PLANT EVOLUTION AT THE INTERFACE OF PALEONTOLOGY AND DEVELOPMENTAL BIOLOGY: AN ORGANISM-CENTERED PARADIGM[1]

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Paleontology yields essential evidence for inferring not only the pattern of evolution, but also the genetic basis of evolution within an ontogenetic framework. Plant fossils provide evidence for the pattern of plant evolution in the form of transformational series of structure through time. Developmentally diagnostic structural features that serve as "fingerprints" of regulatory genetic pathways also are preserved by plant fossils, and here we provide examples of how those fingerprints can be used to infer the mechanisms by which plant form and development have evolved. When coupled with an understanding of variations and systematic distributions of specific regulatory genetic pathways, this approach provides an avenue for testing evolutionary hypotheses at the organismal level that is analogous to employing bioinformatics to explore genetics at the genomic level. The positions where specific genes, gene families, and developmental regulatory mechanisms first appear in phylogenies are correlated with the positions where fossils with the corresponding structures occur on the tree, thereby yielding testable hypotheses that extend our understanding of the role of developmental changes in the evolution of the body plans of vascular plant sporophytes. As a result, we now have new and powerful methodologies for characterizing major evolutionary changes in morphology, anatomy, and physiology that have resulted from combinations of genetic regulatory changes and that have produced the synapomorphies by which we recognize major clades of plants.

Key words: evolutionary pattern and process; ontogeny and phylogeny; paleontology; regulatory genetics.

In a surprisingly simple yet elegant statement, Darwin (1859) characterized biological evolution as "descent with modification." Within a developmental framework, evolutionary modifications can be understood as resulting from alterations of ontogeny, which are derived from modifications of the genome, are mediated via regulatory genetics, and are reflected by modifications in mature structure and function (Gould, 1977; Howell, 1998; Cronk, 2009). Traditional paleontological practice employs transformational series (see Appendix 1 for definitions of terms used in this study) of mature structures to recognize the succession of structural modifications (Fig. 1) and to hypothesize patterns of evolutionary change through time (Fig. 2; Zimmermann, 1938, 1952; Stewart, 1964; Kenrick, 2002). Transformational series allow paleontologists to frame evolutionary hypotheses

(e.g., Florin, 1951), to infer homologies for the purpose of resolving systematic relationships (Tomescu, 2009), and thereby to reconstruct the overall pattern of plant evolution within a phylogenetic framework (Kenrick and Crane, 1997).

Until recently, however, transformational series have not meaningfully contributed to the illumination of evolutionary process. This is because the genetic/developmental bases for modifications of plant structure that are routinely illustrated by transformational series (Zimmermann, 1938, 1952) have remained unknown (Kenrick, 2002), thus leaving untested the evolutionary hypotheses that are represented by specific modifications in transformational series (e.g., Fig. 2). Indeed, hypotheses about the role of transformations in plant evolution lacked specific empirical corroboration within an ontogenetic context until the emergence of molecular genetics and developmental molecular biology in the 1990s (Howell, 1998; Beerling and Fleming, 2007; Cronk, 2009).

The rapidly expanding fields of molecular genetics and genomics now provide an opportunity to marry the methodologies of systematic paleontology and developmental molecular biology by identifying specific genetic modifications that underlie particular evolutionary transformations in the structure and physiology of living and fossil plants, by characterizing the systematic scope of specific genetic modifications on phylogenetic trees, and then by evaluating whether those genetic modifications provide the most parsimonious explanation for the structural transformations displayed (e.g., Rothwell and Lev-Yadun, 2005). Hypotheses can be tested further by determining whether additional species, in the region of the phylogenetic tree where the genetic modification is
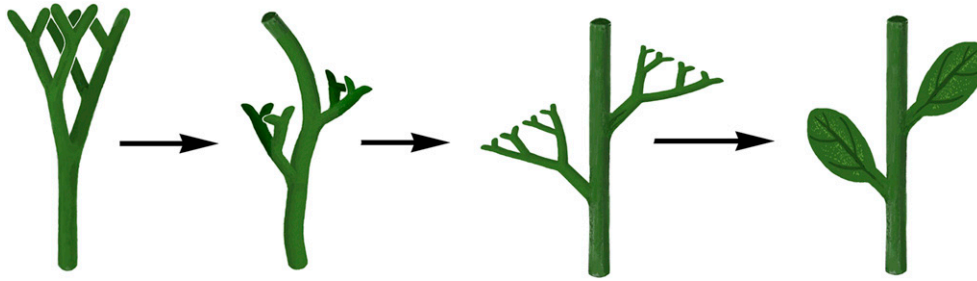
Fig. 1.   Transformational series of morphological variations showing transition from undifferentiated branching axes of basal polysporangiophytes to stem/leaf organography of most crown groups of vascular plants (modified from Sanders et al., 2007).

expected to be present, also show the resulting structural transformation (Rothwell et al., 2008). Although this approach is relatively new (Rothwell and Lev-Yadun, 2005; Sanders et al., 2007), it already is providing evidence for the genetic and regulatory origins of several facets of vascular plant body plans.

Goals of the present study are to review recently developed approaches and methodologies that have resulted from the simultaneous analysis of data from the fossil record and molecular genetics, to summarize a few recent studies that employ these approaches, and to highlight the potential for this emerging discipline of "paleo-evolutionary/developmental plant biology" to meaningfully improve our understanding of plant evolution and phylogeny. To this end, we explain the theoretical foundations of

evolutionary approaches and scientific methodologies for such studies, review specific examples that emphasize the potential of the fossil record to help uncover the developmental regulatory origins of modifications in mature plant structure and function, and highlight the potential for future studies to formulate and test hypotheses of plant evolution.

A PALEONTOLOGICAL/ONTOGENETIC VIEW OF HOW EVOLUTION WORKS

From a developmental/paleontological perspective, evolution can be envisaged as proceeding by the successive alteration
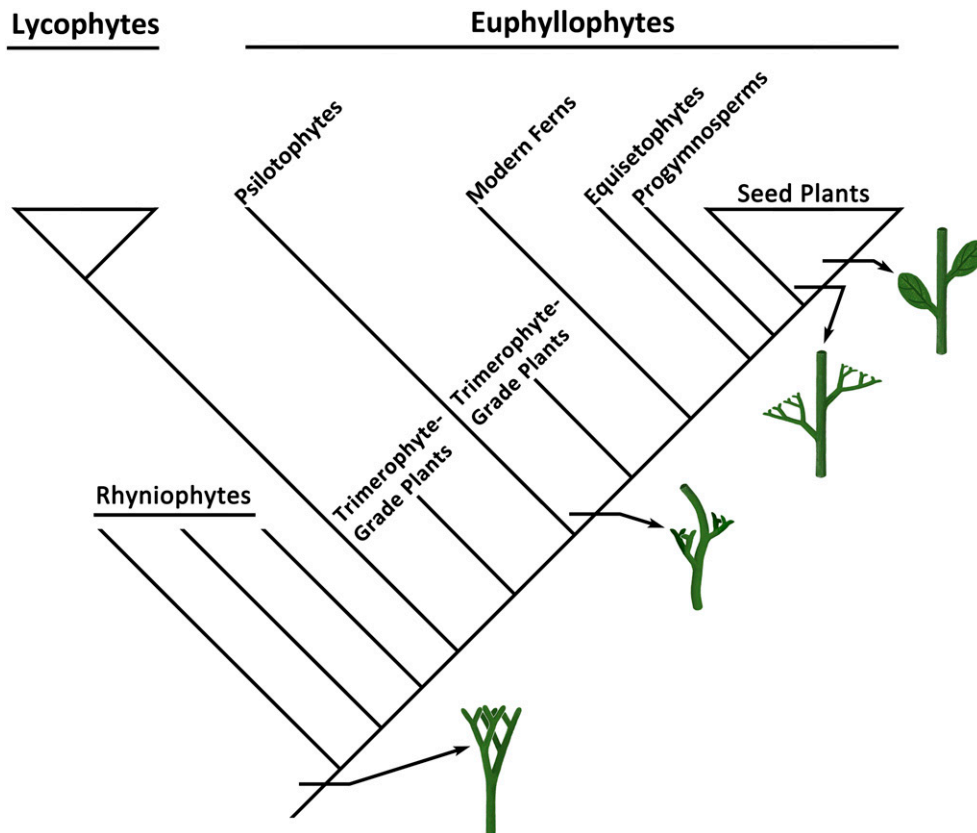

Fig. 2.   Morphologies from Fig. 1 mapped on phylogenetic tree of vascular plants to illustrate general evolutionary changes that have produced the stem/leaf organography of seed plants. See Fig. 6 for more specific parallel changes during leaf evolution in seed plants and other euphyllophyte clades. Tree simplified from Rothwell (1999).

of ontogeny through a progression of generations of organisms, which results in successive alterations of phenotype (e.g., transformational series in Fig. 1). This scenario represents the developmental basis for Darwin's "descent with modification" (Darwin, 1859). Although Darwin's (1859) hypothesis is staunchly organismal in focus, that which evolves (i.e., has continuity and changes through time) is neither an individual organism nor a population of organisms, but the DNA of the genome (diagonal line in Fig. 3), which is characterized through molecular genetics and underlies organismal speciation. As a genetically based developmental program, the genome can be regarded as the biological analogue of computer software. When a genome (i.e., the developmental program) is altered by mutation or recombination, the development (i.e., course of the developmental program) and phenotypes of future organisms with the altered genome (i.e., outcome of the developmental program) are thereby also altered (e.g., each morphology of the transformational series in Figs. 2 and 3).

