thalloid [5•,65,66•]. Although branched, their simple, undifferentiated axes lacked tracheids and probably had determinate growth like a bryophyte sporophyte, with all branch tips terminating in sporangia [22,26]. Longitudinal polarity was initiated early in embryogeny, probably through an auxin-mediated mechanism, between a foot primordium and a pole of vegetative growth characterized by an early-established apical cell (Figure 4). In subsequent development, elongation of the sporophyte axis was a result of growth driven by basipetal polar auxin transport (possibly PIN-mediated) and arising from the apical cell, in a meristematic area maintained by class 1 KNOX genes [28•] — possibly repressing PRC2 gene expression. Branching of the sporophyte axis may have been mediated by a mechanism responsible for the temporal modulation of auxin homeostasis. Vegetative growth and branching, potentially indeterminate, were arrested by the transition to a reproductive growth program and sporangium formation at the branch tips, probably induced by PRC2 gene expression.

"It's the journey, not the destination" …

… reads a popular paraphrase of Ralph Waldo Emerson's famous quip on the meaning of life. Even more so when you don't know the destination. We don't know what answers await at the destination of our search into the origins of the embryophyte sporophyte body plan, but this journey of discovery is sure to reveal along the way many new aspects of morphological evolution in plants. Only by understanding the developmental changes that led to the early polysporangiophyte sporophyte will we be able to resolve the basic body plan homologies across land plants and, ultimately, reach a better understanding of embryophyte phylogeny and evolution. The road to this greater understanding passes through bryophyte development. At present, we know little about the detailed sequence of anatomical, cellular, and molecular level events of sporophyte development in the extant embryophyte relatives of the polysporangiophytes, the bryophytes. Liverwort and hornwort sporophyte development is largely a *terra incognita* in dire need of model systems. Even in mosses, development of a model system characterized by more extensive sporophyte development may provide deeper insights into developmental mechanisms. Studies of *P. patens* have nevertheless started to provide some answers which are inspiring more pointed questions.

A detailed characterization of the development of *P. patens* sporophytes with multiple sporangia obtained by disruption of *TEL* and *LFY* loci or of auxin movement might be insightful. Testing whether sporophyte branching in each of these cases is initiated during vegetative growth or after the transition to sporangial development, combined with detailed documentation of TEL and LFY expression patterns throughout wild type sporophyte development, may provide clues to regulation of branching in the early polysporangiophyte sporophyte. A better understanding of mechanisms that control branching could also come from uncovering the factors that induce branching in the apogamous PRC2 deletion sporophytes, as well as from characterization of the detailed anatomy of

Figure 4



Development of an early polysporangiophyte sporophyte as predicted by the apical growth hypothesis. Early embryogeny followed a vegetative developmental program, with early initiation of longitudinal polarity between a foot primordium and a pole of vegetative growth comprising an early-established apical cell. Vegetative elongation of the sporophyte axis was a result of growth arising from the apical cell (with PRC2 and class 1 KNOX-mediated maintenance of the meristematic area) and driven by basipetal polar auxin transport. Branching of the sporophyte axis could have been mediated by temporal modulation of auxin homeostasis. Elongation and branching, potentially indeterminate, were arrested by transition to a reproductive growth program, probably induced by PRC2 gene expression, and sporangium formation. The resulting sporophyte was small, physically attached and physiologically dependent on the thalloid gametophyte, and all its branches terminated in sporangia.

branching in this and other cases of sporophyte branching. Tracing the detailed expression patterns of markers of vegetative and reproductive growth, such as KNOX1 and PRC2, throughout sporophyte development in the different *P. patens* mutants and deletion backgrounds may also provide insights into causes and mechanisms of branching, as well as indeterminacy. And, if polar auxin transport is not PIN-mediated in mosses, what is the mechanism driving it? Genomic comparisons of fully sequenced plant model systems — *P. patens*, *Selaginella moellendorffii*, and *A. thaliana* — for other candidate genes that regulate indeterminate apical growth of the sporophyte, auxin transport and homeostasis, branching, and the transition to reproductive growth in bryophytes will provide additional directions of investigation.

