
Structural Fingerprints of Development at the Intersection of Evo-Devo and the Fossil Record

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Abstract

The plant body preserves diagnostic structural features that develop as the result of specific regulatory genes and growth regulators. When recognized in extinct species, those features serve as structural *fingerprints* for the regulatory programs

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by which they were produced. We review the contributions of the fossil record to understanding the evolution of plant development in a temporal (geologic time) and a structural perspective (morphology, anatomy), and we highlight major topics in plant evolution in which integration of data from fossil and living plants has yielded significant resolution. Up to the present, the most ubiquitous growth regulator, auxin, has been documented as essential to the regulation of secondary growth and wood formation not only in seed plants, but also in several other major groups in which living species are no longer characterized by secondary growth. Additional fingerprints of growth regulation reveal the occurrence of gravitropic responses in fossils that extend back in time 400 million years and explain the evolution of equisetacean reproductive morphologies, living and extinct, by the interaction of modular regulatory programs. Still other fingerprints document parallel evolution of stem/leaf organography in several clades of living plants (e.g., ferns, *Equisetum*, and seed plants) and of substantial rooting systems that facilitated evolution of giant trees in extinct lycophytes and seed plants. Future application of techniques for identifying and interpreting the significance of structural fingerprints to a much broader spectrum of developmental processes holds tremendous potential for the paleontological record to substantially illuminate and enhance understanding of systematics and evolution within the context of plant development.

Keywords

Anatomy · Auxin · Body plan · Developmental regulation · Fossil · Leaf · Morphology · Paleo-evo-devo · Phytomer · Rhizomorph · Root · Secondary growth · Strobilus · Structural fingerprint

Introduction

Paleontology has a long history of illuminating patterns of evolution, but not the processes that underpin evolution. Until relatively recently, evolutionary processes have been investigated primarily within the realm of classical and population genetic theory. Nevertheless, our understanding of such processes has remained frustratingly incomplete. This situation has begun to change with the rise of molecular biology (ca. 1980s), which is providing a platform for a rapidly increasing number of techniques by which a deeper understanding of gene regulatory processes is being forged. The relatively new discipline of developmental molecular biology, in particular, presents exciting potential for the rapid advancement of knowledge on the processes that underpin evolution at the organismal level.

Developmental molecular studies characterize evolution within the context of differential developmental trajectories under the control of gene regulation, including the activities of developmental gene networks and growth regulators. This fruitful approach also provides, for the first time, an opportunity for ontogenetic studies of extinct plants to begin to contribute to our growing understanding of evolutionary processes (Rothwell et al. 2014; Spencer et al. 2015; Tomescu et al.

2017). The rationale that underlies such paleontological studies is simple. In plants there are ontogenetically diagnostic structural features that result from the activity of specific regulators of development (genes, hormones), and such features can be regarded as *fingerprints* for the specific regulatory pathways by which they have developed (Rothwell et al. 2014). Furthermore, by mapping on phylogenetic trees of living plants the earliest occurrences of genetic regulatory pathways that produce such fingerprints, the tempo of evolution of structural innovations can be documented and correlated with the evolution of gene regulation (e.g., Langdale 2008; Harrison 2016). As is also true for the emerging discipline of paleogenomics, when employed as reciprocal hypothesis tests, these combined approaches comprise powerful methodologies for integrating pattern and process in plant evolution.

The purpose of this contribution is to characterize plant paleo-developmental evolutionary biology, to explain the rationale for and scope of such studies, to highlight studies that integrate patterns of plant evolution and the fossil record with rapidly developing understanding of the role of regulatory genetics in organismal ontogeny, and thereby to illuminate the developmental foundations of plant evolution in an updated perspective of F.O. Bower's and W.N. Stewart's upward outlook.

Beyond Principles: What Has the Inclusion of Data from the Fossil Record Contributed to Evo-Devo Plant Biology

Fossils provide direct evidence for the process of evolution. As bearers of morphological and anatomical characters, fossils are best integrated into evolutionary studies within an evo-devo framework. Inclusion of fossils in evolutionary hypotheses pre-dated and foreshadowed the modern evo-devo paradigm. Classic transformational series, such as those proposed for the evolution of the conifer bract-scale complex or the sphenopsid sporangiophore, were elaborated based on fossils long before the rise of evo-devo molecular biology. Such paleontological data illustrate morphological (and, implicitly, developmental) change through time, the very agenda of evo-devo.

The types of data contributed by fossils range from basic observations on the shape or position of organs, to interpretations of plant development, and to comparative datasets including complex anatomical or morphological relationships between plant parts, tissues, or cells. Crucial for the latter are anatomical and morphological fingerprints that allow for the recognition of developmental and physiological processes in extinct plants and, thus, can bridge the gap between molecular biology and hundred-million-year old fossils. These different types of data illuminate diverse aspects of the evolution of plant features including growth patterns and dynamics (topology, tempo, and modes of meristematic growth; developmental domain partitioning; tissue-level positional patterning of cells and cell types); mechanisms of growth regulation and growth responses; organization of the plant body; and reproductive biology. In turn, these diverse plant features and their temporal (stratigraphic), taxonomic, and phylogenetic context address several categories of

knowledge relevant to the evo-devo agenda: tempo and mode of evolution (minimum ages for the evolution of specific features, processes, or regulators; sequence of character evolution), evidence for homology, and phylogenetic relationships.

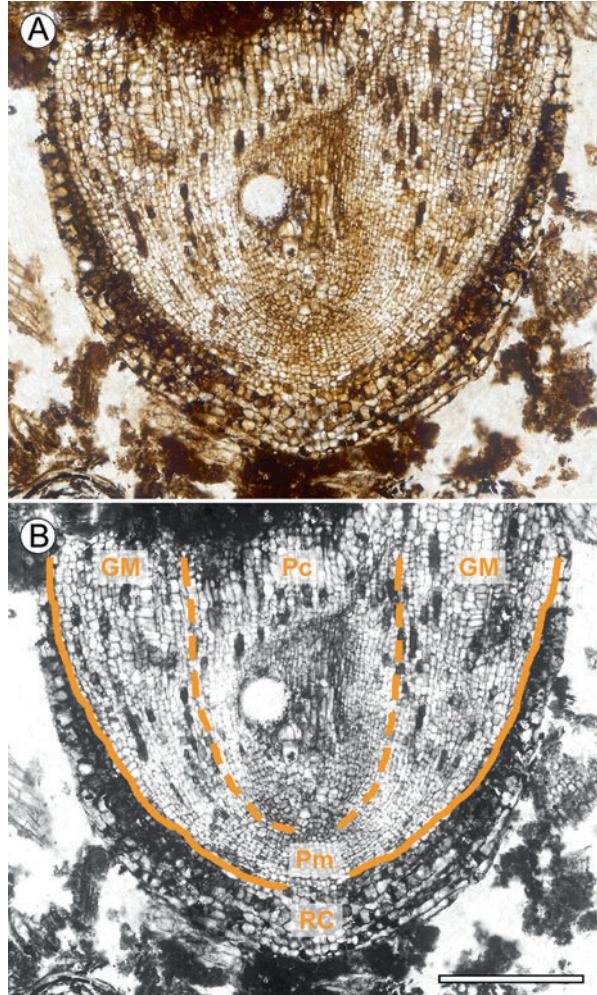
Growth Patterns and Dynamics

The precisely structured anatomy of plants is the result of spatially and temporally coordinated sequences of cell division, growth, and differentiation. One aspect of such developmental sequences is the early partitioning of meristematic tissues into domains with distinct developmental trajectories, i.e., developmental domain partitioning, such as the specification of protoderm versus ground meristem versus procambium in apical meristems. In the root apical meristem, another aspect of developmental domain partitioning involves the early establishment of the Körper (body) and Kappe (cap) domains, characterized by distinct patterns of cell division. The two domains cover different extents of the root apical meristem and give rise to different tissues of the root in different plant lineages; therefore, this partitioning bears a phylogenetic signal. Importantly, because they are identified based on patterns of cell division, the Körper and Kappe domains can be recognized in fossils with anatomical preservation, and not just in live, developing plants. This has allowed for recognition of a type of gymnospermous Körper-Kappe organization in a Carboniferous (ca. 320 Ma) root apical meristem (Fig. 1) that is different from those of all extant gymnosperms (Hetherington et al. 2016a) and, thus, reveals structural diversity previously unaccounted for, that could be used in phylogenetic inference.

Plant reproductive structures are often produced as a result of expression of a reproductive regulatory module in meristems otherwise responsible for vegetative growth. Reproductive regulatory modules likely conserved across embryophytes involve *LEAFY* genes, the AP2 gene subfamily, MIKC MADS-box genes, and Polycomb group genes (Tomescu et al. 2017). In all known cases, the reproductive growth mode is activated in apical meristems. However, Paleozoic and Mesozoic sphenopsid fossils have recently been shown to exhibit patterns of size and positioning of reproductive structures (sporangiophores) consistent with activation of a reproductive regulatory module in intercalary meristems. This has implications for the topology and mode of meristematic growth, suggesting that growth in reproductive mode can be effected not only by apical meristems, but also by intercalary meristems. This is the first example of reproductive growth arising from intercalary meristems, a mode of growth that could not have been predicted from the modern flora alone, and which has deep implications for the homology and evolution of sphenopsid reproductive structures (see section “[The *Equisetum strobilus*: A Case of Reciprocal Illumination](#)”).

Also associated with intercalary meristematic growth, rapid internode elongation that exceeds the tensional capacity of mature protoxylem cells generates rhexigenous protoxylem lacunae. Such lacunae found in *Equisetum* and grasses (Fig. 2) indicate that rapid growth from intercalary meristems evolved independently in distant plant lineages. The lacunae also provide a fingerprint for this topology (position) and tempo of meristematic growth that can be identified in the fossil record. If the

Fig. 1 (a). Apical meristem of a Carboniferous (ca. 320 Ma) gymnosperm root. (b). Same image as in a, with the root cap (RC), promeristem (Pm), and primary meristems (GM – ground meristem; Pc – procambium) indicated. Solid line separates the Kappe domain (represented in this root by the root cap) and Körper domain (everything else); dashed line separating procambium from the rest of root corresponds to Körper/Kappe boundary in extant gymnosperm roots. Scale = 400 μ m. Image courtesy of Alexander Hetherington



Givetian (ca. 385 Ma) plant *Ibyka* (Fig. 3) does indeed include rhexigenous protoxylem lacunae (as opposed to areas of incomplete preservation of protoxylem parenchyma), such rapid intercalary meristematic growth may have evolved as early as the Middle Devonian.