If genetic changes are not readily reversible (i.e., following organismal speciation), then a permanent evolutionary change has occurred and the raw material for future evolution is permanently altered (Gould, 2002). Increases in species diversity occur only by permanent modification to (i.e., speciation among) the organisms that are living at any given time. In contrast to increases in species richness that result primarily from biological mechanisms, the fossil record reveals that reductions in species richness result primarily from catastrophic extinctions of varying intensities that have occurred periodically throughout geologic time (Knoll, 1984; Koebrel and MacLeod, 2002). By their very nature, such catastrophic extinctions are independent of the forces of natural selection and of the theoretical fitness that natural selection may impart to any given individual, population of individuals, or species within any particular community or ecosystem (Gould, 2002). As a result, organismal evolution is primarily a contingency-based chaotic system rather than the "probability game" that underlies traditional theoretical and population genetic views of evolution.

The conceptual outline for this view of biological evolution (Fig. 3) can be illustrated as a trajectory in time (*y*-axis in Fig. 3) along which successive genomic changes (diagonal line in Fig. 3) lead to modified ontogenies of organisms (blue vertical lines below transformational series of cartoons in Fig. 3), which produce successively changed phenotypes (i.e., transformational series of cartoons in Fig. 3). Organismal ontogeny may be further altered and/or constrained by the environment of development, wherein dysfunctional phenotypes are eliminated (red X at the top of some ontogeny lines in Fig. 3) by natural selection. Within this context, Darwinian natural selection serves as an evolutionary filter, rather than a driving force in evolution (Rothwell, 1987).

STRUCTURAL FINGERPRINTS OF DEVELOPMENTAL REGULATION

Even though nucleotide sequences of most fossils are not available for analysis and experimental manipulation of extinct organisms is not possible for directly testing evolutionary hypotheses within an ontogenetic framework, numerous studies of extinct plants have demonstrated that the diagnostic features of organismal development that are hypothesized to underlie evolution (Gould, 1977; Rothwell, 1982, 1987, 1995) can be preserved by the morphology and anatomy of fossils (e.g., Eggert, 1961; Delevoryas, 1964; Rothwell, 1971; Boyce and Knoll, 2002; Boyce, 2008). More recently, structural evidence for the regulatory mechanisms of plant development also has been recognized from fossils (Rothwell and Lev-Yadun, 2005; Bateman et al., 2006; Sanders et al., 2007). Such evidence may be viewed as "fingerprints" of the developmental regulatory mechanisms by which phenotypes are produced (e.g., Figs. 4, 5).
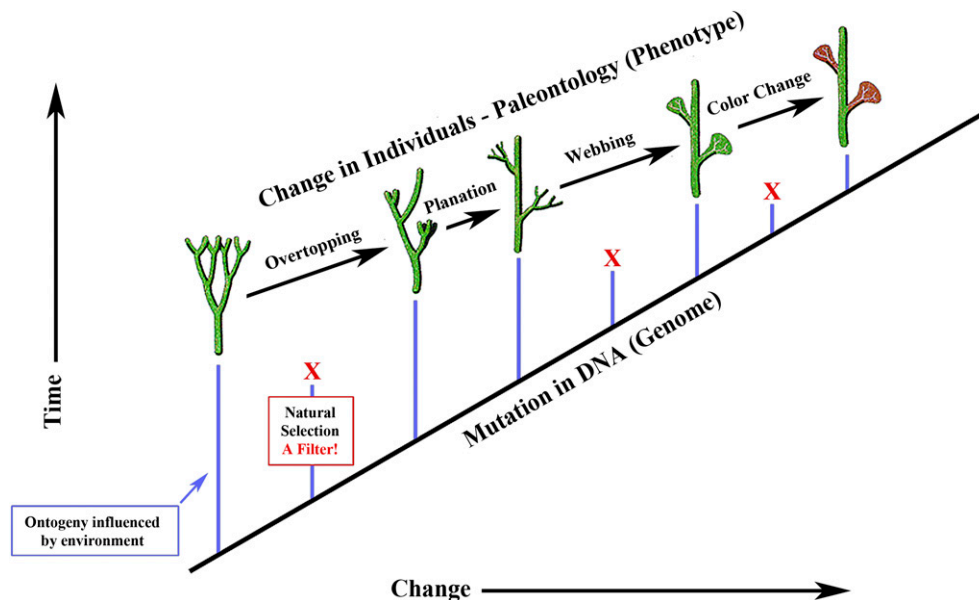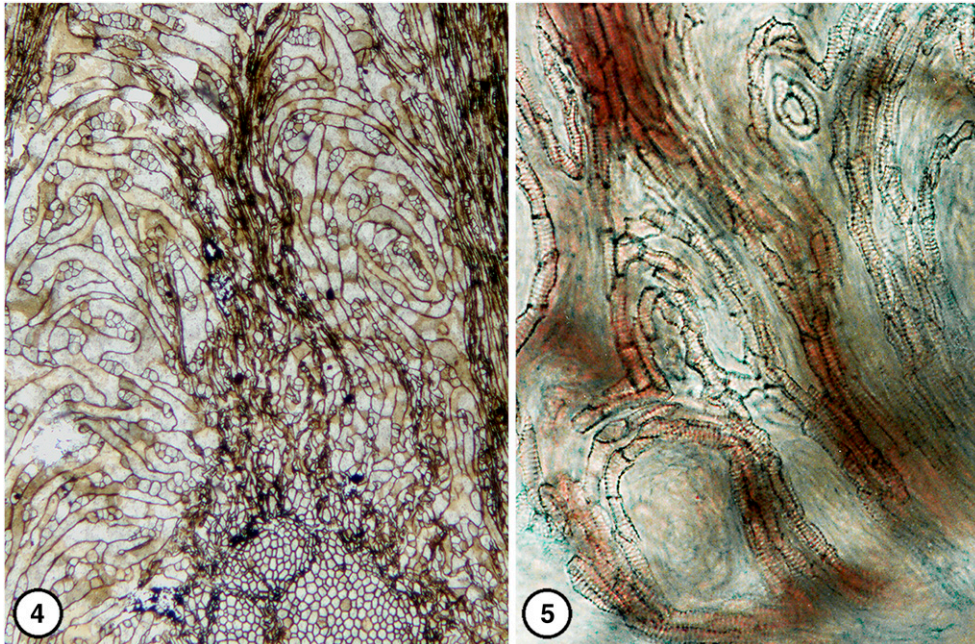

Fig. 3. Diagrammatic representation of evolution within a developmental framework. Transformational series of morphologies modified from Sanders et al. (2007). Transformations identified by traditional telomic process names as proposed by Zimmermann (1938, 1952) and Stewart (1964). See text for additional details.

Figs. 4, 5.   Tangential longitudinal sections of secondary xylem with tracheary element swirls that serve as structural fingerprints for polar auxin patterning of cambial activity in wood development. **4.** *Arabidopsis thaliana* (Wyatt et al., 2010). **5**. The progymnosperm *Archaeopteris* (i.e., *Callixylon* wood; Rothwell and Lev-Yadun, 2005).

Through the combined analysis of developmental fingerprints, transformational series that reflect evolutionary modifications, and the systematic distribution of genetic modifications, the evolutionary origins of major plant body plans (Rothwell, 1995; Tomescu, 2011) are beginning to be related to changes in ontogeny that are understood in terms of regulatory genetics for living counterparts of the fossils under investigation (e.g., Rothwell and Lev-Yadun, 2005; Bateman et al., 2006; Sanders, 2007; Sanders et al., 2007; Rothwell et al., 2008; Tomescu, 2009; Tomescu et al., 2014). Moreover, recent advances in our understanding of regulatory genetics and genomics are allowing variations in body plans across the entire spectrum of vascular plant systematics to be correlated with specific developmental pathways that are understood for living plants (e.g., Sanders et al., 2007, 2009, 2011; Beerling and Fleming, 2007; Dolan, 2009; Tomescu, 2009), thus serving as tests for paleontologically formulated evolutionary hypotheses within a combined ontogenetic/systematic framework (Tomescu et al., 2014).

CONCEPTUAL FOUNDATIONS OF PALEO-EVOLUTIONARY/DEVELOPMENTAL PLANT BIOLOGY

Methodologies employed in this paper include formulating a hypothesis from analysis of a given data set and then testing that hypothesis by expanding the data set and reanalyzing to determine whether the results continue to support the original hypothesis. When corroborated by tree-based determination for the systematic span of a specific gene or gene function, growth regulator, or developmental pathway, this "a-posteriori" method provides a meaningful test for an evolutionary hypothesis. To those scientists who almost exclusively employ experimental methods of hypothesis testing in their studies, some of the approaches that we elaborate upon in this paper may seem unsettlingly exotic.

However, our scientific methodology has been practiced within natural history investigations for more than 200 yr (Gould, 2002), and the same approach now can be effectively applied to evolutionary studies in the genomic age.