At the beginning of this review, we identified several missing pieces of this evolutionary puzzle. Identification of these provides an opportunity to develop testable hypotheses that expand our understanding of the role of developmental changes in the evolution of the branching polysporangiophyte sporophyte, and also for characterizing the subsequent evolution of stem/leaf/root organography shared by nearly all modern vascular plants. The use of genetic and genomic tools to test such hypotheses has great promise for moving beyond gene-by-gene morphological changes to encompass the transcendent properties of developmental systems that result from the combined influence of numerous genetic changes. The journey ahead is long, but behind the many unknowns hide just as many exciting avenues to explore.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Kenrick P, Crane PR: *The Origin and Early Diversification of Land Plants*. Washington, DC: Smithsonian Institution Press; 1997.

2. Kenrick P, Crane PR: **Water-conducting cells in early fossil land plants: implications for the early evolution of tracheophytes**. *Bot Gaz* 1991, **152**:335-356.

3. Stewart WN, Rothwell GW: *Paleobotany and the Evolution of Plants*. 2nd edn. Cambridge: Cambridge University Press; 1993.

4. Cronk QCB: *The Molecular Organography of Plants*. Oxford: Oxford University Press; 2009.

5. • Gerrienne P, Gonez P: **Early evolution of life cycles in embryophytes: a focus on the fossil evidence of gametophyte/sporophyte size and morphological complexity**. *J Syst Evol* 2011, **49**:1-16.
Review that brings together within an evolutionary framework the latest direct fossil evidence for early land plant morphology. The authors use a detailed phylogenetic context of bryophytes and early polysporangiophytes to propose a sequence of progressive change in the size ratio of the gametophyte and sporophyte phase, and in the morphological complexity of the two phases. They also propose a thalloid morphology for the gametophytes of early polysporangiophytes.

6. •• Ligrone R, Duckett JG, Renzaglia KS: **The origin of the sporophyte shoot in land plants: a bryological perspective**. *Ann Bot* 2012, **110**:935-941 http://dx.doi.org/10.1093/aob/mcs176.
The inferred sister-group relationship between hornworts and polysporangiophytes provides the background for the hypothesis proposing that the polysporangiophyte sporophyte evolved by sterilization of a hornwort-like sporophyte. Some of the ideas presented are complemented by ideas included in [7••].

7. •• Ligrone R, Duckett JG, Renzaglia KS: **Major transitions in the evolution of early land plants: a bryological perspective**. *Ann Bot* 2012, **109**:851-871 http://dx.doi.org/10.1093/aob/mcs017.
Review of bryophyte morphologic and developmental characters that inform ideas about the evolution of embryophytes, vascular plants, and the respective innovations which allowed them to thrive on land. Some of the ideas presented are complemented by ideas included in [6••].

8. • Qiu Y-L, Taylor AB, McManus HA: **Evolution of the life cycle in land plants**. *J Syst Evol* 2012, **50**:171-194.
A look at the evolution of the land plant life cycle in terms of adaptive advantages of the gametophyte and sporophyte phases, morphological transitions in the two phases (phylogenetic trends and change in size, nutritional mode and reproductive output), the origin of the sporophyte phase and the evolution of sporangium-bearing structures; also endorses a sister-group relationship between hornworts an polysporangiophytes.

9. • Mishler BD, Churchill SP: **A cladistic approach to the phylogeny of the "bryophytes"**. *Brittonia* 1984, **36**:406-424.
The first morphological cladistic analysis of embryophyte phylogeny, yielded embryophyte monophylly, paraphylly of bryophytes with respect to polysporangiophytes and supports a moss-polysporangiophyte clade.

10. Edwards D, Davies ECW: **Oldest recorded *in situ* tracheids**. *Nature* 1976, **263**:494-495.

11. Edwards D, Feehan J: **Records of *Cooksonia*-type sporangia from late Wenlock strata in Ireland**. *Nature* 1980, **287**:41-42.