Alveolar megagametophyte cellularization, a type of tissue-level positional patterning of cells, is the result of a specific sequence of cell wall construction relative to position in the gametophyte, which leaves a conspicuous anatomical fingerprint (Fig. 4a). This fingerprint can be used to infer homology of process and regulatory mechanisms that can be traced into the fossil record. Seed plant megagametophytes that exhibit alveolar cellularization (Fig. 4b, c) extend back to the Famennian (Late Devonian, ca. 160 Ma), indicating that this feature shared by living and extinct seed plants represents a synapomorphy for the clade.

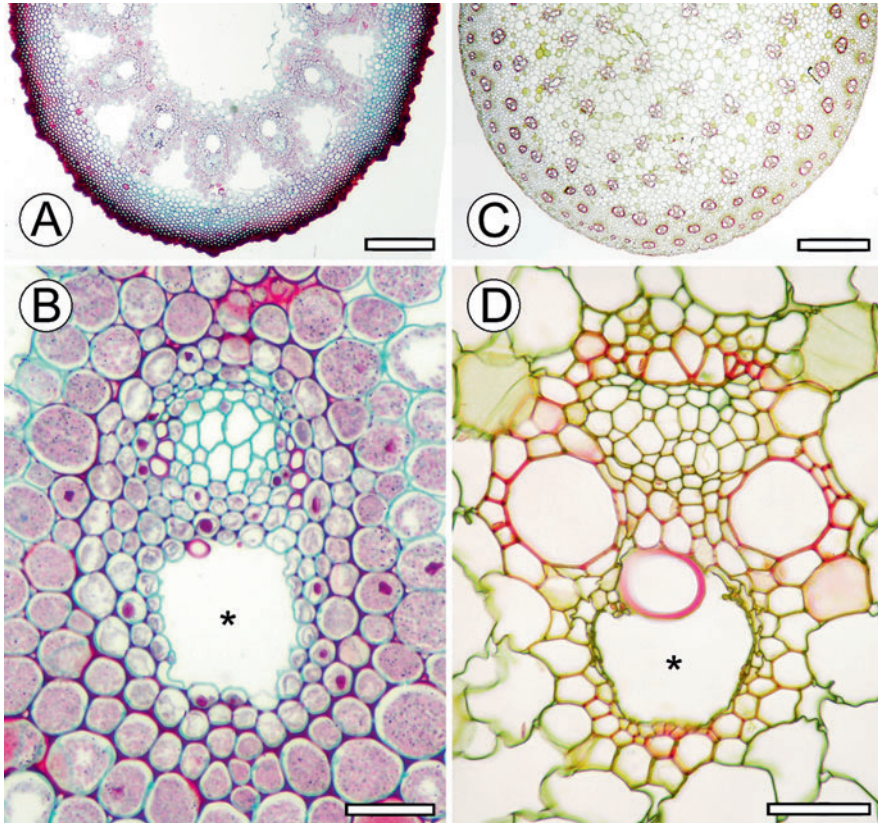


Fig. 2 Rhexigenous protoxylem lacunae (asterisks) produced by rapid internode elongation due to growth from intercalary meristem located at base of internode. (a, b) *Equisetum*. (c, d) *Zea*. Cauline vascular bundles in B and D shown with phloem at top and xylem at bottom. Scales = 500 μm (a); 75 μm (b); 750 μm (c); 50 μm (d)

In a different type of approach, the morphology and anatomy of fossil plants have inspired modeling studies of growth dynamics whose implications can be used to generate hypotheses about genetic regulatory mechanisms. Dynamics of apical meristematic growth in terms of growth rates and frequency of branching, taxis and angle of branching, meristem size, and growth determinacy, as illustrated by Silurian and Devonian tracheophytes, have been modeled by Niklas (1997 and references therein) and Stein and Boyer (2006). These studies have shown that variations in only a small set of parameters can generate a wide diversity of plant branching architectures. They also indicate that a shared set of underlying developmental regulators may be responsible for all this diversity, and point to specific developmental processes and domains to be studied by molecular biology in order to identify these regulators.

In another modeling approach, Stein (1993) used data from living plants – concerning the role of auxin in shaping the vascular system – to model features of

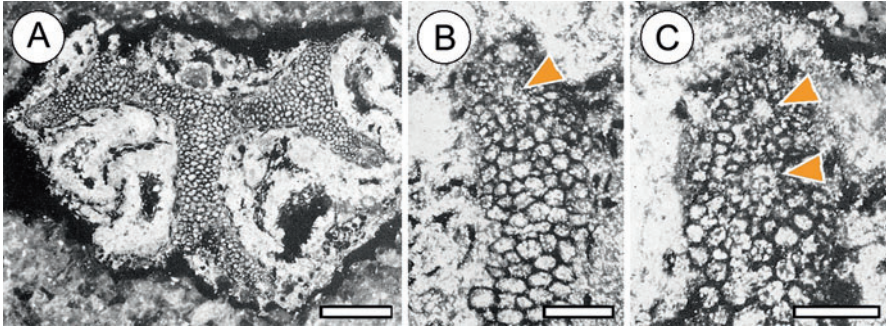


Fig. 3 Protoxylem lacunae of possible rhexigenous origin in the Givetian (ca. 385 Ma) plant *Ibyka amphykoma*. (a). Cross section of main axis with deeply lobed xylem; lacunae are small light areas close to tips of xylem lobes. (b, c) Details of xylem lobes with protoxylem lacunae at tips (arrowheads); protoxylem starting to divide (b) and already divided (c) radial direction showing divergence of lateral trace. Scales = 500 μm (a); 200 μm (b, c). Published by permission of Botanical Society of America (American Journal of Botany 60(4)/1973, p.375)

extinct plants, namely, stelar architecture, another feature reflecting developmental domain partitioning (vascular vs. ground tissues) and tissue-level positional patterning of cells (protoxylem vs. metaxylem). Comparisons between stelar configurations predicted by this model and the xylem architecture of fossil plants can be used, like in the case of branching architecture, to identify the model parameters responsible for xylem architecture of those plants. In turn, these parameters can be used to infer the functions of putative developmental regulators responsible for xylem architecture and formulate testable hypotheses for studies of the evolution of stelar architecture. Interestingly, whereas Stein's model was successful in predicting stelar architecture in aneurophyte-like plants (Fig. 5), it was less successful in generating stelar architectures comparable to those of cladoxyloids. This result could be indicating fundamental differences between cladoxyloids and progymnosperms in terms of regulatory programs controlling vascular architecture, consistent with the view that the two groups represent phylogenetically distinct lineages.

Fundamental Plant Growth Responses

Much of plant response to external stimuli consists of modulation of the location, direction, and rate of growth. Gravitropic growth represents a fundamental and conspicuous plant growth response. The fossil record provides a minimum age for gravitropic responses, demonstrating positive gravitropism in below-ground plant parts and negative gravitropism in above-ground parts no later than the Early Devonian (Lochkovian), ca. 415 Ma ago (Matsunaga and Tomescu 2017). These Early Devonian plants exhibit organs which grow downward into the substrate or in directions opposite those of the parts bearing reproductive structures or leaves.

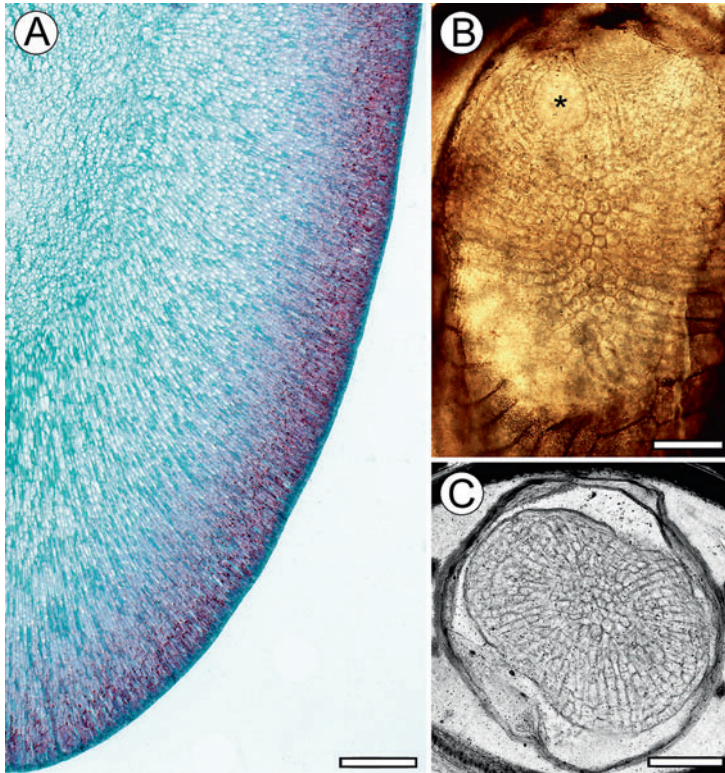


Fig. 4 Alveolar megagametophyte cellularization in extant and fossil seed plants. **(a)** Longitudinal half of chalazal portion of extant *Ginkgo* megagametophyte; alveolar cellularization recognized by radial cell files oriented perpendicular to megagametophyte surface. **(b, c)** Longitudinal and transverse sections (respectively) of megagametophytes of Late Carboniferous (ca. 305 Ma) pteridosperm (seed fern) *Gnetopsis elliptica*, displaying alveolar cellularization (asterisk marks archeogonium). Scales = 1 mm **(a)**; 200 μ m **(b)**; 250 μ m **(c)**. **(b and c)** courtesy of Jean Galtier

Furthermore, rhizomatous axes and cormose bases of some Early Devonian plants only developed rhizoids on portions that were in contact with the substrate (e.g., the rhizoids of *Nothia aphylla*; see section “[Gravitropism](#)”). Like in the downward growing organs, this polarization of rhizoid positioning implies the presence of gravity signal transduction mechanisms.