A growing body of evidence confirms that orthologous and paralogous genes and gene families (Fitch, 1970) underlie comparable structural and physiological phenotypes across a broad systematic spectrum of vascular plants (e.g., Cooke et al., 2002; Floyd and Bowman, 2007; Tomescu, 2011; Pires and Dolan, 2012). When mapped onto a phylogenetic tree, the position of the lowest occurrence of a specific gene, gene family, or growth regulator may be used to hypothesize that the gene also codes for the same phenotypic character in other species that occur in more distal regions of the tree (e.g., Fig. 6). This hypothesis can be tested by assaying for a gene or gene family in any or all of the species that occur in more distal positions on the tree to confirm whether both the gene and the phenotype that it produces are present. For living species, such tests can be performed by standard molecular genetic techniques. For fossil plants, where genes are not directly accessible, a hypothesis can be tested by assaying for the structural fingerprint (i.e., developmentally diagnostic phenotype) that is known to result from the activity of the specific gene or gene family under consideration. By this methodology, hypotheses of gene occurrence and gene action can be tested in a reciprocal fashion to progressively expand understanding within the context of a systematically based paleontological/ontogenetic view of evolution.

RESULTS FROM RECENT STUDIES

Paleontological record of polar auxin regulation in wood development—An early application of structural fingerprints in evolutionary studies of fossil plants employs the search for
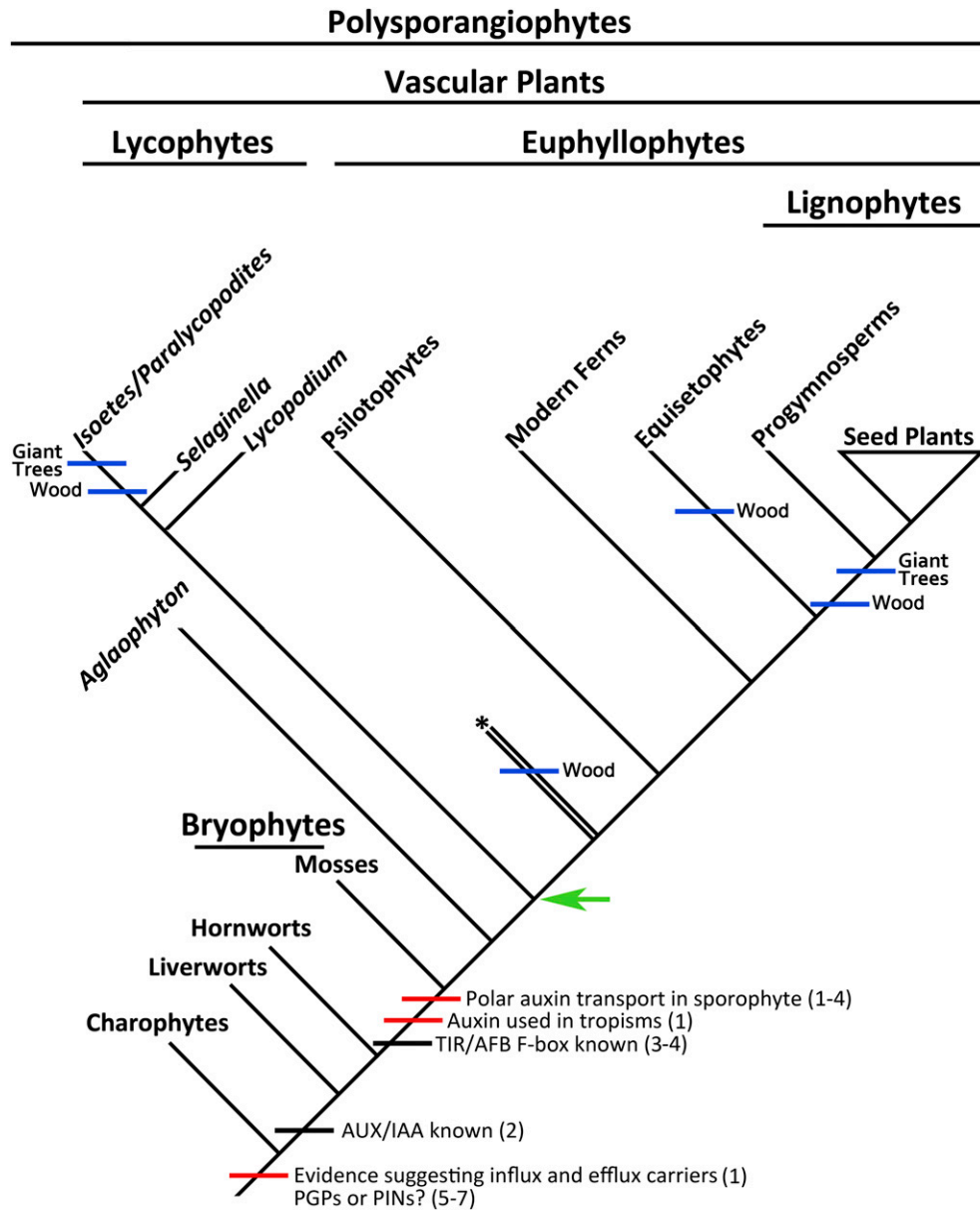
Fig. 6.   Generalized phylogenetic tree of embryophytes showing hypothesized systematic distributions of earliest occurrences of physiological processes (red hash marks), structural features (blue hash marks), and genes (black hash marks) illustrating methods of hypothesis formulation and testing. All hash marks represent specific genes, physiological process, and structural features that are either hypothesized or demonstrated to be present in all taxa that are derived above them on the tree. Green arrow = most recent common ancestor of lycophytes and euphyllophytes. The closely spaced parallel lines identified by an asterisk represent the Lower Devonian trimerophyte-grade plants of uncertain relationships in which secondary xylem recently has been documented (Gerrienne et al., 2011; Hoffman and Tomescu, 2013). Composite tree simplified from Rothwell (1999) and Cooke et al. (2002). See text for additional details. (1) Cooke et al., 2002; (2) Ross and Reid, 2010; (3) Shinohara et al., 2011; (4) Dolan, 2009; (5) Blakeslee et al., 2005; (6) Křeček et al., 2009; (7) De Smet et al., 2011.

circular-patterned secondary xylem elements (i.e., swirls) positioned acroscopically with respect to branch junctions in the stem wood of a Paleozoic progymnosperm (Fig. 4). Such swirls have been demonstrated to result from auxin whirlpools formed when obstructions, such as branches or broad leaf trace rays disrupt polar auxin flow in the cambium of plants where auxin regulates vascular tissue development (Sachs and Cohen, 1982). The discovery of such swirls in the Late Devonian progymnosperm *Archaeopteris* Dawson (Fig. 4) that are comparable to those in similar positions of seed plants (e.g., *Arabidopsis thaliana*

L., Fig. 5; Wyatt et al., 2010) shows that the patterning of wood development in progymnosperms also was under polar auxin transport regulation (Rothwell and Lev-Yadun, 2005; Fig. 4), thus strengthening the hypotheses that secondary vascular tissue of progymnosperms is homologous to that of seed plants (Beck, 1960) and strongly supporting the monophyly of lignophytes (i.e., progymnosperms + seed plants; Kenrick and Crane, 1997; Fig. 6).

In a phylogenetic context, sporophytic polar auxin transport regulation (Fig. 6) extends from far below the node where

progymnosperms (i.e., *Archaeopteris*; "Progymnosperms" in Fig. 6) occur on the embryophyte tree (Cooke et al., 2002; Lau et al., 2008; Williams, 2012). Wood production recently has been demonstrated to occur in some trimerophytes as early as ca. 400 Ma (i.e., Early Devonian plants near the base of the euphyllophyte clade; asterisk in Fig. 6; Gerrienne et al., 2011; Hoffman and Tomescu, 2013). Furthermore, the occurrence of physiological evidence for polar auxin transport (which is part of the molecular tool kit for patterning in secondary vascular tissue production) even lower on the tree than those plants (Fig. 6), implies that the genetic basis (PGPs and/or PINs) for this developmental mechanism could have been present in the ancestors of all vascular plants, and therefore available for the parallel recruitment to wood production in lycophytes and in two or more clades of euphyllophytes (Fig. 6).

Although wood is produced only by seed plants within the living flora, abundant wood is present in numerous fossil representatives of isoetalean lycophytes (i.e., Lepidodendrales) and equisetophytes (e.g., Calamitales; Stewart and Rothwell, 1993). Because the known systematic distribution of polar auxin transport encompasses both of those clades (Cooke et al., 2002; Fig. 6), it is probable that the evolution and development of wood in all of the extinct woody species was under polar auxin regulation as well. That hypothesis was recently tested by examining the Paleozoic wood of both the lepidodendralean lycophyte, *Paralycopodites* Morey et Morey and the calamitalean equisetophyte, *Arthropitys* Goeppert for xylem swirls, which were found to be present in stems at acroscopic locations in relation to branch bases in the wood of both (Rothwell et al., 2008). Those discoveries strongly support the hypothesis of a shared polar auxin transport mechanism involved in wood patterning. However, because the most recent common ancestor of lycophytes and euphyllophytes (green arrow in Fig. 6) did not produce secondary tissues, this tree-based assessment of the genetic basis for tissue evolution provides strong evidence for separate but parallel origins of wood in each clade via a common polar auxin regulatory pathway (Rothwell et al., 2008).