12. Gensel PG: **The earliest land plants**. *Annu Rev Ecol Evol Syst* 2008, **39**:459-477.

13. Steemans P, Le Herisse A, Melvin J, Miller MA, Paris F, Verniers J, Wellman CH: **Origin and radiation of the earliest vascular land plants**. *Science* 2009, **324**:353.

14. Goffinet B: **Origin and phylogenetic relationships of bryophytes**. In *Bryophyte Biology.* Edited by Shaw AJ, Goffinet B. Cambridge: Cambridge University Press; 2000:124-149.

15. Mishler BD, Kelch DG: **Phylogenomics and early land plant evolution**. In *Bryophyte Biology,* 2nd edn. Edited by Shaw AJ, Goffinet B.Cambridge: Cambridge University Press; 2008:173-197.

16. Rothwell GW, Nixon KC: **How does the inclusion of fossil data change our conclusions about the phylogenetic history of euphyllophytes?** *Int J Plant Sci* 2006, **167**:737-749.

17. Rothwell GW, Crepet WL, Stockey RA: **Is the anthophyte hypothesis alive and well? New evidence from the reproductive structures of Bennettitales**. *Am J Bot* 2009, **96**:296-322.

18. •• Kato M, Akiyama H: **Interpolation hypothesis for origin of the vegetative sporophyte of land plants**. *Taxon* 2005, **54**:443-450.
The hypothesis proposes that the bryophyte sporophyte is a footed sporangium and that the polysporangiophyte sporophyte evolved from a bryophyte-type sporophyte by sterilization of the sporangium and intercalation of a vegetative axial organ in early embryo development.

19. Smith GM: *Cryptogamic Botany, Volume two. Bryophytes and Pteridophytes*. New York, NY: McGraw-Hill; 1955.

20. Campbell DH: *The Structure and Development of Mosses and Ferns*. 1st edn. London: Macmillan; 1895.

21. Bower FO: *Primitive Land Plants*. London: Macmillan; 1935.

22. Rothwell GW: **The fossil history of branching: implications for the phylogeny of land plants**. In *Experimental and Molecular Approaches to Plant Biosystematics. Monographs in Systematic Botany*, Vol 53. Edited by Hoch PC, Stephenson AG. St. Louis, MO: Missouri Botanical Garden; 1995:71-86.

23. • Stewart WN: **An upward outlook in plant morphology**. *Phytomorphology* 1964, **14**:120-134.
An underappreciated lesson in thinking about morphological evolution, which discusses all major aspects of plant morphology and the widely reaching implications of the telome theory. Although the telome theory is now largely outdated, the outlook on evolution is inspiring.

24. Qiu YL, Li L, Wang B, Chen Z, Dombrovska O, Lee J, Kent L, Li R, Jobson RW, Hendry TA, Taylor DW, Testa CM, Ambros M: **A nonflowering land plant phylogeny inferred from nucleotide sequences of seven chloroplast, mitochondrial, and nuclear genes**. *Int J Plant Sci* 2007, **168**:691-708.

25. Chang Y, Graham SW: **Inferring the higher-order phylogeny of mosses (Bryophyta) and relatives using a large, multigene plastid data set**. *Am J Bot* 2011, **98**:839-849.

26. Edwards D, Wellman C: **Embryophytes on land: the Ordovician to Lochkovian (Lower Devonian) record**. In *Plants Invade the Land. Evolutionary and Environmental perspectives.* Edited by Gensel PG, Edwards D. New York, NY: Columbia University Press; 2001:3-28.

27. Bower FO: *The Origin of a Land Flora. A Theory Based Upon the Facts of Alternation*. London: Macmillan; 1908.

28. • Bowman JL: **Walkabout on the long branches of plant evolution**. *Curr Opin Plant Biol* 2013, **16**:70-77.
Review of the major features of land plant morphological evolution and potential developmental regulators involved in their evolution, with a discussion of hypotheses concerning the origin of the vascular plant shoot apical meristem. An attempt to reconstruct ancestral characters in land plants suggests that ancestral thalloid or axial gametophytes are similarly parsimonious and that origin of the vascular plant shoot apical meristem from a transient bryophyte apical meristem is most parsimonious.