Fossils also provide information on the tempo and mode of evolution of positive gravitropism (see section “[Sequence of Character Evolution](#)”). Most of the Early Devonian plants exhibiting positively gravitropic responses do not have stem-leaf-root organization, which indicates that positive gravitropism pre-dates the evolution of roots. Additionally, fossils illustrate positively gravitropic organs that are not roots, such as undifferentiated axes of Early Devonian polysporangiophytes or rooting organs with stem homology. These indicate that gravitropic responses and root identity are not necessarily coupled (Matsunaga and Tomescu 2017).

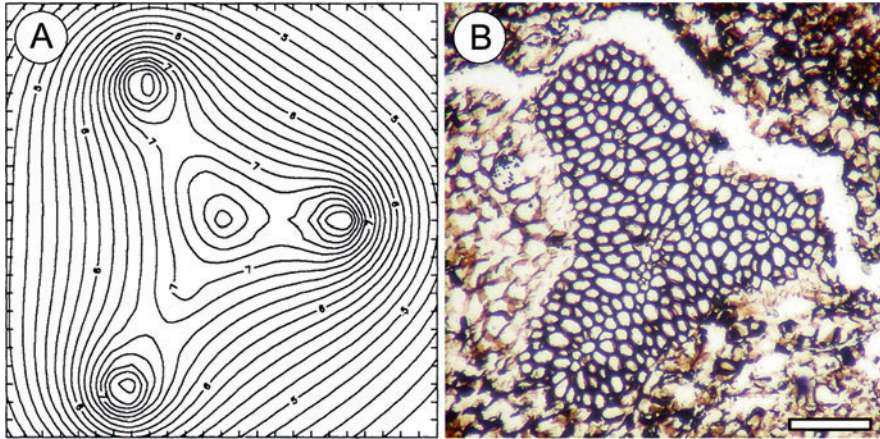


Fig. 5 Model based on small number of parameters controlling auxin dynamics at apical meristem and responsiveness of target tissue to auxin concentrations (a) predicts stele anatomy of Early Devonian (ca. 400 Ma) euphylllophyte probably related to progymnospermous lignophytes (b). Scale = 150 μm . (a) published by permission of University of Chicago Press (International Journal of Plant Sciences 154(2)/1983, p. 247)

Underground interactions between plants are another type of growth response elicitors. These interactions include kin recognition that directs growth toward minimizing interference between the roots of conspecifics, closely related or clonal individuals, to avoid competition and maximize resource exploitation. The fossil record illustrates kin recognition-driven growth responses, as shown by roots that curve away from one another or follow parallel trajectories, such as the Early Permian (ca. 280 Ma) in situ roots *Pinnatiramosus*. Similar patterns of growth orientation in root-bearing axes of the Early Devonian *Sengelia* suggest that underground kin recognition had evolved to direct rooting system development in lycophytes as early as 410 Ma ago.

Homology and Sporophyte Body Plans

Allowing access to the rich extinct diversity within plant clades, the fossil record includes morphologies and anatomical features that are absent among modern plants. Such features can be crucial in understanding the homology of plant structures or the origin of body plans, especially in lineages whose living representatives are isolated at the tips of long phylogenetic branches. The extant lycophyte *Isoetes* has a highly derived morphology that was understood, in terms of homologies, only through studies of the extensive fossil record of its clade, the rhizomorphic lycophytes. The studies emphasizing comparative anatomy, morphology, and embryogeny assembled a body of evidence that supports deep shoot homology of the lower corm of *Isoetes* and leaf homology of the “rootlets” attached to it, along with severely

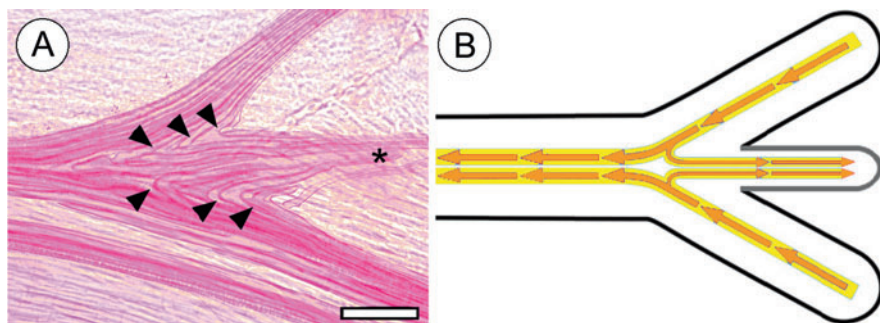


Fig. 6 *Selaginella*. Arching (U-shaped) tracheids (arrowheads) that connect steles of main stem (bottom right), side branch (top right), and rhizophore (asterisk) in (a) represent anatomical fingerprint for reversal of polar auxin transport associated with rhizophore development: from basipetal transport in stem and branch, to acropetal in rhizophore (b); yellow strips = vascular tissue; orange arrows = polar auxin transport. Scale = 200 μm

diminished elongation growth and branching capacities of the main axes, compared to extinct relatives (e.g., *Lepidodendrales*; see section “[Lepidodendrolean Rooting Structures](#)”).

In the same realm of body plan and organ homologies of the lycophyte sporophyte, a recent study has taken a comparative anatomy approach to address hypothesized homology relationships between the rhizophore of *Selaginella* and rooting structures associated with branching points in fossil early lycophytes and zosterophylls (Matsunaga et al. 2017). That study revealed an anatomical fingerprint for a reversal of polar auxin transport associated with rhizophore development: basipetal polar auxin transport in shoots to acropetal in the rhizophore. This fingerprint, in the form of arching (U-shaped) tracheids (Fig. 6), could be sought for in fossils, to test for presence of similar polar auxin transport patterns and to illuminate the homologies of early lycophyte rooting structures.

Another classic puzzle of plant morphology involves the origin and homologies of the *Equisetum* strobilus and sporangiophore. In an example of reciprocal illumination, understanding of vegetative meristematic growth in *Equisetum* can be used to formulate evo-devo hypotheses on reproductive development that can be tested based on information from fossil sphenopsids. This leads to a generalized model explaining variations in sphenopsid reproductive morphology, which, combined with information on reproductive developmental anatomy in extant *Equisetum*, provides an explanation for the origin of the strobilus and a hypothesis of sporangiophore homology (Tomescu et al. 2017; see section “[The Equisetum Strobilus: A Case of Reciprocal Illumination](#)”).

The typical sporophyte organization in modern tracheophytes comprises three basic types of vegetative organs: stems, leaves, and roots (i.e., stem-leaf-root organography). Leaves and roots each share minimal sets of defining features that render them comparable across the entire breadth of tracheophyte diversity. An outlook on tracheophyte morphological evolution within a phylogenetic context

that excludes the fossil record can easily take these features and their ubiquity in extant plants as indicating that leaves and roots are each homologous across all vascular plants. Conversely, inclusion of the fossil record in such a broad outlook plays a crucial role in resolving major aspects of the evolution of this basic body plan and the homologies of leaves and roots. Specifically, Late Silurian and Devonian tracheophytes characterized by simple body plans (undifferentiated branching axes bearing sporangia) form paraphyletic grades at the base of major branches of tracheophyte phylogeny, demonstrating that stem-leaf-root organography evolved independently in different lineages (Rothwell et al. 2014). This implies that neither leaves nor roots are homologous across different lineages. Furthermore, several lines of evidence reveal that leaves and roots almost certainly evolved independently more than twice (Boyce and Knoll 2002; Tomescu 2009; see section “[Euphyllophyte Leaf Evolution](#)”).

Sequence of Character Evolution

Plant phylogenies can be used to infer the mode of morphological evolution. Character distribution on phylogenetic trees can be and has been used to infer sequences of character evolution and ancestral character states. However, because phylogenetic trees represent hypotheses of relationships, sequences of character evolution predicted based on them are just as hypothetical. This is particularly evident in systematic trees that exclude extinct taxa (Rothwell and Nixon 2006). Within this context, fossils provide the only direct means for testing sequences of character evolution. Presence or absence of structures and anatomical features in fossils of different ages within a lineage provide direct evidence for the order of appearance of those features. An example is the sequential evolution of characters in organs we call leaves, during their independent parallel evolution in ferns and seed plants. Fossils demonstrate that whereas seed plants evolved determinate growth and broad pinnules before adaxial-abaxial polarity in the leaves, in filiclean fern leaves evolution of adaxial-abaxial polarity preceded broad pinnules and determinacy (Sanders et al. 2009; see section “[Euphyllophyte Leaf Evolution](#)”).

Lycophyte rooting structures are diverse and so are their homologies, some of which are not fully resolved (Rothwell and Erwin 1985; Tomescu 2011; Matsunaga et al. 2017). The oldest unequivocal lycophyte roots were described in the Early Devonian plant *Sengelia*, which produced roots on specialized axes of the branching system that are stem homologs. *Sengelia* rooting systems consist of horizontal or downward-growing root-bearing axes with laterally diverging roots. In all cases, the roots expand in a horizontal plane, irrespective of the orientation of subtending root-bearing axes. These observations indicate that, in lycophytes, root identity was uncoupled from positive gravitropism, a feature fundamentally associated with modern plant roots – in *Sengelia*, the organs that exhibit a gravitropic response are the root-bearing axes and not the roots. The roots of *Sengelia* also provide evidence for the sequence of character evolution: roots acquired positive gravitropism after they evolved as distinct organs, in lycophytes (Matsunaga and Tomescu 2017).