Polar auxin regulation and the parallel evolution of bipolar growth in giant seed plant and lycophyte trees—Polar auxin patterning of secondary vascular tissue is currently the most extensively documented aspect of growth regulation in extinct plants, but it is only one of several vital functions that auxin performs in plant development (Abel and Theologis, 2010). Additional functions that recently have been demonstrated from plant fossils are the establishment of polarity and gravitropic responses that facilitate bipolar growth and the production of substantial rooting systems by giant trees (Sanders et al., 2011). Although trees of relatively modest proportions (i.e., 5–10 m tall) are found in several clades of ferns and equisetophytes (e.g., Stein et al., 2007), throughout all of plant history giant trees (i.e., >50 m tall) have evolved only in lignophytes (i.e., seed plants and their progymnospermous sister groups, including *Archaeopteris*; Fig. 6) and Paleozoic fossil lycophytes (i.e., isoetaleans, including *Paralycopodites* [Fig. 8]; Rothwell, 1995; Fig. 6). Such giant trees are up to an order of magnitude larger than all other plants, and their exceptional size has been made possible by a combination of secondary growth, bipolar growth, and some facets of branching (Trivett, 1993; Rothwell, 1995).

In seed plants, embryonic polarity is initiated before the first division of the zygote to produce a two-celled embryo (Fig. 7A; He et al., 2007). The root–shoot axis is regulated by auxin,

which is transported basipetally beginning in the globular stage of embryogeny (red arrow in Fig. 7B), then from the cotyledons, down the hypocotyl toward the radicle (red arrow in Fig. 7C; Howell, 1998; Blakeslee et al., 2005; Wabnik et al., 2013), and later from sites of future leaf primordia on the flanks of the shoot apical meristem (red oval in Fig. 7C). Bipolar growth first becomes obvious during seed germination when the root meristem (i.e., radicle) of the cotyledonary embryo grows out to establish a rooting system that shows positive gravitropism, and the shoot meristem (i.e., epicotyl or plumule) grows out to establish a shoot system with negative gravitropism (Fig. 7). Thus, polar auxin transport from the shoot apical meristem to the root apical meristem (Muday and Rahman, 2008) indirectly mediates gravitropic responses. As a result, bipolar growth is a fundamental aspect of the lignophyte body plan.

By contrast, the rooting systems of isoetalean lycophytes (including *Isoetes* and Paleozoic trees of the Lepidodendrales such as *Paralycopodites*; Fig. 11) do not develop from the radicle of a cotyledonary embryo and are not homologous to the true roots of either seed plants or other lycophytes (Eames, 1936; Phillips, 1979; Stubblefield and Rothwell, 1981; Sanders et al., 2011). Rather, isoetaleans grow from an embryo similar to those of living pteridophytes, which typically has a foot region for absorbing nutrients from the gametophyte, a shoot apical meristem that establishes unipolar growth of the plants, and an embryonic root and sometimes also a suspensor (Eames, 1936). In the Isoetales no embryonic root meristem is produced (Phillips, 1979; Stubblefield and Rothwell, 1981; Rothwell and Erwin, 1985; Fig. 8A). From a variety of developmental stages of fossilized *Lepidocarpon* Scott embryos (i.e., the megasporangiate cone of the isoetalean tree, *Lepidophloios* Sternberg) we know that some lepidodendralean embryos had only a foot region and a growing shoot meristem; Phillips, 1979; Fig. 8A). Somewhat larger *Lepidocarpon* embryos reveal that the shoot meristem forked precociously (Figs. 8B, 9) to produce two branches (Figs. 8, 10) with dissimilar internal anatomy (Fig. 10) and growth trajectories (Phillips, 1979; Stubblefield and Rothwell, 1981). One branch is negatively gravitropic (Fig. 8C) and has the exarch protostele that characterizes stems of the Lepidodendrales (Eggert, 1961; Fig. 10 at s). The other branch is not negatively gravitropic, has a pith, and the endarch xylem maturation (Fig. 10 at r) that characterize *Stigmaria* Brongniart, the mature lepidodendralean rooting system (Frankenberg and Eggert, 1969). Along with other features of *Stigmaria*, these comprise extensive morphological, anatomical, and developmental evidence to demonstrate that giant lycophyte trees were rooted by a highly modified shoot system termed a rhizomorph (Frankenberg and Eggert, 1969; Rothwell and Erwin, 1985), rather than by true roots homologous to those of either seed plants or other lycophytes (Stewart, 1947; Frankenberg and Eggert, 1969; Eggert, 1972; Stubblefield and Rothwell, 1981; Rothwell and Erwin, 1985; Sanders et al., 2011; Coudert et al., 2013; Figs. 7, 8). Therefore, bipolar growth is secondarily derived in the body plan of arborescent lycophytes (Fig. 8).

As with the regulatory genetics of wood patterning, development of growth architecture in giant lycophyte trees such as *Paralycopodites* (Fig. 11) is recorded by tracheary element swirls adjacent to the positions where polar auxin flow was impeded in the cambium of stems and rhizomorphic rooting systems (Sanders et al., 2011). In stems such swirls are positioned acroscopically with respect to branch bases, indicating the normal basipetal polar auxin flow (black arrowheads in Fig. 12; Sanders et al., 2011). In the rhizomorphic *Stigmaria* rooting
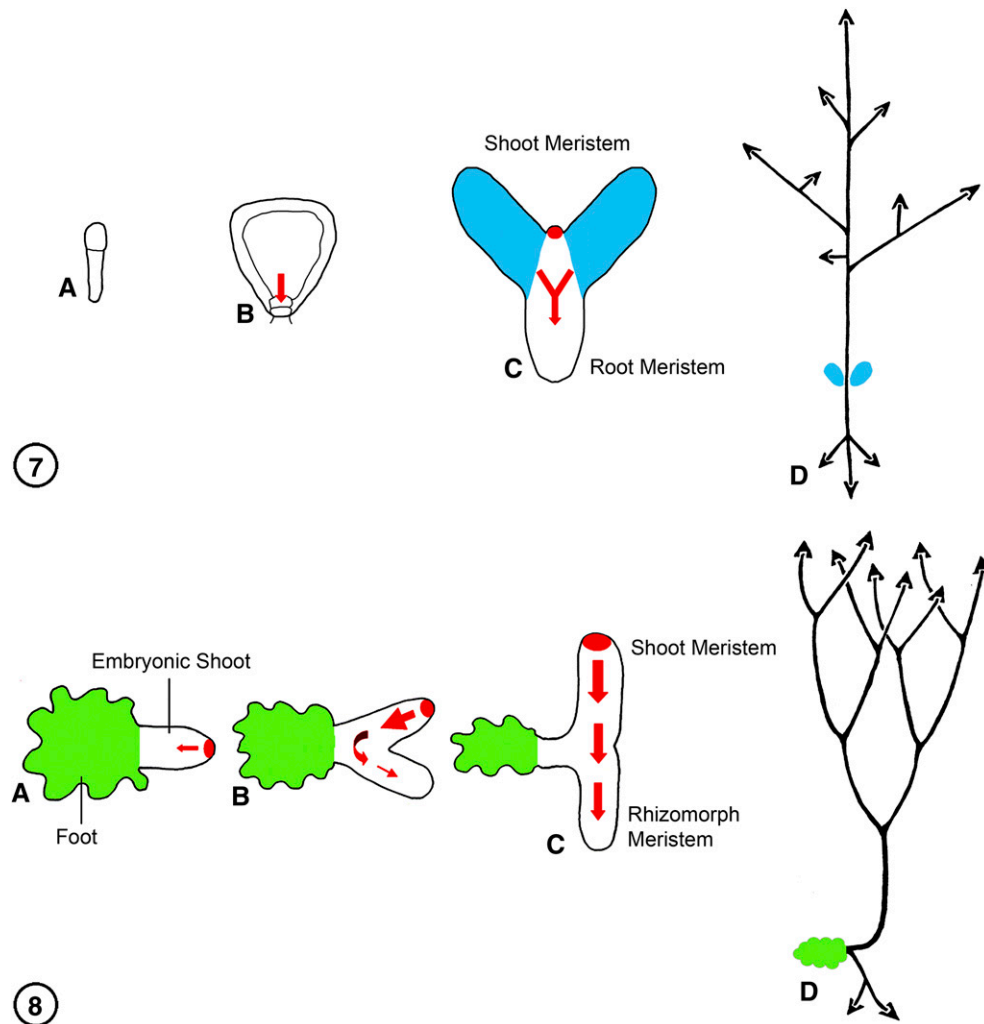
Figs. 7, 8.   Developmental/evolutionary origin of bipolar growth in **7.** Seed plants with cotyledonary embryos and true rooting systems, and **8.** Lycophyte trees with pteridophytic embryos and rhizomorphic (shoot derived) rooting systems. Cotyledons are in blue, and embryonic foot is in green. Red ovals indicate meristematic sites of auxin accumulation and red arrows show parallel polar auxin flow. **7A.** Two-celled embryo with well established polarity. **7B**. Globular stage of embryo at initiation of polar auxin flow (arrow). **7C.** More mature embryo with cotyledons and both shoot and root meristems that impart bipolar growth. **7D.** Diagram of bipolar growth in the architecture of seed plants; meristems indicated by arrowheads. **8A.** Young *Lepidocarpon* embryo with foot region and embryonic shoot apical meristem; polar auxin is hypothesized to have been initiated at this stage of growth. **8B.** Larger embryo after precocious fork in shoot apex, with polar auxin hypothesized to have been initiated only in one branch, and to have been transported toward the apex of the other (arrows). **8C.** Somewhat larger embryo with negatively gravitropic shoot meristem and rhizomorph meristem that is not positively gravitropic. **8D**. Diagram of secondarily derived bipolar growth in the architecture of arborescent lycophytes. Fig. 7A–C drawn after Howell, 1998; Fig. 8A–C modified from Sanders, 2007 and Sanders et al., 2011; Fig. 7D and 8D modified from Rothwell, 1995.