29. Crum H: *Structural Diversity of Bryophytes*. Ann Arbor, MI: University of Michigan Herbarium; 2001.

30. Moss B: **The apical meristem of *Fucus***. *New Phytol* 1967, **66**:67-74.

31. Gifford EM Jr: **Concept of apical cells in bryophytes and pteridophytes**. *Annu Rev Plant Phys* 1983, **34**:419-440.

32. Millar AJK, Wynne MJ: ***Valeriemaya* gen. nov. (Rhodophyta), with a discussion of apical organizations within the Delesseriaceae**. *Br Phycol J* 1992, **27**:131-143.

33. • Graham LE, Cook ME, Busse JS: **The origin of plants: body plan changes contributing to a major evolutionary radiation**. *Proc Natl Acad Sci U S A* 2000, **97**:4535-4540.
An overview of streptophyte phylogeny and the evolution of major morpho-anatomical characters and changes provides the broader context for a discussion of embryophyte evolution.

34. Leitgeb H: **Ueber verzweigte Moossporogonien**. *Mitteilungen des naturwissenschaftlichen Vereins für Steiermark* 1876, **13**:3-20.

35. • Györffy I: **Monstruoses Sporophyton von Tetraplodon bryoides aus Suomi**. *Annales Societatis Zoologicae-Botanicae Fennicae Vanamo* 1929, **9**:299-319.
Although old, this paper includes a comprehensive list and proposes a typology for the different types of sporophytic teratologies encountered in bryophytes.

36. Györffy I: **Musci monstruosi Transsilvanici. 1. Monstruose Catharinaea haussknechtii aus Siebenburgen**. *Erdelyi Muzeum-Egyesulet (Fundo Rockefelleriano Adiuvante Editum 9)* 1934, **1934**:345-348.

37. Vivancos J, Spinner L, Mazubert C, Charlot F, Paquet N, Thareau V, Dron M, Nogue F, Charon C: **The function of the RNA-binding protein TEL1 in moss reveals ancient regulatory mechanisms of shoot development**. *Plant Mol Biol* 2012, **78**:323-336.

38. Tanahashi T, Sumikawa N, Kato M, Hasebe M: **Diversification of gene function: homologs of the floral regulator FLO/LFY control the first zygotic cell division in the moss *Physcomitrella patens***. *Development* 2005, **132**:1727-1736.

39. Fujita T, Sakaguchi H, Hiwatashi Y, Wagstaff SJ, Ito M, Deguchi H, Sato T, Hasebe M: **Convergent evolution of shoots in land plants: lack of auxin polar transport in moss shoots**. *Evol Dev* 2008, **10**:176-186.

40. •• Okano Y, Aono N, Hiwatashi Y, Murata T, Nishiyama T, Ishikawa T, Kubo M, Hasebe M: **A polycomb repressive complex 2 gene regulates apogamy and gives evolutionary insights into early land plant evolution**. *Proc Natl Acad Sci U S A* 2009, **106**:16321-16326.

An analysis of apogamous *Physcomitrella* sporophytes produced by deletion of PRC2 genes; these exhibit indeterminate growth from a persistent apical cell expressing class 1 KNOX genes, as well as dichotomous branching. Expression of the PRC2 genes in the apogamous sporophytes arrests meristematic activity and turns on the reproductive growth program, consistent with PRC2 expression patterns in wild-type sporophytes.

41. Mosquna A, Katz A, Decker EL, Rensing SA, Reski R, Ohad N: **Regulation of stem cell maintenance by the Polycomb protein FIE has been conserved during land plant evolution**. *Development* 2009, **136**:2433-2444.

42. •• Sakakibara K, Nishiyama T, Deguchi H, Hasebe M: **Class 1 KNOX genes are not involved in shoot development in the moss *Physcomitrella patens* but do function in sporophyte development**. *Evol Dev* 2008, **10**:555-566.