Early Devonian strata have yielded euphyllophytes as old as 407 Ma that exhibit secondary growth (wood production) from a vascular cambium (Gerrienne and Gensel 2016) (euphyllophytes are the sister clade to lycophytes and include psilotophytes, ferns, sphenopsids, and seed plants, along with diverse related lineages). These fossils provide a minimum age for the evolution of this important structural feature. Furthermore, the fact that these early wood producers have simple sporophyte organization (undifferentiated axes) indicates that secondary growth pre-dates the evolution of complex body plans with stem-leaf-root differentiation, in euphyllophytes. The small size of these wood-producing sporophytes suggests that secondary growth evolved primarily in response to selective pressures related to maximizing hydraulic conductance and not mechanical stiffness (Gerrienne and Gensel 2016; see sections “[Developmental Regulation](#)” and “[Secondary Growth](#)”).

Developmental Regulation

Plant fossils exhibit combinations of characters unknown in modern plants and preserve anatomical and morphological fingerprints for developmental processes and physiological mechanisms. Aside from implications for sequences of character evolution (see section “[Sequence of Character Evolution](#)”), when considered in their stratigraphic (temporal) and taxonomic context, these types of data provide glimpses into the systems biology of developmental regulation and its evolution. In many instances, the resulting perspectives inform understanding of the modularity of developmental regulatory networks, hierarchy of regulatory modules, synchronization in developmental processes, or relationships between physiology and development.

In isoetalean lycophytes, “rootlets” borne on the corm base (in *Isoetes*) branch apically and dichotomously and produce root cap-like structures at their tips (Fig. 7a), as well as root hairs (Fig. 7b). Fossil members of the clade provide developmental and structural evidence pointing to shoot homology of the corm base and leaf homology of the “rootlets” (Rothwell and Erwin 1985; see section “[Lepidodendrale Rooting Structures](#)”). Together, these indicate that development of these leaf homologs also involves expression of a shoot- or root-specific developmental program (for apical dichotomous branching) and of two developmental programs that are widely believed to be root-specific among living plants (for root cap and root hair production). Two implications of the expression in leaf homologs of developmental programs not usually associated with leaves are that (1) these programs include conserved gene regulatory networks that are modular, and (2) expression of these regulatory modules is independent of organ identity. In the case of root hairs, this is to be expected, given evidence available on shared developmental regulators between bryophyte gametophyte rhizoids and angiosperm sporophyte root hairs; this implies that such rooting structures are fundamentally homologous (deep homology) across embryophytes and independent of life cycle phase, let alone organ identity.

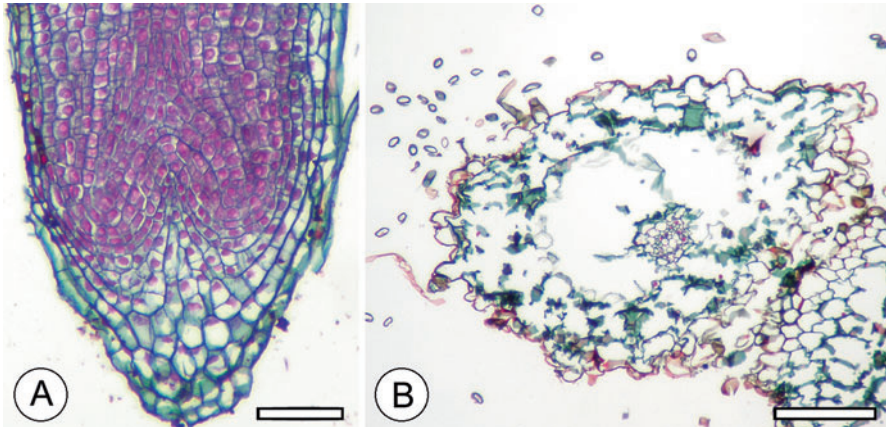


Fig. 7 Appendages (“rootlets”) of *Isoetes* corm base (rhizomorph) bear structures typical of roots – a protective cap on the apical meristem (a) and absorptive hairs (b) – even though they are leaf homologs; note incipient isotomous branching of the “rootlet” apical meristem. Scales = 75 μ m

In light of the homologies of the body plan of *Isoetes*, as resolved by data from the fossil record, the presence of root cap-like structures in the “rootlets” of this plant provides interesting phylogenetic perspectives. These root cap-like structures, present on organs that are not root homologues, could imply that the root cap pre-dates roots and evolved on less specialized axes with rooting function, *if* rhizomorphic lycophytes evolved directly from ancestors devoid of roots. However, because among modern tracheophytes the root cap is known exclusively in roots, another possible explanation, namely, that *Isoetes* descends from ancestors that had true roots with root caps, seems more probable. This hypothesis has implications for lycophyte phylogeny and character evolution, consistent with previous ideas that the clade of root-less lycophytes that includes *Isoetes* (rhizomorphic clade) occupies a derived position in the lycophyte clade.

In equisetacean sphenopsids, the fossil record yielded several Paleozoic fossils exhibiting character combinations that fill important gaps in terms of morphological evolution between modern *Equisetum* and ancestral forms. Considered in the developmental context provided by modern *Equisetum*, these fossils were crucial in the development of hypotheses that explain the morphology of equisetacean reproductive structures as the result of a hierarchic system of modular regulatory programs. Nested within this set of hypotheses are also implications for the developmental program of the sporangiophore, which may represent a conserved regulatory module responsible for the development of basic fertile lateral branching systems, and for timing of the evolution of this module, which may have preceded the evolution of stem-leaf-root organography (Tomescu et al. 2017; see section “[The *Equisetum* Strobilus: A Case of Reciprocal Illumination](#)”).

At the scale of the sphenopsid group, the presence of intercalary meristems at the base of each internode in Equisetaceae, and in fossil Calamitaceae and

Sphenophyllales, represents an anatomical fingerprint for a shared set of developmental regulators that could imply common ancestry and, thus, inform phylogeny. Furthermore, the presence of whorled appendages in all fossil members of this group that also share the same type of apical meristematic organization as *Equisetum* suggests another shared regulatory program. This program, responsible for developmental synchronization of merophytes in a primordial ring (Tomescu et al. 2017), could then be traced back to the common ancestor of the group, somewhere in the Late Devonian – Early Carboniferous (ca. 380–325 Ma ago).

In modern tracheophytes, secondary growth from a vascular cambium is known only in seed plants and *Isoetes*. The fossil record has revealed that this mode of growth is shared by the extinct seed-free progymnosperms, which form a clade with the seed plants (the lignophyte clade), and that other groups – lepidodendrolean lycophytes, sphenopsids, zygopterid fens – had also evolved secondary growth. In this context, identification of auxin swirls as anatomical fingerprints for polar auxin regulation of cambial growth points to auxin-related processes of secondary growth regulation shared across the euphyllophyte-lycophyte divide and among euphyllophytes (Rothwell et al. 2008; see section “Secondary Growth”). This has implications for the evolution of developmental regulation, because the distribution of secondary growth across tracheophyte phylogeny points to parallel evolution of this developmental pathway in different lineages. This would imply that the shared auxin-related regulatory module pre-dates the evolution of secondary growth and may have been involved in more basic developmental pathways. However, discoveries of Early Devonian basal euphyllophytes exhibiting secondary growth (Gerrienne and Gensel 2016) may indicate just the contrary, namely, that all (or most) euphyllophytes share a regulatory program for secondary growth inherited from a common ancestor but expressed only in some of its descendants. Clearly, better understanding of secondary growth regulation and continued exploration of the fossil record can bring resolution to these questions.

For another auxin-related growth response, gravitropism, fossils contribute support to a hypothesis integrating physiology, development, and homology. Plant roots exhibit typically positive gravitropic responses. Lepidodendrolean lycophytes demonstrate positive gravitropism in the downward-growing rhizomorph, an organ with shoot homology (Rothwell and Erwin 1985). Interestingly, lepidodendrolean rhizomorphs exhibit acropetal polar auxin transport (Rothwell et al. 2014), just like roots and like the *Selaginella* rhizophore, another organ that is not a root homolog but has rooting functions. These observations could imply that acropetal polar auxin transport is independent of organ identity (homology) and is more generally associated with positive gravitropic responses in a diverse array of organs that have absorption and anchoring roles (Matsunaga et al. 2017).

Life Cycles, Reproductive Systems

The fossil record has provided direct and indirect evidence for the timing and mode of evolution of reproductive biology and plant life cycles. For instance, fossils provide

the only evidence that sphenopsids, the clade that includes homosporous *Equisetum* as its sole living representative, evolved heterospory as early as the Early Mississippian, ca. 350 Ma ago. The fossil record also reveals that heterospory originated independently, in different variants, in several exclusively extinct plant lineages (to the extent that it has been regarded as the most iterative key innovation in the evolutionary history of plants) and provides minimum ages for those independent origins. None of these aspects could have been gleaned by the study of extant plants alone, irrespective of the methods of investigation or inference employed.

In the same vein, the fossil record contains evidence on first occurrences of plant structures associated with reproduction. Considered in a systematic context, these features can provide characters for phylogenetic analyses that inform understanding of the evolution of plant reproductive biology. Middle Pennsylvanian (ca. 310 Ma) callistophytalean seed fern ovules preserve evidence for a pollination drop mechanism and branched pollen tubes formed by the developing macrogametophyte. These features similar to those of extant gymnosperms are, consequently, known to have arisen early among basal gymnosperm groups. Late Permian (ca. 250 Ma) glossopterid seed ferns exhibit a novel combination of reproductive characters in which sperm with a helical flagellate band, similar to that of extant cycads and *Ginkgo*, is associated with pollen tubes simpler than those of other living or extinct gymnosperms.

Going back to the origins of land plants, the spore record provides minimum dates for fundamental embryophyte characters. Ordovician (Darriwilian, ca. 460 Ma) spores recovered in tetrads with characteristic configuration provide the oldest evidence for simultaneous meiosis (cytokinesis). Furthermore, the ultrastructure of these spores indicates that sporoderm development involved active secretion by a sporangial tapetum early in embryophyte evolution (Taylor et al. 2017).