system of the same plant, such disruptions occur adjacent to wide lateral appendage trace rays (black arrowheads in Fig. 13). However, such swirls aren't located acroscopically with respect to the disruption, as would be the case if auxin were being transported from the apical meristem of the rhizomorphic rooting axis. Rather, in *Stigmaria* they occur in a basiscopic position with respect to the disruptions (Fig. 13), as is the case for the true rooting systems of seed plants, where auxin is transported toward the root apical meristem (Fig. 7C). Although the isoetalean rhizomorph is a highly modified shoot system, it has the polar auxin transport patterning of a root, and not that of a shoot, thus illustrating parallel evolution of a common pattern of auxin transport in rooting structures with contrasting homologies and evolutionary histories. These methods for

recognizing the regulatory genetics of wood patterning in extinct tree-sized lycophytes provide a template for conducting future paleobotanical studies to decipher the growth architecture of additional extinct plants that also have no living structural counterparts.

Parallel evolution of euphyllophyte leaves—Contrary to the proposal that there has been a single origin of the euphyllophyte leaf (e.g., Schneider et al., 2002; figs. 17–14 of Evert and Eichorn, 2013; Schneider, 2013), phylogenetic analyses that include both living and fossil species (e.g., Rothwell, 1999; Rothwell and Nixon, 2006), evolutionary studies of fossil plants (Stewart and Rothwell, 1993; Boyce and Knoll, 2002; Boyce, 2005) and developmental studies (Friedman et al., 2004; Sanders et al.,
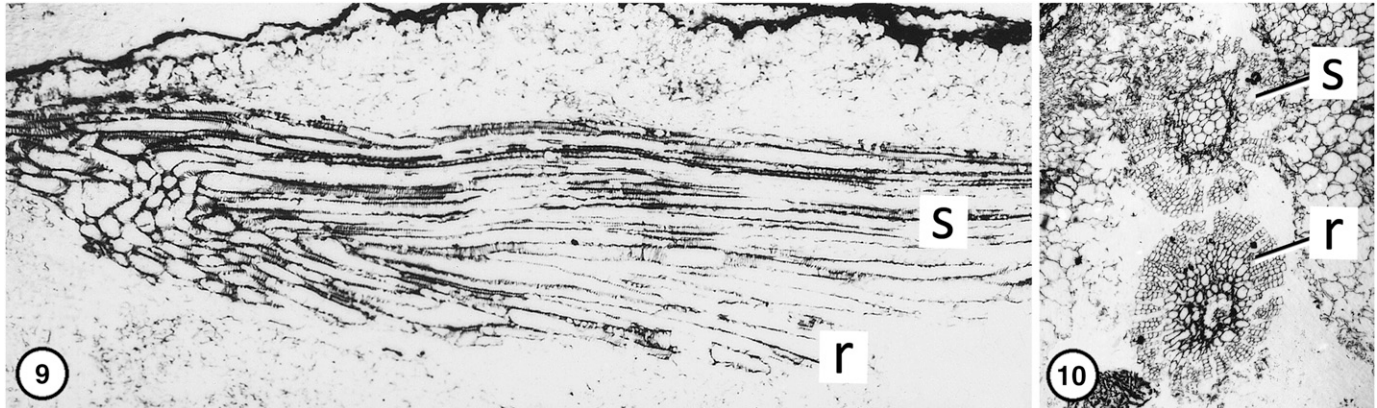
Figs. 9, 10. Fossil embryos within sporangia on sporophylls of Middle Pennsylvanian age fossil megasporangiate cone of arborescent lycophyte, *Lepidocarpon*. **9.** Longitudinal section of embryo with precocious branching of shoot showing primary and secondary xylem tracheids of stem (s) and rhizomoprh (r). University of Illinois slide no. 8149, ×36. **10.** Cross section of embryo distal to level of precocious shoot branching, showing exarch stele and wood of stem (s) above, endarch stele with pith and wood of rhizomorph (r) below. University of Illinois slide no. 7799, ×22. Photographs courtesy of the late Dr. James M. Schopf.

2007; Tomescu, 2009; Pires and Dolan, 2012) each reveal that there have been multiple origins of euphyllophyte leaves from lateral branching systems of trimerophyte-grade plants (e.g., Gensel, 1984). Early structural/developmental fossil evidence for the latter focuses on parallel patterns of change in leaf venation used as structural fingerprints for changes in meristematic activity in leaf laminae, which are based on observations of venation and meristematic growth in the leaves of living plants (Boyce and Knoll, 2002).
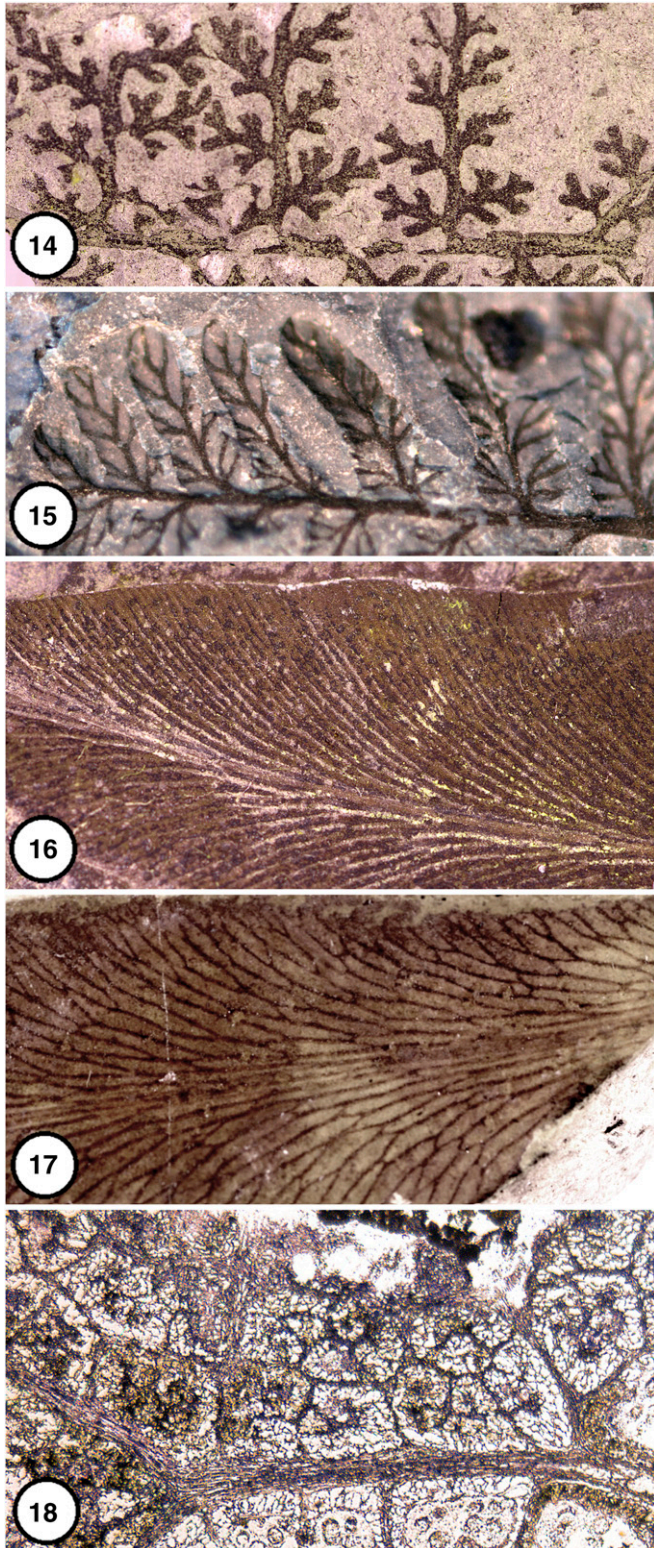
Data developed in an extensive survey of the fossil record that spans the interval when well-differentiated stem/leaf organography of euphyllophytes first can be recognized (i.e., Late Devonian through Early Carboniferous; Stewart and Rothwell, 1993; Taylor et al., 2009) document parallel modifications in four separate clades of euphyllophytes (Boyce and Knoll, 2002). Those fossils reveal that the most ancient representatives of equisetophytes, modern (i.e., crown-group) ferns, progymnosperms, and seed plants are all derived from trimerophyte-grade plants with subordinate laterals (Fig. 19; Gensel, 1984). They all have leaves with essentially linear terminal segments, one vein in each segment, and terminal/marginal vein endings (Fig. 14), which correspond to the leaves of living plants with exclusively terminal/marginal meristems (Boyce and Knoll, 2002). Successively more recent representatives of each clade show progressive modifications to broader laminae with divergent venation and marginal vein endings (Figs. 15, 19) that are correlated with a transition to diffuse internal meristematic activity, including convergent venation and marginal vein endings (Figs. 16, 19), reticulate venation with marginal vein endings (Figs. 17, 19), and, lastly, reticulate venation with internal vein endings (Figs. 18, 19; Boyce and Knoll, 2002, and references cited therein). Not all of those structural modifications have been attained in the leaves of all clades (Fig. 19; table 1 of Boyce and Knoll, 2002), with only a few ferns and seed plants showing the entire range of venation modifications and presumably, therefore, of meristematic modifications (Fig. 19).