Study of class 1 KNOX gene expression patterns in *Physcomitrella* sporophytes shows that they are associated with meristematic activity in the apical cell and seta meristem and they are repressed when the reproductive developmental program is initiated. It is hypothesized that class 1 KNOX genes regulate the frequency of cell division and cell growth in mosses.

43. Floyd S, Bowman JL: **Gene expression patterns in seed plant shoot meristems and leaves: homoplasy or homology?** *J Plant Res* 2010, **123**:43-55.

44. Champagne CEM, Ashton NW: **Ancestry of KNOX genes revealed by bryophyte (*Physcomitrella patens*) homologs**. *New Phytol* 2001, **150**:23-36.

45. Singer SD, Ashton NW: **Revelation of ancestral roles of KNOX genes by a functional analysis of Physcomitrella homologues**. *Plant Cell Rep* 2007, **26**:2039-2054.

46. • Sakakibara K, Ando S, Yip HK, Tamada Y, Hiwatashi Y, Murata T, Deguchi H, Hasebe M, Bowman JL: **KNOX2 genes regulate the haploid-to-diploid morphological transition in land plants**. *Science* 2013, **339**:1067-1070.

Aposporic gametophytes produced by deletion of class 2 KNOX genes in *Physcomitrella* sporophytes indicate that KNOX2 transcription factors repress the haploid developmental program in the sporophyte phase; thus, KNOX2 may have had a critical role in the establishment of the alternation of generations in embryophytes.

47. • Cooke TJ, Poli D, Cohen JD: **Did auxin play a crucial role in the evolution of novel body plans during the Late Silurian–Early Devonian radiation of land plants?** In *The Evolution of Plant Physiology. From Whole Plants to Ecosystems*. Edited by Hemsley AR, Poole I. Amsterdam: Elsevier; 2004:85-107.

Overview of auxin regulation of plant body plans in the context of morphological evolution in land plant sporophytes. Data on auxin transport in the different embryophyte lineages indicate that auxin is a master regulator of plant development and that major changes in auxin action underlie the morphological radiation of embryophytes.

48. Poli D, Jacobs M, Cooke TJ: **Auxin regulation of axial growth in bryophyte sporophytes: its potential significance for the evolution of early land plants**. *Am J Bot* 2003, **90**:1405-1415.

49. Paponov IA, Teale WD, Trebar M, Blilou I, Palme K: **The PIN auxin efflux facilitators: evolutionary and functional perspectives**. *Trends Plant Sci* 2005, **10**:170-177.

50. Mravec J, Skůpa P, Bailly A, Hoyerová K, Křeček P, Bielach A, Petrášek J, Zhang J, Gaykova V, Stierhof Y-D, Dobrev PI, Schwarzerová K, Rolčík J, Seifertová D, Luschnig C, Benková E, Zažímalová E, Geisler M, Friml J: **Subcellular homeostasis of phytohormone auxin is mediated by the ER-localized PIN5 transporter**. *Nature* 2009, **459**:1136-1140.

51. • Pires ND, Dolan L: **Morphological evolution in land plants: new designs with old genes**. *Philos Trans R Soc B: Biol Sci* 2012, **367**:508-518.

Review of the major features of morphological evolution in land plants and the evolution of correlated genes and developmental mechanisms. The developmental toolkit of the earliest land plants is shown to have included many of the gene families that control development in extant plants, which suggests that morphological evolution involved subfunctionalization and neo-functionalization of pre-existing genes and redirection of pre-existing mechanisms, against a background of progressive diversification of transcription factor families.

52. • Sanders HL, Langdale JA: **Conserved transport mechanisms but distinct auxin responses govern shoot patterning in *Selaginella kraussiana***. *New Phytol* 2013, **198**:419-428.

Study of patterns of polar auxin transport in *Selaginella* demonstrates that basipetal auxin transport in shoots is a character shared across vascular plants (but with different morphogenetic roles) and suggests that it may be a plesiomorphic feature of embryophyte sporophytic axes.

53. •• Boyce CK: **How green was *Cooksonia*? The importance of size in understanding the early evolution of physiology in the vascular plant lineage**. *Paleobiology* 2008, **34**:179-194.