Observations on Late Silurian – Early Devonian (430–410 Ma) polysporangiophyte sporophytes allowed for assessment of their nutritional status, with implications for sporophyte-gametophyte relationships early in the evolution of the group. The size and anatomy of these early sporophytes, and comparisons with extant plants taking into account physiology, demonstrate that the earliest polysporangiophytes sporophytes could not have sustained photosynthetic activity at levels high enough to ensure their nutritional independence. These sporophytes were nutritionally dependent on the gametophytes, like the sporophytes of extant bryophytes. Such observations provide a glimpse into the sequence of character evolution, demonstrating that the branched sporophyte pre-dates independence of the sporophyte from the gametophyte.

The gametophytes of the earliest embryophytes have been elusive, with only equivocal hints available to date of what their morphology may have been. However, the stable carbon isotope chemistry and internal structure of thalloid carbonaceous fossils scattered throughout the Silurian and Early Devonian, combined with experiments simulating fossilization on extant thalloid organisms, indicate that at least some of those fossils are plants. These fossils demonstrate thalloid gametophytes in early embryophytes and corroborate hypotheses that early polysporangiophyte gametophytes may have had thalloid morphology (Tomescu et al. 2014). This perspective on polysporangiophyte gametophyte morphology is at odds with

predictions based on phylogenetic studies that place mosses, which have leafy gametophytes, as the sister group of polysporangiophytes.

Morphological evolution in the gametophytes of early polysporangiophytes has a story of which we have uncovered only some parts, and those thanks to the fossil record. Unlike the thalloid gametophytes of early polysporangiophytes, the next oldest known gametophytes, belonging to Early Devonian (Lochkovian–Pragian, ca. 408 Ma) protracheophytes and tracheophytes of the Rhynie chert, exhibit morphologies with no counterpart in the modern flora. Their morphology is similar to that of sporophytes, with axial organization, branched architecture, and vascular tissues. The only approximation to this morphology in living plants are the subterranean gametophytes of *Psilotum* and *Tmesipteris*, which are also axial, can branch, and can be vascularized. However, compared to these, Rhynie chert gametophytes were larger, highly branched, and developed above-ground. Currently, it is not clear how polysporangiophyte gametophytes evolved from a basic thalloid morphology to the axial forms seen in the Rhynie chert, and then back to the primarily thalloid forms seen in living seed-free vascular plants. These present questions in evo-devo whose answers will require additional data from the fossil record.

The Early Devonian Rhynie chert plants also provide the oldest direct evidence of anisospory (segregation of micro- and megaspores within the same sporangium), a reproductive system currently known only in a subset of bryophytes, in a few *Isoetes* species and, potentially, in *Equisetum* and *Ceratopteris*. Specifically, gametophytes of the Rhynie chert protracheophyte *Aglaophyton* are found forming dense populations, when preserved in situ. Because *Aglaophyton* spores were dispersed as masses representing the contents of whole sporangia, such dense gametophyte tufts probably represent the product of individual sporangia. Although spore size shows no bimodal distribution within *Aglaophyton* sporangia and gametophytes are exclusively unisexual, the gametophyte tufts always include mixtures of both sexes. These have been interpreted as indicating anisospory of *Aglaophyton* and other Rhynie chert plants (Taylor et al. 2005). Like in the case of gametophyte morphology, it is not yet clear what the anisospory of Rhynie chert plants means for the complex picture of plant life cycle evolution.

Emblematic Case Studies

Gravitropism

Tropisms play a major role in plant development and evidence is accumulating that tropic responses influenced growth of the earliest land plants. Fossils from the Early Devonian reveal that rooting organs of lycophytes appeared long before those of the other major clade of vascular plants (euphyllophytes), for which roots are not known until the Middle Devonian. Evidence for tropic responses in the aerial and rooting structures of Early Devonian lycophytes is provided by several plants, including *Drepanophycus* and *Sengelia* (Matsunaga and Tomescu 2017). These plants have leafy axes that extend in one direction (horizontally or upwards) and smaller smooth

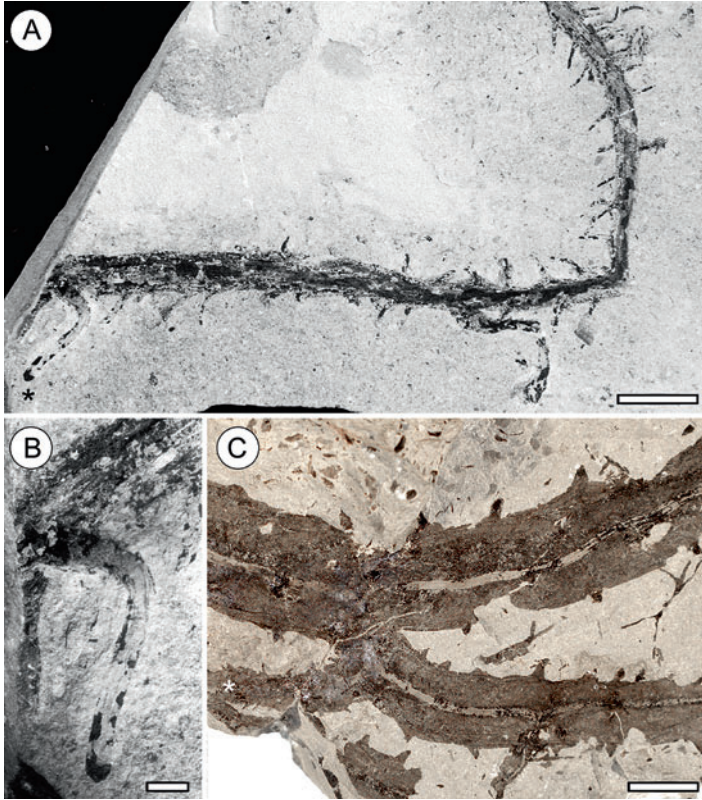


Fig. 8 Evidence for gravitropic responses in the rooting structures of Early Devonian (ca. 410 Ma) lycophytes. (a, b) *Drepanophycus spinaeformis* with smooth root-like axis (asterisk in a) pointing away from direction of stem growth; (b) is detail of (a). (c) K-branching in *Sengelia radicans*; main stem (top) produces side branch that forks very close to its base, producing a branch stem (bottom right) and a rooting axis (bottom left; asterisk), which develops in a direction opposite to growth direction of main stem and branch stem. Scales = 10 mm (a, c); 2 mm (b). (a and b courtesy of Patricia G. Gensel; c courtesy of Patrick S. Herendeen and Kelly K.S. Matsunaga)

axes that bend in the opposite direction to root the plant, suggesting negative and positive gravitropism in the aerial and rooting organs, respectively (Fig. 8). Similar dichotomy in growth direction, reflecting gravitropic responses, is present in the undifferentiated axes of Early Devonian zosterophylls, which produce branches with upward growth and others with downward growth; e.g., *Zosterophyllum* (Fig. 9a, b), *Bathurstia*, and *Sawdonia*.

Additional evidence for tropic responses derives from Early Devonian Rhynie Chert plants preserved in growth position, with excellent cellular detail. Plants such as *Rhynia*, *Aglaophyton*, *Horneophyton*, and *Nothia* had no stem-leaf-root differentiation, but did have aerial axes that grew both laterally and upward, suggesting negative gravitropism. These plants also have bulges on the lower surface of laterally growing axes and the base of upward growing axes, from which tufts of rhizoids

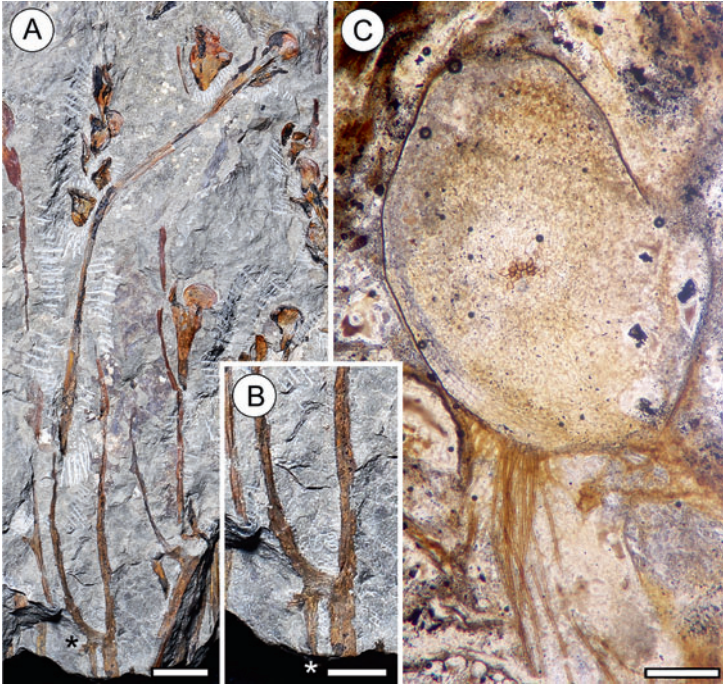


Fig. 9 Evidence for gravitropic responses in Silurian-Early Devonian (ca. 420–410 Ma) tracheophytes. (a) *Zosterophyllum qujingense* displaying K-branching (asterisk) similar to that of *Sengelia* (Fig. 8c) but expressed in undifferentiated naked axes. (b) Detail of A; one of two axes of K-branch (asterisk) diverges away from upright aerial axes. (c) Cross section of rhyniophyte axis bearing tuft of rhizoids on lower side that was in contact with substrate. Scales = 5 mm (a); 3 mm (b); 500 μ m (c). (a and b courtesy of Jinzhuang Xue)

diverge (Fig. 9c). Such rhizoids either extend or bend downward, demonstrating positive gravitropism similar to that of rhizoids on living plants and their green algal close relatives, in which the direction of rhizoid growth is controlled by a statolith mechanism. Because this tropic response is driven by the same type of statolith mechanism in both extant vascular plants and charophycean green algae, it is reasonable to infer that the molecular toolkit for the regulation of gravitropic growth was present in the earliest vascular plants, as illustrated by rhyniophytes.