Although leaf meristems have been studied in only a spattering of non-angiospermous plants, and we currently know almost nothing about the regulatory genetics underlying the structural modifications documented by Boyce and Knoll (2002), this fossil-based hypothesis of leaf blade meristematic evolution is concordant with the correlated canalization in vein and leaf patterning documented for a wide spectrum of flowering plants (Dengler and Kang, 2001; Reinhardt et al., 2003; Koenig et al., 2009; Chitwood et al., 2014). It also provides a focus for
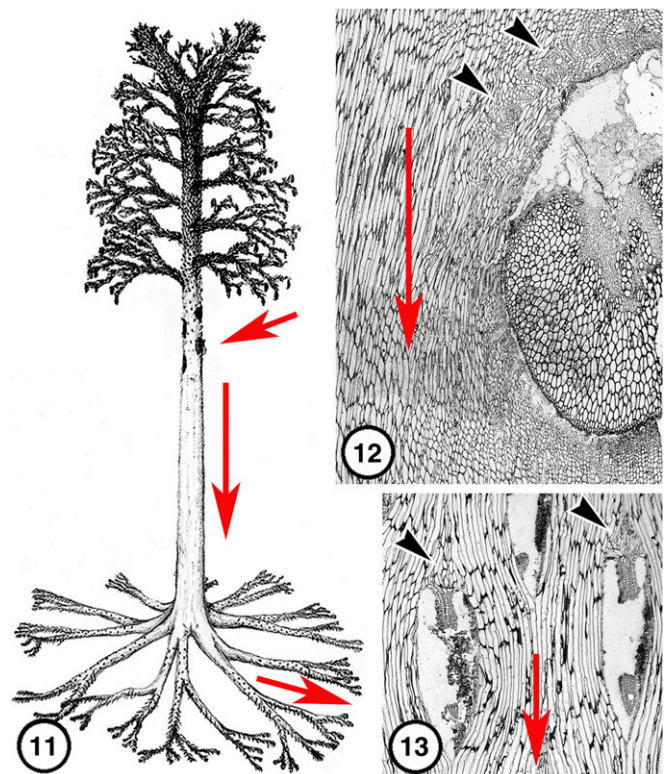
Figs. 11–13. Bipolar growth in lycophyte tree, *Paralycopoidites*. **11.** Plant reconstruction with red arrows indicating polar auxin flow. Modified from Sanders et al. (2011). **12.** Tangential section through wood of stem, showing tracheid swirls on acroscopic side of branch (arrowheads) and straight rows of tracheids below branch, revealing basipetal flow of auxin (red arrow). **13.** Tangential section through wood of rhizomorph, showing distorted tracheids (arrowheads) on basiscopic side of broad appendage rays and straight rows of tracheids on apical side (toward base of photograph). Arrow indicates acropetal auxin flow in this rooting system.

future hypothesis testing in a fashion that is similar to that for the simplification of euphyllophyte leaf morphology through the downregulation of the *KNOTTED1* gene (Hareven et al., 1996; Sanders et al., 2007 and references cited therein).

Independent origins and parallel evolution of leaves in several euphyllophyte clades also are evidenced by changes in overall leaf structure, which can be correlated with changes in regulatory genetics through time. Leaves are widely recognized to be one of three fundamental vegetative organs of vascular plants. They are produced at nodes on stems and typically subtend axillary buds/branches in seed plants (Bower, 1919). From a structural perspective, leaves may be distinguished from stems by (1) determinate vs. indeterminate growth, (2) bilateral vs. radial symmetry of external morphology, (3) presence vs. absence of adaxial/abaxial patterning of internal anatomy, and (4) presence vs. absence of lamina with veins. A comparative examination of the most ancient anatomically preserved representatives of seed plants and leptosporangiate ferns reveals that not all of those distinctions are present in the body plans of basal members of each clade (Sanders et al., 2009). In other words, not all of the modifications of lateral branch systems (e.g., Gensel, 1984) associated with the evolution of leaves in modern plants had evolved in those ancient fossil plants.

Leaves of the Upper Devonian seed plant *Elkinsia polymorpha* Rothwell, Scheckler et Gillespie, a pteridosperm (seed fern), are highly dissected and fern-like, with determinate growth and laminar pinnules (Serbet and Rothwell, 1992). They also have relatively broad pinnules and adaxial/abaxial patterning at the base of the frond, but not in the most distal regions (Sanders et al., 2007). By contrast, fronds of the filicalean fern *Psalixochlaena antiqua* (Kidston) Good retain some indeterminacy in growth and lack significant lamina of the pinnules, but show adaxial/abaxial patterning of internal anatomy throughout the frond (Sanders et al., 2007). This difference in the combination of stem-like and leaf-like features shows that leaf characters evolved in a different order in seed plants and leptosporangiate ferns—determinate growth and broad pinnules before adaxial/abaxial patterning in *Elkinsia polylmorpha* vs. adaxial/abaxial patterning before broad pinules and determinacy in *Psalixochlaena antiqua*, thus providing additional evidence for the parallel evolution of leaves in seed plants and ferns (Sanders et al., 2007).

Further evidence for multiple origins of euphyllophyte leaves is provided by a developmental/systematic outlook on extant lineages. Fern and seed plant leaves exhibit contrasting trajectories in terms of developmental anatomy (Kaplan and Groff, 1995). Many fern leaves grow from an apical cell for a significant part of their development and mature acropetally, with the leaf tip typically maintaining a meristematic state throughout development. This development is consistent with the venation patterns for the most ancient euphyllophyte leaves studied by Boyce and Knoll (2002) and discussed earlier in the present paper. In contrast, leaves of most flowering plants have limited apical meristematic activity, rely more on diffuse growth, and

Figs. 14–18. Venation variations that reflect parallel evolution of meristematic activity in the pinnules of several euphyllophyte clades. **14.** Linear laminar segments with one vein per segment and marginal vein endings. Putative lygionpterid seed fern (gymnosperm), *Sphenopteris* Brongniart sp. from the Lower Pennsylvanian of Illinois. **15.** Divergent venation with marginal vein endings. Leptosporangiate fern, *Senftenbergia oregonensis* (Arnold) Hillier et Rothwell from the Pennsylvanian of Oregon. **16.** Convergent

venation with marginal vein endings. Medullosan seed fern (gymnosperm), *Neuropteris scheuchzeri* Hoffman from the Middle Pennsylvanian of Illinois. **17.** Reticulate venation with marginal vein endings. Gymmosperm *Glossopteris* Brongniart sp. from the Permian of Australia. **18.** Reticulate venation with internal vein endings. Leptosporangiate fern *Hausmannia morinii* Stockey, Rothwell et Little from the Lower Cretaceous of British Columbia, Canada.
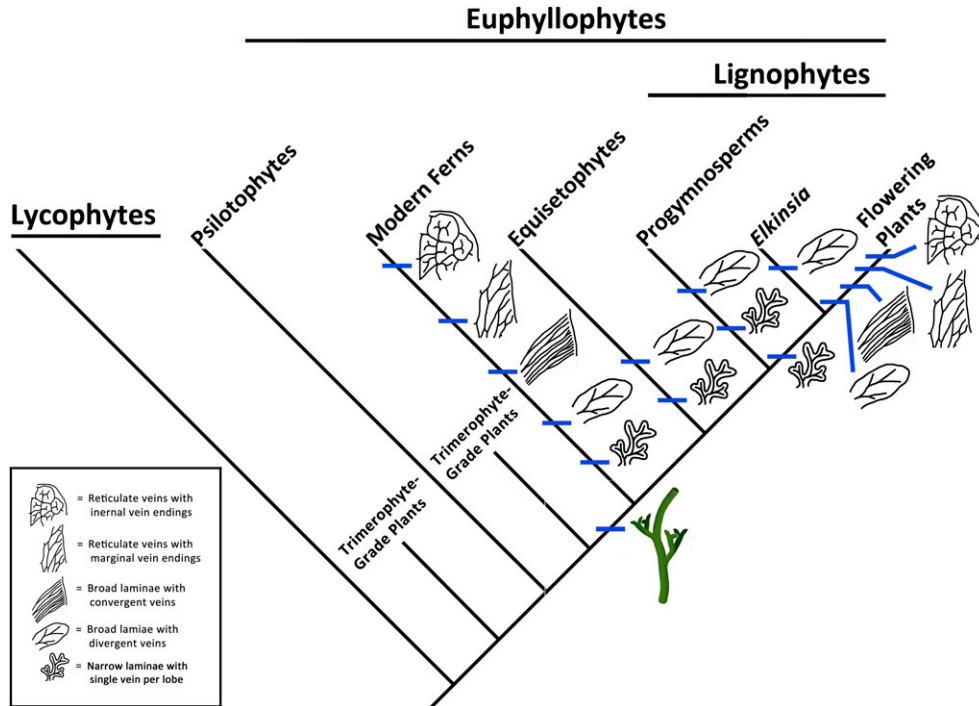
Fig. 19.   Variations in leaf characters illustrating evolutionary changes in the development of euphyllophyte leaves mapped on phylogenetic tree of vascular plants. Systematic position of morphology hypothesized to be earliest stage in euphyllophyte stem/leaf evolution (Gensel, 1984) illustrated by green figure. Parallel evolution of pinnule venation morphologies documented for several euphyllophyte clades from the fossil record and hypothesized to have resulted from parallel evolutionary changes in meristematic activity (Boyce and Knoll, 2002) are mapped on the appropriate branches. Tree simplified from Rothwell (1999). See text and legend for details.

have complex maturation that proceeds initially in an acropetal direction but is completed during a final phase of basipetal tissue differentiation (e.g., Aloni, 2001; Kang and Dengler, 2004; Donner and Scarpella, 2009).