Morphometric analysis of early polysporangiophyte sporophytic axes and consideration of the physiological implications of their anatomy demonstrates that the smallest such axes were obligate dependents on the gametophytes and suggests that this is a plesiomorphic character of polysporangiophytes, congruent with the growth model hypothesized in [22].

54. Gensel PG, Edwards D (Eds): *Plants Invade the Land. Evolutionary and Environmental Perspectives*. New York, NY: Columbia University Press; 2001.

55. Tomescu AMF: **Megaphylls, microphylls and the evolution of leaf development**. *Trends Plant Sci* 2009, **14**:5-12.

56. Kenrick P: **The telome theory**. In *Developmental Genetics and Evolution.* Edited by Cronk QCB, Bateman RM, Hawkins JA. London: The Systematics Association; 2002:365-387.

57. Stahl Y, Grabowski S, Bleckmann A, Kuhnemuth R, Weidtkamp-Peters S, Gustavo Pinto K, Kirschner GK, Schmid JB, Wink RH, Hülsewede A, Felekyan S, Seidel CAM, Simon R: **Moderation of *Arabidopsis* root stemness by CLAVATA1 and ARABIDOPSIS CRINKLY4 receptor kinase complexes**. *Curr Biol* 2013, **23**:362-371.

58. Benfey PN: **Is the shoot a root with a view?** *Curr Opin Plant Biol* 1999, **2**:39-43.

59. Sugimoto K, Jiao YL, Meyerowitz EM: ***Arabidopsis* regeneration from multiple tissues occurs via a root development pathway**. *Dev Cell* 2010, **18**:463-471.

60. • Chatfield SP, Capron R, Severino A, Penttila P-A, Alfred S, Nahal H, Provart NJ: **Incipient stem cell niche conversion in tissue culture: using a systems approach to probe early events in *WUSCHEL*-dependent conversion of lateral root primordia into shoot meristems**. *Plant J* 2013, **73**:798-813.

High throughput methods are used to study the lateral root primordium to shoot meristem conversion in *Arabidopsis* and show overlap in gene expression patterns with callus-based shoot regeneration; WUS is required for lateral root primordium to shoot meristem conversion. The article also reviews evidence which suggests that cells with root pericycle-like identity and their meristematic derivatives are a most developmentally plastic cell population.

61. Steeves TA, Sussex IM: *Patterns in Plant Development*. Cambridge: Cambridge University Press; 1991.

62. Deveaux Y, Toffano-Nioche C, Claisse G, Thareau V, Morin H, Laufs P, Moreau H, Kreis M, Lecharny A: **Genes of the most conserved WOX clade in plants affect root and flower**

development in Arabidopsis. *BMC Evol Biol* 2008, **8** http://dx.doi.org/10.1186/1471-2148-8-291.

63. Miwa H, Tamaki T, Fukuda H, Sawa S: **Evolution of CLE signaling. Origins of the CLV1 and SOL2/CRN receptor diversity**. *Plant Signal Behav* 2009, **4**:477-481.

64. Nardmann J, Reisewitz P, Werr W: **Discrete shoot and root stem cell-promoting WUS/WOX5 functions are an evolutionary innovation of angiosperms**. *Mol Biol Evol* 2009, **26**:1745-1755.

65. Tomescu AMF, Rothwell GW: **Wetlands before tracheophytes: thalloid terrestrial communities of the Early Silurian Passage Creek biota (Virginia)**. In *Wetlands Through Time*. Edited by Greb SF, DiMichele WA. Boulder, CO: Geological Society of America; 2006:41-56.

66. • Strother PK: **Thalloid carbonaceous incrustations and the asynchronous evolution of embryophyte characters during the Early Paleozoic**. *Int J Coal Geol* 2010, **83**:154-161.
Study of Early Devonian land plant fossil assemblages that shows co-occurrence of axial sporophytes with thalloid compressions interpreted as gametophytes and suggests that the earliest polysporangiophytes had thalloid gametophytes, as hypothesized in [5•] and congruent with the findings presented in [65].