It will be interesting to learn from future fossil discoveries what anatomical structures were responsible for the positive and negative gravitropic responses of the undifferentiated axes of zosterophylls or the root-bearing axes of plants such as *Sengelia*. Because statolith-based mechanisms are ubiquitous as gravity sensing systems across embryophytes and their green algal relatives, at both the cell level (rhizoids) and in multicellular organs (roots, shoots), it is safe to assume that the axes of zosterophylls and *Sengelia* had the same type of mechanism. In roots of extant angiosperms, amyloplast statoliths are located in cells of the root cap columella. We do not know whether the positively gravitropic axes of zosterophylls and *Sengelia*

had root cap-like structures. Considering that zosterophylls did not possess stem-leaf-root differentiation and that the root-bearing axes of *Sengelia* are not root homologs, it is likely that none of these axes had root cap-like structures, unless regulation of root cap development is independent of organography (as it appears to be in *Isoetes*). If a root cap was absent from these positively gravitropic axes, could such axes have housed statoliths in boundary layers (e.g., endodermis, starch sheath) like those responsible for negative gravitropism in the shoots of extant angiosperms? If so, how would the statolith-based mechanisms responsible for positive gravitropism in the below-ground axes of zosterophylls and for negative gravitropism in the above-ground axes of those plants have been different? Could they have differed only in the polarity of the response to a gravitropic stimulus sensed in a shared type of structure? And would these tropic responses have involved redistribution of polar auxin fluxes, as seen in positive and negative gravitropic responses of angiosperm roots and shoots? Answers to all of these questions and their integration into a more complete picture of the evolution of gravitropism will also require understanding of the incompletely explored structural, developmental, and physiological underpinnings of gravitropism in many lineages of extant seed-free plants (the roots and shoots of lycophytes, ferns, *Equisetum*, or the rhizophores of *Selaginella*).

Polar Auxin Transport

Auxin is among the most prominent of growth regulators, and polar auxin transport from developing leaf primordia in the apical meristem toward the base of the stem, and then toward the apical meristem(s) of the root system, regulates a wide spectrum of developmental processes. Among the most important of those processes is the patterning of primary vascular architecture and of tracheary elements in the secondary xylem.

Secondary Growth

Vascular tissue differentiation of living plants is under the control of several plant growth regulators, including gibberellins, cytokinins, and ethylene, among which auxin is the most prominent. Moreover, at least some aspects of polar auxin transport have been identified as far down the green lineage as bryophytes and charophycean algae.

Polar auxin flow within the vascular cambium of seed plants patterns the axially elongated tracheary elements of the secondary xylem, which typically follow a straight course. However, when obstacles such as buds, branches, and wounds impede polar auxin flow, auxin whirlpools form in the cambial zone, inducing the differentiation of characteristic circular patterns of tracheary elements above the obstacles (Rothwell et al. 2008). Similar circular patterns have been identified at the same positions in the wood of the Upper Devonian progymnosperm *Archaeopteris* (Fig. 10a) and serve as structural fingerprints for polar auxin regulation. This recognition revealed that polar auxin flow and auxin regulation also affect wood patterning in species from the fossil record and established the existence of structural

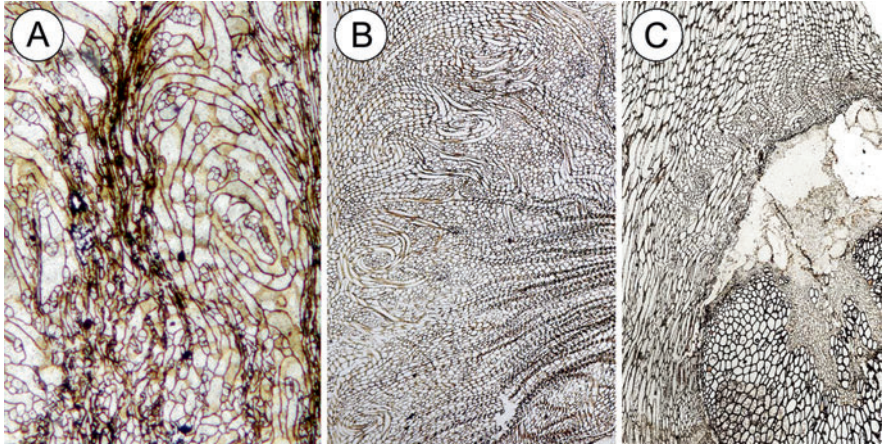


Fig. 10 Swirls formed by tracheids in wood (in tangential longitudinal view) as structural fingerprints for polarized transport of auxin (polar auxin flow) in vascular cambium. Demonstration of such auxin swirls, well documented in extant seed plants, in the wood of archaeopteridalean progymnosperms (**a**; *Callixylon*), calamitalean sphenopsids (**b**; *Arthropitys*), and lepidodendralean lycopsids (**c**; *Paralycopodites*) supports hypothesis of shared regulatory role for polar auxin in development of secondary xylem, in all three lineages

fingerprints for the regulatory mechanisms of secondary growth in extinct plants. Subsequent studies documented similar patterns in the wood of extinct sphenopsids and lycophytes (Fig. 10b, c) (Rothwell et al. 2008; see also section “[Lepidodendralean Rooting Structures](#)”).

Current knowledge of early vascular plants suggests that the most recent common ancestors of lycophytes, sphenopsids, and lignophytes (i.e., seed plants + progymnosperms) were plants without secondary growth. In this context, such fingerprints demonstrating polar auxin flow regulation of cambial activity suggest that wood evolved separately (i.e., parallel evolution) in each of these groups, even though the different evolutionary pathways may have involved the same ancestral regulatory pathways associated with polar auxin transport (Rothwell et al. 2008). However, discoveries of Early Devonian plants that produced wood as early as 407 Ma ago (Gerrienne and Gensel 2016) push the origin of secondary growth very close to the base of the euphyllophyte clade, suggesting that euphyllophytes may share a common ancestor that had evolved the basic toolkit for cambial growth (see also sections “[Sequence of Character Evolution](#)” and “[Developmental Regulation](#)”). If lycophytes and euphyllophytes share the same polar auxin flow-related regulation of wood patterning, future studies of basal euphyllophytes with secondary growth from the Lower Devonian will reveal the same fingerprints for auxin regulation of wood production.

Lepidodendralean Rooting Structures

Among vascular plants, giant trees have evolved in only two major groups, modern seed plants and extinct lepidodendralean lycophytes, where that stature has been achieved by the evolution of substantial rooting systems. At the same time, we also

recognize that the rooting structures of the two groups have distinctly different homologies and arose by divergent evolutionary pathways, and that those differences are understood only because the lycophytes have a rich fossil record.

In seed plants, giant trees are supported by a rooting system that arises from the radicle of a cotyledonary embryo, thus establishing bipolar growth via a system of true roots. By contrast, lycophytes do not have cotyledonary embryos and the only living descendent of the giant lepidodendraleans is the tiny quillwort, *Isoetes*. Interestingly, *Isoetes* appears to have distinctly unusual structure and growth, unless interpreted with reference to extinct relatives (Rothwell and Erwin 1985). Among lycophytes, the fossil record reveals that giant lepidodendralean trees are rooted by a shoot that is modified for rooting (known as a rhizomorph), rather than by a system of true roots. As recently emphasized by Hetherington et al. (2016b), homologies of the lepidodendralean rooting system are with the shoot system, as originally hypothesized more than a century ago.

Interest in similarities between the developmental morphology and anatomy of *Isoetes* and the lepidodendralean rooting system (e.g., the fossil genera *Stigmaria* and *Protostigmaria*) was rekindled by Stewart (1947), who emphasized that *Stigmaria* axes have anatomical features that agree more closely with stems than roots. Stewart also detailed that the leaf-like anatomy and arrangement of stigmarian lateral appendages, referred to as stigmarian rootlets, compare closely to both the leaves and rootlets of *Isoetes*. Likewise, the elongated branched rooting systems of *Stigmaria* and the cormose lobed rooting systems of *Protostigmaria* and *Isoetes* are now recognized as growth variations of a common organography (Rothwell and Erwin 1985). However, such paleontological evidence was not fully understood or appreciated until much later, despite several, additional paleontological discoveries. Frankenberg and Eggert (1969) reconstructed the overall morphology and anatomy of stigmarian rooting systems, reemphasizing both anatomical and developmental similarities of stigmarian axes to the lepidodendralean stems. The authors further demonstrated that stigmarian rootlets abscised as if they were leaves, and provided additional support for anatomical and developmental similarities between stigmarian appendages and *Isoetes* leaves. Concurrently, Jennings (1975) recognized that some lepidodendralean trees were rooted by a cormose *Isoetes*-like rooting system, thus strengthening the homologies between living and extinct rhizomorphic lycophytes. Subsequent characterizations of both embryogeny and apical development for related lycophytes clarified meristematic activity and embryogeny in the clade, and laid the groundwork for a comprehensive summary of homologies among lycophyte shoots and stigmarian rooting systems (Rothwell and Erwin 1985).

Most recently, the developmental significance of the overwhelming morphological, anatomical, developmental, and embryological evidence that stigmarian rooting systems of lepidodendralean lycophytes (and *Isoetes*) are homologous to the above-ground shoot systems (Rothwell et al. 2014) has been explained by the discovery of fingerprints for polar auxin patterning of xylem in such plants. Polar auxin swirls are now known to occur in the wood of both the stems and stigmarian rooting axes of lepidodendraleans, but polar auxin flows in opposite directions in these above- and below ground systems. In stems of the lepidodendralean *Paralycopodites*, such

swirls occur above branches, demonstrating basipetal auxin flow from shoot apices. By contrast, such swirls are located, in *Stigmaria* rhizomorphs, on the basiscopic side of stigmarian rootlet traces, indicating acropetal polar auxin flow, toward the apices of rhizomorphs (Fig. 11). These data reveal that basipetal auxin transport was lost during evolution of the stigmarian and protostigmarian rooting systems of tree-sized lycophytes, and confirm that both gravitropic response and polar auxin flow are independent of the homologies of organs in vascular plants.