Data on the molecular genetic pathways controlling leaf development indicate a disparity in the mechanisms regulating leaf-defining features (determinacy and adaxial/abaxial polarity) between ferns and seed plants, supplying additional evidence for independent origins of leaves in the two groups (Tomescu, 2009). For example, whereas in seed plants leaf development is initiated by mutually antagonistic interactions between transcription factors responsible for shoot apical meristem identity and maintenance (*KNOX1*), and determinacy-inducing transcription factors (ARP) with an expression domain limited to the leaf primordium, in ferns the two classes of transcription factors are expressed in both shoot apical meristem and leaf primordia (Bharathan et al., 2002; Harrison et al., 2005). Furthermore, *YABBY* family genes that repress *KNOX* expression in the leaf primordium (Floyd and Bowman, 2006) and act in adaxial/abaxial polarity establishment in seed plants (Goldshmidt et al., 2008) have not been detected in ferns (Floyd and Bowman, 2007). Therefore, although the genetic determinants of leaf adaxial/abaxial polarity are unknown in ferns, this aspect of leaf development is different from that of seed plants in at least one respect, consistent with independent origins of leaves in the two lineages.

We anticipate that future paleontological studies focusing on additional comparative combinations of morphological and anatomical characters will provide a basis for generating additional hypotheses for the evolution of stem/leaf organography

in divergent clades of vascular plants. When coordinated with the continued characterization of leaf meristems and of genetic regulatory mechanisms associated with leaf development, data from rapidly developing transcriptomic techniques have exciting potential for testing such fossil-based hypotheses of leaf evolution across the entire spectrum of vascular plants.

Evolutionary origin of the ancestral vascular plant body plan—The deep origins of embryophytes (including bryophytes, polysporangiophytes and vascular plants [tracheophytes]) have been the subject of much debate fuelled by the perceived paucity of the fossil record of early land plants. Premid-Silurian (i.e., >430 Ma) rocks are devoid of axial fossils, which have been equated with plant presence in the traditional view influenced by the dominance of axial sporophytes of vascular plants in modern floras. However, approached with a different search image (Tomescu and Rothwell, 2006), older rocks going as far back in time as the Late Ordovician (ca. 450 Ma ago) have proved to preserve a wealth of terrestrial fossils with thalloid growth habit, some of which are comparable to bryophyte-grade plant gametophytes (Tomescu et al., 2009, 2010). Vascular plants evolved from such bryophyte-grade ancestors (Mishler and Churchill, 1984) with long-lived gametophytes and inconspicuous ephemeral sporophytes, but rose to dominate terrestrial ecosystems by virtue of their vascularized branched sporophytes with indeterminate growth and modular construction.

The origin of the tracheophyte sporophyte gains resolution from a working hypothesis based on data from extant plants and developmental genetics, which receives further support from

paleontology. Evolution of the vascular plant sporophyte occurred in two stages (Kenrick and Crane, 1997), beginning with the origin of branched sporophytes (i.e., polysporangiophytes) and followed by the evolution of true vascular tissue to produce vascular plants. The origin of polysporangiophytes has been explained by several hypotheses comprising modification of development in an ancestral bryophyte-type sporophyte (Tomescu et al., 2014). Some hypotheses have proposed intercalation of a novel vegetative organ between the bryophyte foot and the sporangium (Kato and Akiyama, 2005), while others have argued for transient sterilization of an early embryonic pole with initial sporangial identity (Ligrone et al., 2012a, b). Recent progress in developmental genetics, in conjunction with classic observations of developmental anatomy and teratological specimens, has provided the basis for an alternative hypothesis (Tomescu et al., 2014), which explains evolution of the vascular plant sporophyte by a delay of the transition to reproductive development in the embryo, with prolongation of vegetative growth from an apical meristem and branching (Figs. 20–25).

The presence, in many extant bryophytes, of an embryogenetic phase with apical growth (Fig. 20C, D) as well as teratological branched sporophytes, demonstrate potential for indeterminate growth and branching. Apogamous sporophytes

of *Physcomitrella patens* (Hedw.) Bruch et Schimp. produced by deletion of polycomb repressive complex 2 (PRC2) genes, such as *PpCLF,* exhibit prolonged vegetative growth and branching (Okano et al., 2009; Figs. 24, 25). Along with *P. patens* sporophytes that produce multiple sporangia as a result of disruption of different genetic loci or inhibition of polar auxin transport (Tanahashi et al., 2005; Fujita et al., 2008; Vivancos et al., 2012), these findings corroborate developmental anatomy and teratological observations and point to putative mechanisms for acquisition of growth indeterminacy and modularity.

The recently developed hypothesis of polysporangiophyte evolution based on this internally consistent set of observations (Figs. 20–25) includes testable changes in genetic regulatory pathways of development (Figs. 20–25) and predicts homology of the vegetative body of the vascular plant sporophyte with the vegetative axis of the bryophyte embryo and the seta of the bryophyte sporophyte (Figs. 20–25; Tomescu et al., 2014). Studies of the oldest vascular plant sporophytes (mid-Silurian– Early Devonian) demonstrate that basal tracheophyte sporophytes were minute, entirely dependent on the gametophytes, and had limited branching (e.g., Fig. 22; Edwards, 2000; Boyce, 2008); thus, they were little more than branched versions (Fig. 23) of bryophyte sporophytes (Fig. 21; Tomescu et al., 2014). This
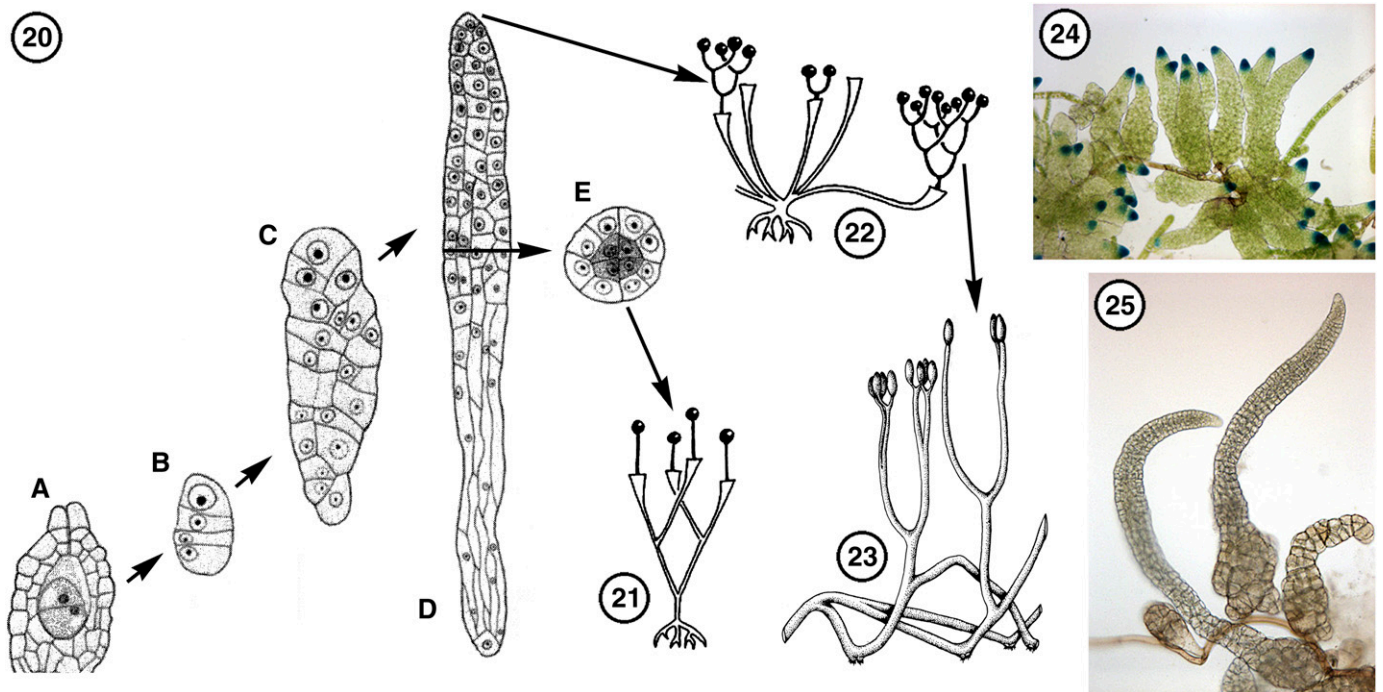
Figs. 20–25.   Illustrations explaining the apical growth hypothesis (Tomescu et al., 2014) for the origin of the ancestral polysporangiophyte body plan. **20A–D.** Developmental/transformational series illustrating early moss-type embryogeny (represented by *Physcomitrium cyathicarpum* Mit), with transient growth from apical cells (adapted from Lal and Bhandari, 1968). Embryonic transition to reproductive growth (at e) leads to bryophyte-type sporophytes (Fig. 21) with unbranched seta and single terminal sporangium, whereas continued apical growth leads to polysporangiophyte-type sporophyte with elongated and branched axes, each with a terminal sporangium (Fig. 22). **21.** Bryophyte model of growth (of Rothwell, 1995), with unbranched sporophyte (in black) growing on gametophyte phase of life cycle (in white; from Rothwell, 1995). **22.** Ancestral polysporangiophyte morphology (i.e., cooksonioid model of growth of Rothwell [1995]) with elongated and branched seta (in black) that remains attached to and dependent upon the gametophyte (in white; from Rothwell, 1995). **23.** Lower Devonian nonvascular polysporangiophyte *Aglaophyton major* (Kidston and Lang) D. S. Edwards from the Rhynie Chert that conforms to the cooksonioid model of Rothwell (1995; from Stewart and Rothwell, 1993). **24.** *PpCLF* deletion mutant of apogamous *Physcomytrella patens* sporophyte with branched axes derived from extended period of sporophyte growth by an apical cell. Apical cells highlighted in blue by expression of MKN4-GUS fusion protein. See Okano et al. (2009) for details (photograph courtesy of N. Aono and M. Hasebe). **25.** *PpCLF*-deletion mutant of apogamous *Physcomytrella patens* sporophyte with elongated sporophytic axis derived from extended period of growth by an apical cell. See Okano et al. (2009) for details (photograph courtesy of N. Aono and M. Hasebe).