Euphyllphyte Leaf Evolution

Traditionally, vascular plants have been interpreted as having either microphylls, derived from enations (i.e., lycophytes), or megaphylls derived from modified branching systems (i.e., euphyllphytes). Whereas currently there is general agreement that euphyllphyte and lycophyte leaves originated independently, the evolution of leaves within euphyllphytes has been the subject of discussion and debate for several years.

On one hand, there is the idea that all euphyllphyte leaves have a unique origin. This idea stems from phylogenetic analyses of only living species (Pryer et al. 2001), which recovered a set of relationships whereby all living seed-free euphyllphytes form a clade (referred to as Moniliformopses) that is sister to the seed plants. Because all living euphyllphytes (except for psilotophytes) have leaves, some have called on these relationships to propose that the common ancestor of all living euphyllphytes also had leaves and, therefore, euphyllphyte leaves have a single, common evolutionary origin. On the other hand, the relationships proposed by Pryer et al. (2001) are at odds with the results of analyses that include living and fossil taxa (Rothwell and Nixon 2006), which (1) do not recover ferns, sphenopsids, and psilotophytes as a clade, and (2) show leafless extinct lineages at the base of living ferns, sphenopsids, and seed plants; thus, supporting independent origins of leaves in several euphyllphyte lineages (Tomescu 2009).

Even if the relationships among living euphyllphytes proposed by Pryer et al. (2001) were supported, review of the fossil record shows that (1) the origin, deep phylogeny, and relationships of ferns, sphenopsids, and seed plants are not well understood; and (2) part of the reason for this situation is that the basal, Devonian representatives of these groups are leafless. The original Moniliformopses – as defined by Kenrick and Crane (1997) – consist of three Devonian taxa: *Ibyka*, *Pseudosporochnus*, and *Rhacophyton*. The relationships of these fossil taxa to living ferns and sphenopsids are unresolved, so equivalence between the formally defined Moniliformopses (Kenrick and Crane 1997) and living “Moniliformopses” (Pryer et al. 2001) is uncertain. Furthermore, *Ibyka* and *Pseudosporochnus* are leafless; therefore, the common ancestor of Moniliformopses did not possess leaves, which must have evolved independently more than once in the descendants of the group. Concurrently, there is little doubt today that seed plants are nested within the lignophytes, a clade that includes a paraphyletic grade of extinct leafless progymnosperms at the base and, thus, has a leafless common ancestor. In summary,

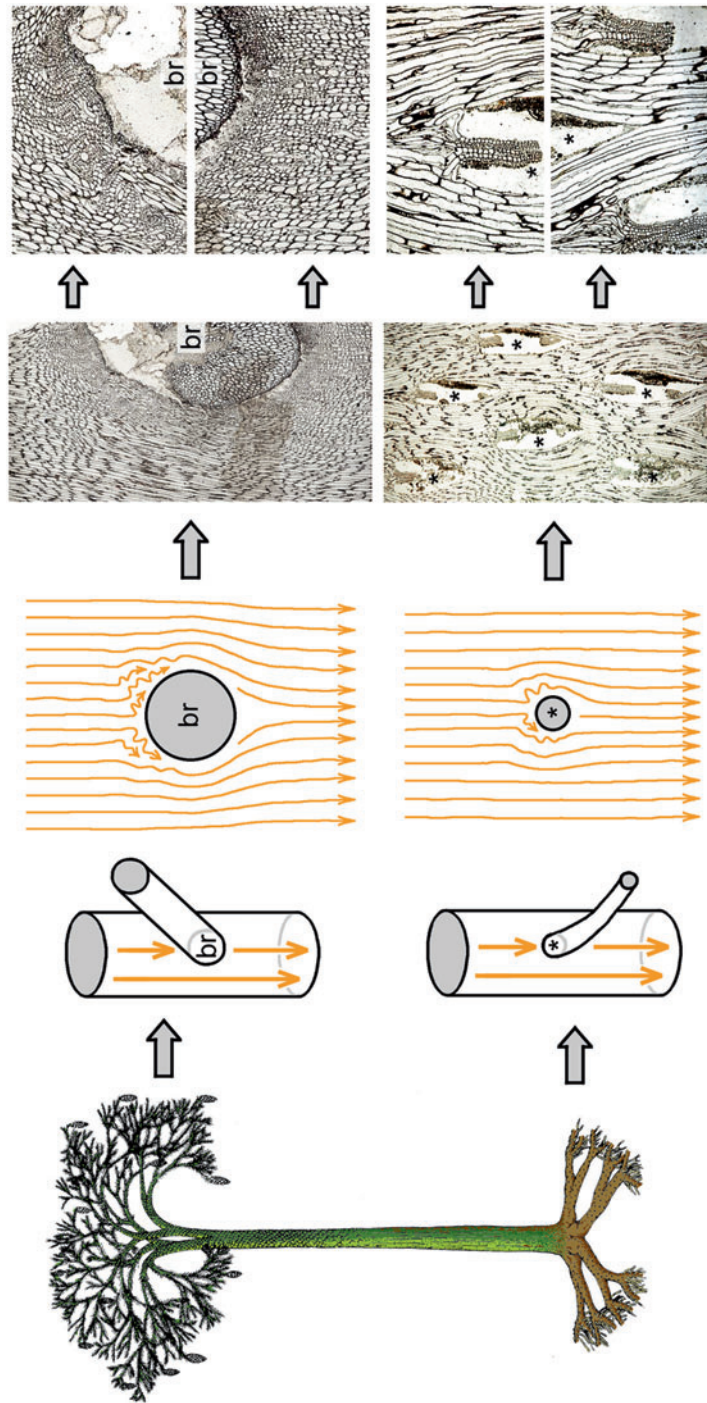


Fig. 11 Polar auxin transport in vascular cambium of arboreal lepidodendralean lycopsid (left), away from shoot tip. An obstacle in the cambium, represented by base of diverging branch (br), disrupts flow of auxin leading to formation of auxin swirls in wood acropetally with respect to branch trace (above trace), but not in a basicopic position (below trace). In positively gravitropic rooting structures (rhizomorphs; bottom panel), subtle auxin swirls are formed in wood basicopically with respect to “rootlet” traces (asterisks), i.e., above traces, but not in acropetal position (below traces). This demonstrates that polar auxin flow is directed acropetally in rhizomorph, i.e., toward tip. Wood anatomy illustrated by *Parahycopodites* (stem) and *Stigmaria* (rhizomorph)

from a phylogenetic perspective, the fossil record unequivocally supports a minimum of three independent origins of leaves among euphyllophytes – in ferns, sphenopsids, and seed plants.

The fossil record also contributes evidence for multiple origins of euphyllophyte leaves in an evo-devo perspective. One line of evidence is the demonstration that leaf evolution followed different trajectories, in terms of sequence of character evolution, in ferns and seed plants (Sanders et al. 2009; see also section “[Sequence of Character Evolution](#)”). Another line of evidence is provided by fossils demonstrating that the evolution of leaf venation followed similar trajectories, from simpler to more complex architectures, in different euphyllophyte lineages (Boyce and Knoll 2002). This corroborates the evidence for leaf evolution from leafless ancestors in each of those lineages, indicating parallel evolution of leaf venation in distinct euphyllophyte lineages, as opposed to inheritance from a common ancestor that had leaves with complex venation.

Partial homology has been proposed for the leaves of different euphyllophyte lineages at the level of their precursor structures, i.e., the lateral branching systems of their leafless Devonian ancestors (Kenrick and Crane 1997). However, because the branching architectures of Devonian tracheophytes cover a continuous range of morphologies from lateral subordinate (overtopped) branching systems all the way to the branched sporophyte axes of the ancestral polysporangiophyte, statements of homology are difficult to formulate, let alone demonstrate, along this morphological continuum. In a similar vein, Boyce and Knoll (2002) hypothesized that the independent origins of euphyllophyte leaves could have been based on modifications of a common underlying developmental system. Tomescu (2009) reviewed the genetic regulation of leaf development and concluded that interactions between shared regulatory genes are too diverse among (and sometimes within) major lineages to support a common underlying regulatory system.

Vasco et al. (2016) proposed another form of deep homology. They demonstrated expression of Class III HD-Zip transcription factors (HD-Zip III) in the sporangia of *Selaginella* (lycophyte), *Psilotum* (psilotophyte), and *Ophioglossum* (fern). Because HD-Zip III genes have also been shown to be expressed in the sporangia of *Physcomitrella* (bryophyte) and *Arabidopsis* (angiosperm), Vasco et al. hypothesized deep homology of leaves across all tracheophyte lineages, resulting from independent co-option of an ancestral sporangium developmental program that involved III HD-Zip III transcription factors. However, it is also possible that the shared expression of HD-Zip III genes in plant sporangia is not directly relevant to leaf homologies. HD-Zip III genes also have a role in vascular tissue development in all tracheophytes (Floyd and Bowman 2010), possibly evolved after duplication of the ancestral HD-Zip III, which regulated sporangium development. Therefore, it is likely that HD-Zip III expression patterns in the leaves of lycophytes and euphyllophytes have more to do with vascular tissue identity and the regulation of radial (and adaxial-abaxial) polarity in vascular tissues, than with leaf identity and homology (Floyd and Bowman 2010).

In summary, in the debate of euphyllophyte leaf evolution, the fossil record adds phylogenetic resolution by revealing leafless taxa at the base of major euphyllophyte

lineages, as well as morpho-anatomical resolution, by showing plants with combinations of characters (determinacy, adaxial-abaxial polarity, venation) that could not be predicted from studies of extant plant diversity alone. When considered alongside the living plants, these reveal patterns of phylogeny and character evolution that support multiple independent origins of leaves among euphyllophytes.