evidence for the gametophyte-dominated life cycle of basal vascular plants and the exclusiveness of thalloid forms in the pre-mid-Silurian terrestrial vegetation, are in agreement with the consistent association of axial vascular plant sporophytes with thalloid compressions interpreted as gametophytes, in Early Devonian plant fossil assemblages (Strother, 2010), thus further strengthening this hypothesis. We anticipate that our rapidly growing understanding of regulatory genetics (e.g., Xu et al., 2014) will continue to broaden the spectrum of developmental correlates available for future tests of similar evolutionary/developmental hypotheses.

CONCLUSIONS

The role of development in organismal evolution was explored vigorously during the 19th century but has received far less attention in succeeding decades. For much of the past century evolutionary studies focused either on evolutionary pattern (as characterized by systematics and paleontology) or else on evolutionary process (which was viewed primarily from the standpoint of population genetics). The return to an emphasis on development in evolutionary studies was heralded by the work of Steven J. Gould, arguably the most influential evolutionary biologist of the late 20th century.

Beginning in the early 1970s, Gould and his colleagues began to test hypotheses regarding evolutionary process with evidence of evolutionary pattern from the fossil record (e.g., Eldredge and Gould, 1972; Gould and Lewontin, 1979). Gould's 1977 book *Ontogeny and Phylogeny* dramatically altered the trajectory of evolutionary studies by laying the groundwork for a modern understanding of evolutionary changes in terms of organismal development. Under the umbrella terms "heterochrony" and "heterotopy" (Gould, 1977), Gould adapted and refined earlier concepts of Haeckel (1905) to give us powerful tools for reintegrating the development of organisms with the evolution of tissues, organs, and organisms through time. However, only after the rise of molecular genetics in the 1990s did details about the causal mechanisms of evolutionary change begin to be understood.

In recent years it has become increasingly clear that while individual genes do transcribe mRNAs for translation to individual proteins, the genetic basis for development requires a much more complex integration of gene action through the activities of pre- and posttranscriptional regulation and growth regulators. Together, these control the developmental pathways that yield mature organisms and produce alterations that result in the changes in the structure and physiology of mature organisms that Gould (1977) originally characterized under the various categories of heterochrony. This brings us back full circle to Darwin's 1859 theory elaborated in *On the Origin of Species* (Darwin, 1859), that biological evolution proceeds by "descent with modification". In other words, Darwin's descent with modification is categorized and characterized by Gould's heterochronic and heterotopic processes, which are given a functional explanation by our increasing understanding of gene regulation.

While the fossil record has traditionally been regarded as incomplete and, hence, unreliable for documenting evolutionary trajectories, continued paleontological exploration has resulted in improved overall density of sampling both stratigraphically and geographically. This improvement in sample collection has proven repeatedly to lead to the uncovering of reliable evolutionary patterns (e.g., Knoll and Bambach, 2000; DiMichele et al., 2001; Boyce and Knoll, 2002; Leslie et al., 2012). Likewise, as we have shown,

such data appear to be increasingly congruent with documented effects of genetic changes in development, evidenced from the results of experiments with living species, thus allowing the paleontological data to serve both as powerful tests for evolutionary hypotheses, and as reasonable explanations for the modes and patterns of evolution that have produced modern plant forms. As more genomes become sequenced throughout the phylogenetic spectrum, they will undoubtedly provide a wealth of additional genetic information for testing hypotheses constructed from the fossil record.

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Appendix 1. Glossary of terms used in the study.

Acroscopic—Position on a structure or on a lateral organ of a main axis that faces toward the apex of the main axis.

Apogamous sporophytes—Development of sporophytic morphology without fertilization, such that apogamous sporophytes typically have a haploid genotype.

Axial sporophyte—Sporophyte body plan comprising an axis with longitudinal polarity and radial symmetry that bears one or several sporangia.

Basiscopic—Position on a structure or on a lateral organ of a main axis that faces toward the base (away from the apex) of the main axis.

Endarch xylem maturation—Pattern of development where the protoxylem (i.e., first tracheids or vessel elements of the xylem to mature) differentiates at the interior of the xylem and/or toward the center of the plant organ. Protoxylem cells are easily recognized because of their small diameters and annular or helical secondary wall thickenings.

Exarch xylem maturation—Pattern of development where the protoxylem (i.e., first tracheids or vessel elements of the xylem to mature) differentiates at the exterior of the xylem and/or toward the periphery of the plant organ.

Fingerprints (of regulatory genetic pathways)—Developmentally diagnostic structural features of plants that are known to be produced by a specific gene or genetic regulatory pathway.

Gravitropism—Directed growth of a plant in response to gravity; positive gravitropism = growth toward the pull of gravity; negative gravitropism = growth away from the pull of gravity.

Heterochrony—Evolutionary alteration that results from a change in the relative timing (e.g., onset, duration) of developmental events or processes and leads to changes in size or shape of a plant structure or organ or of timing of a physiological event.

Heterotopy—Evolutionary alteration that results from a change in the location of a developmental event or process and leads to changes in the position of an organ or structure on the plant body with respect to the ancestral morphology.

Isoetaleans—Members of order Isoetales of subdivision Lycophytina sensu Kenrick and Crane (1997), consisting of plants that are heterosporous, woody, and that are rooted by a rhizomorph. Isoetales s.l. includes Paleozoic trees traditionally assigned to the Lepidodendrales, Mesozoic plants traditionally assigned to the Pleuromeiales, and small living and fossil plants that traditionally have been assigned to the Isoetales s.s.

Lignophytes—A clade of vascular plants in which sporophytes produce wood from a bifacial vascular cambium and that includes both seed plants and progymnosperms.

Orthologous genes—Two genes from two different species that derive from a single gene in the last shared common ancestor.

Paralogous genes—Genes that derive from a single gene that was duplicated within a genome.

Pith—The central region of plant axes made up of ground tissue and that is surrounded by the vascular tissues of the stele.

Polysporangiophytes—A clade of plants whose sporophytes branch to produce more than one sporangium, including all vascular plants plus a small grade of the most ancient species that have no true vascular tissue.

Progymnosperms—Extinct lignophytes that reproduce by free spores, rather than seeds.

Protostele—Vascular tissue that occupies the center of stems and roots of several clades of vascular plants; often appearing round or star-shaped in cross sections. Stems with protosteles do not produce leaf gaps.

Pteridophytes—Plants that reproduce by free spores, including progymnosperms, lycophytes, and several other clades and grades of nonseed plants.

Pteridosperms (= seed ferns)—Extinct gymnosperm-grade of seed plants with fern-like leaf morphology.

Rhizomorph—Rooting system of isoetalean lycophytes that has been derived from a modified shoot system and that is not homologous to the roots of other plants. The rhizomorph allows isoetalean lycophytes to have bipolar growth, facilitating the growth of giant trees among Paleozoic Lepidodendrales.

Synapomorphy—A shared derived character that defines a clade.

Tracheophytes (= vascular plants)—Embryophytes that produce true xylem and phloem; recognized in the paleontological record by thick-walled tracheary elements that function as water-conducting cells.

Transformational series—Intergrading morphologies that infer successive modifications, including evolutionary changes.

Trimerophytes—Systematic grade of ancient vascular plants of the Devonian that lack stem/leaf differentiation of the shoot system and that form the basal members of Euphyllophytina sensu Kenrick and Crane (1997).