The *Equisetum* Strobilus: A Case of Reciprocal Illumination

The strobilus of *Equisetum*, a highly condensed structure, and the sporangiophores it comprises have presented a puzzle in terms of evolution and homology for many years. *Equisetum* is the only living representative of the sphenophytes, a diverse clade with a rich fossil record, and as such provides an excellent example of a long phylogenetic branch on which homology issues can only be resolved by querying the fossil record. At the same time, information from fossils is only relevant in the context of development and developmental regulation, as understood based on studies of extant plants (including *Equisetum*), in an example of reciprocal illumination between data on fossils and living plants (Fig. 12).

Transformational series assembled during the mid-twentieth century based on the sphenophyte fossil record suggest that both the leaves and the sporangiophores of *Equisetum* evolved from lateral branching systems. This implies equivalence between leaves and sporangiophores and, consequently, equivalence of their locations on shoots, which were regarded as *nodes* for both types of organs. However, a subset of sphenophyte fossils demonstrate quite the contrary by possessing whorls of sporangiophores attached along *internodes*. These seemingly irreconcilable interpretations, based on two distinct datasets, led to a deadlock in homology interpretations that was not resolved until the realization that a node-internode view may not be the appropriate paradigm within which to interpret homologies of *Equisetum* strobilus structure (Tomescu et al. 2017), cleared the way for a solution to the conundrum.

Studies of vegetative development in extant *Equisetum* provide a framework for hypothesis generation (Fig. 12) by showing that shoot development in this genus owes to the combined activity of the apical meristem (which generates phytomers) and intercalary meristems (responsible for growth of individual phytomers by internode elongation). Our growing understanding of the molecular regulatory mechanisms responsible for meristematic growth suggests that plant meristems of all types are equivalent in their fundamental capacities (Tomescu et al. 2017). These include the capacity to transition to reproductive growth, and molecular programs regulating this transition in meristems are shared broadly among tracheophytes. Together, these developmental capabilities of living plants suggest the hypothesis that the switch to a reproductive developmental program in the intercalary meristems could lead to production of sporangiophore whorls along internodes, as has been observed in fossil equisetaleans. Predictions based on this hypothesis for the development internodal sporangiophore whorls were tested against the anatomy and morphology of fossils. These tests confirm that extinct sphenophytes had the same

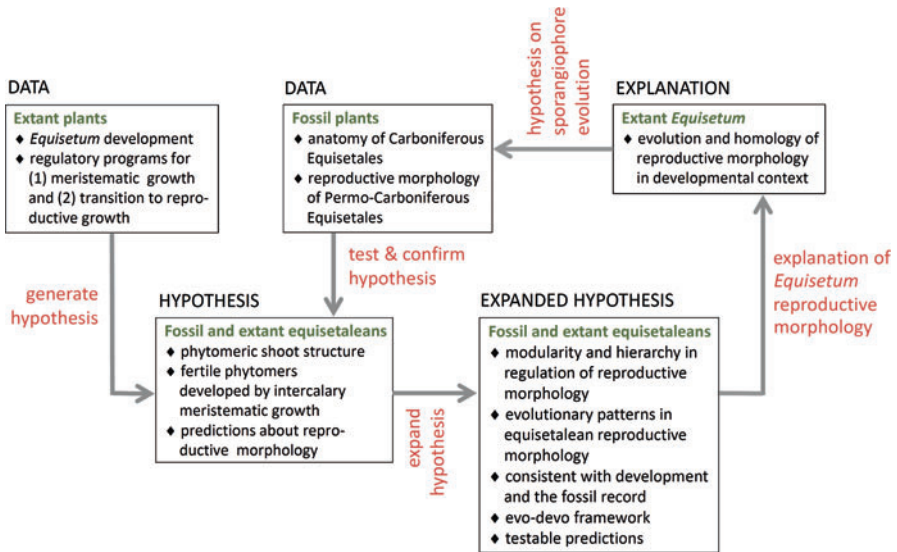


Fig. 12 Reciprocal illumination in elucidation of the origin and evolution of equisetacean reproductive morphology (Tomescu et al. 2017). Hypothesis generated by data from living plants was tested and confirmed by data from fossil record. This provided framework for subsequent hypotheses that included additional data from living *Equisetum* and fossil plants to offer novel explanation for origin of *Equisetum* strobilus and fossil plant reproductive morphologies. This new understanding of *Equisetum* strobilus was then used to formulate further hypotheses about evolution and the deep fossil record, namely, explaining origin and evolution of equisetalean sporangiophore

mode of shoot development involving apical and intercalary meristems, and support the hypothesis that internodal sporangiophore whorls are the product of intercalary meristematic growth.

Confirmation of this *intercalary reproductive growth hypothesis* provides a new framework for formulating additional hypotheses to explain the origin of the *Equisetum* strobilus, as well as the array of different reproductive morphologies documented in extinct equisetaleans. These hypotheses integrate information on development of the *Equisetum* strobilus and observations on fossil equisetalean morphology and propose that independent regulatory modules (gene regulatory networks) are expressed in a hierarchic sequence, leading to determinate apical growth in reproductive mode, and to repression of node-internode differentiation and of intercalary meristematic activity within fertile regions.

Considered within an evo-devo framework, this set of hypotheses on developmental regulation of the *Equisetum* strobilus, in turn, offers solutions for the homology and evolution of the equisetalean sporangiophore, relevant to the deep fossil record of sphenophytes (Fig. 12). Specifically, because sporangiophore development seems to be independent of nodal/internodal identity, it is proposed that the sporangiophore followed an independent evolutionary trajectory that bypassed the evolution of shoots with node-internode structure (including leaves) and whose beginnings may have pre-dated these structures (Tomescu et al. 2017). Accordingly,

the sporangiophore could represent the direct expression of a conserved regulatory module originally responsible for development of fertile lateral branching systems, a module that underwent its own evolution, which included heterotopic change, from expression along undifferentiated axes, to expression on specialized shoot segments, the internodes.

Summarizing this case of reciprocal illumination: a hypothesis generated by data from living plants was tested and confirmed by data from the fossil record. This provided a framework for subsequent hypotheses that included additional data from living *Equisetum* and fossil plants, to offer a novel explanation of the origin of the *Equisetum* strobilus and fossil reproductive morphologies. This new understanding of the *Equisetum* strobilus was then used to formulate further hypotheses about evolution and the deep fossil record, explaining the origin and evolution of the equisetalean sporangiophore.

Conclusions and Future Outlook

Fossils are quintessential witnesses of evolution. Study of the fossil record has contributed tremendously toward resolving plant evolution, systematics, and phylogeny, and gaining a fuller understanding of the role and workings of development in evolution. Living biodiversity represents only a small fraction of the diversity of life that spans Earth's history; therefore, most of the history of plant life is revealed exclusively by the fossil record. The fossil record provides access to an extensive diversity of plant structure that allows for higher resolution in the understanding of evolutionary processes and events in deep time.

Understanding the indispensable role of fossils in addressing questions of plant evolution and phylogeny also provides a powerful argument for a much wider systematic spectrum of genomic sequencing (i.e., a species of Lycopodiaceae, *Psilotum*, *Equisetum*, a species of Ophioglossales, a species of Marattiales). Only with such data available to test hypotheses of phylogeny will we be able to resolve currently recalcitrant relationships among seed plants, euphyllophytes, ferns, and in several regions of the angiosperm clade.

Plant fossils are invaluable in documenting the pattern of evolution (for tissues, organs, modes of growth, life cycles, etc.), which illuminates structural and developmental homologies and provides a test for hypotheses that have been generated from other disciplines. Focused queries of data from the physiology, developmental molecular biology, and comparative developmental anatomy of extant plants will identify additional fingerprints like the ones discussed here. These fingerprints provide as many additional bridges over the gaps that separate living plants, in which development, physiology, genetics, and molecular biology can be studied directly, from the fossils, in which only morphology and anatomy can be observed.

Conversely, the fossil record provides data for the formulation of hypotheses that can be tested with genetic and developmental regulatory experiments. Pressing questions that are currently apparently insoluble and could be addressed by these methodologies regard patterns of evolution for plant vegetative organs (e.g., stele

types, axillary branching, intercalary growth), fertile organs, and life cycles (e.g., the seed, the flower, the fruit, heterospory, angiospermous fertilization). It would be interesting to test, for example, if enhanced polar auxin transport in *Isoetes* would lead to elongation and branching in the rhizomorph and stem, and to more active secondary growth, lepidodendralean-style. Or if discovery and silencing of the regulatory module that represses node-internode differentiation in *Equisetum* sporangiophore phytomers would lead to reproductive morphologies like those of extinct *Peltotheca*. And if further induction of indeterminacy in the fertile shoots of such plants would produce *Cruciaetheca*-like morphologies. We could also test whether abaxialized leaves of loss-of-function HD-Zip III mutants would grow and branch like the undifferentiated axes of early polysporangiophytes, if they were induced into indeterminacy.

The examples highlighted here encompass only a small number of insightful studies where hypotheses generated either from the fossil record or from regulatory developmental genetics serve as reciprocal hypothesis tests. Nevertheless, they demonstrate the exciting potential for such approaches to dramatically improve our ability to address many evolutionary questions that have thus far eluded resolution through the application of either paleontological, systematic, or regulatory genetic/developmental approaches alone. These examples also highlight the value of developmental fingerprints for employing data from the fossil record to enhance our understanding of the role of regulatory genetics in the evolution of plant structure and the origin of major clades. Because these techniques have thus far been applied to such a small number of studies distributed across a narrow sample of potentially fruitful approaches, we are optimistic that the rapidly expanding application of coordinated developmental genetic–paleontological studies will, for the first time, allow us to address some of the most poorly understood events and processes of evolutionary biology.

Cross-References

- ▶ [A Process-based Approach to the Study of Flower Morphological Variation](#)
- ▶ [Developmental homology](#)
- ▶ [Evo-devo of the Origin of Flowering Plants](#)
- ▶ [Macroevolution](#)
- ▶ [Methods and Practices in Paleo-evo-devo](#)
- ▶ [Novelty and Innovation](#)
- ▶ [The Evolution of Branching in Land Plants: Between Conservation and Disparity](#)

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