Chapter 10
Reciprocal Illumination and Fossils Provide Important Perspectives in Plant Evo-devo: Examples from Auxin in Seed-Free Plants

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10.1 Introduction

Understanding the evolutionary origins of modern plant form is a central goal of plant evolutionary biology. Data relevant to understanding morphological evolution come from varied fields of study including paleontology, physiology, comparative anatomy and morphology, and developmental genetics. Only integration of all these data can provide a holistic understanding of plant evolution that spans plant phylogeny and deep evolutionary time. Studies on development are particularly important for understanding macroevolutionary patterns because all mature structures are formed through tightly regulated developmental processes, and these processes can differ depending on the homology of structures and the phylogenetic affinities of taxa. Development can therefore be used as an additional source of data, along with morphological and molecular data, for understanding evolution. When applied broadly to living and extinct representatives of a group, anatomical, physiological, and molecular aspects of development can thus provide novel insights on important aspects of plant evolution, including the origins of different growth forms, body plans, and major morphological innovations such as leaves, roots, and seeds.

Hormones such as auxins, cytokinins, and gibberellins are key regulators of a vast array of developmental processes (e.g., Durbak et al. 2012; Lacombe and Achard 2016). Auxins are particularly interesting because they have numerous documented roles in nearly every major aspect of plant development (Benjamins and

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Scheres 2008). Indole-3 acetic acid (IAA) is the predominant form of auxin in the plant body (Finet and Jaillais 2012). Once auxin enters a cell either through diffusion or active transport by influx carriers, they can only exit the cell via auxin efflux carriers in the cell membrane known as PIN proteins (Jones 1998). Polarization of PIN protein position within the cell establishes a directional movement of auxin through the cell; at the tissue scale this directional movement is referred to as polar auxin transport (Benková et al. 2003; Friml et al. 2003).

From the perspective of understanding major morphological transitions of embryophytes, polar auxin transport is especially intriguing in several respects: (1) auxin is involved in numerous aspects of embryonic and postembryonic development, and auxin transport networks have an integral role in coordinating growth and development on a whole-plant scale (Cooke et al. 2004; Benjamins and Scheres 2008; Leyser 2011); (2) on a smaller scale, auxin accumulation and polar auxin transport have important functions in initiation and development of leaves, roots, and vascular tissues (Reinhardt 2005; Blilou et al. 2005; Miyashima et al. 2013); and (3) the molecular mechanisms underlying polar auxin transport appear to be conserved across vascular plants (Sanders et al. 2011; Bennett 2015). A recent synthesis of PIN protein evolution indicates that PIN proteins have undergone relatively little structural change throughout plant phylogeny, suggesting that changes in the PIN proteins themselves are unlikely to have directly driven morphological evolution. However, it remains unclear whether changes in the regulatory networks controlling the timing, position, and duration of auxin signaling can explain some of the major morphological transitions of embryophytes (Bennett 2015).

To date, most of the available data on auxin action come from studies of seed plants, particularly angiosperms. However, because significant evolutionary time separates angiosperms from the earliest vascular plants, these data may not be entirely applicable to seed-free plants. Understanding differences in the roles of auxin in development between angiosperms and seed-free lineages is therefore critical for developing and testing hypotheses on morphological evolution. Here, we summarize current knowledge of the roles of auxin, particularly polar auxin transport, in the development of seed-free tracheophytes. This survey encompasses data from extant and fossil plants, and we suggest hypotheses to be tested and directions for future research.

10.2 Auxin in the Seed Plant Sporophyte

Polar auxin transport through the plant body is essential for establishing the overall axial organization of the plant and the patterning of vascular tissues (Berleth 2001; Scarpella and Meijer 2004; Sawchuk and Scarpella 2013; Lucas et al. 2013; Harrison 2016; Campbell and Turner 2017). Our detailed knowledge of the drivers and effects of polar auxin transport in the plant sporophyte come almost exclusively from *Arabidopsis*. This means that as soon as we leave the angiosperm (and certainly the seed plant) realm, we are walking on terra incognita in terms of almost everything

except for the most basic notions on the presence and sometimes the direction of polar auxin transport. Without trying to present a comprehensive review of polar auxin transport in angiosperm development, we review here its major aspects to provide context for a survey of polar auxin transport in seed-free plants. In doing this, we deliberately left out the interactions of auxin with genes and with other hormones, because there is little comparative data available on these aspects for the seed-free plants.

10.2.1 Embryogenesis

Auxin polarization is present as early as the asymmetric division of the zygote, which generates auxin-transporting and auxin-responsive daughter cells (Friml et al. 2003). This generates a steady auxin activity gradient responsible for the establishment and maintenance of polarity in the embryo (Friml et al. 2003; Aida et al. 2004). Initially, polar transport of auxin produced at the base of young embryos is directed toward the apical cell of the proembryo (Friml et al. 2003; Robert et al. 2013). The apical auxin response maximum thus generated contributes to the specification of apical embryonic structures, leading to localized apical auxin biosynthesis and subsequent reversal of auxin transport polarity within the embryo (Aida et al. 2004). The resulting basipetal (toward the base) polar auxin transport in the embryo specifies the root pole (radicle) and contributes positional information for establishment of the root stem cell niche (Aida et al. 2004; Moller and Weijers 2009; Robert et al. 2013).

Interestingly, looking at spruce (*Picea*) embryos, Larsson et al. (2008) showed that polar auxin transport is essential for the correct patterning of apical and basal parts of the embryos. They also demonstrated that auxin transport inhibitors generate phenotypes comparable with those of auxin response and transport mutants in *Arabidopsis*, suggesting that the role of polar auxin transport in embryogenesis is conserved between angiosperms and gymnosperms.

10.2.2 Shoot Apical Meristem

At the shoot apical meristem, auxin gradients are necessary for leaf initiation and phyllotactic patterning. The sites of new leaf primordia are specified by localized auxin maxima in the tunica (Reinhardt et al. 2000, 2003; Reinhardt 2005). The maxima are generated by the patterns of auxin transport in this superficial layer and are positioned according to constraints imposed by pre-existing primordia (Reinhardt 2005). Phyllotactic patterns result from the interplay between (1) meristem size, (2) spatial perturbations in the field of auxin gradients in superficial meristem layers induced by the positioning of pre-existing primordia that act as sinks (initially) or sources (subsequently) (Reinhardt 2005; Smith et al. 2006), and (3) differential

modulation of the elastic properties of cell walls across the same superficial layers of the meristem (Kierzkowski et al. 2012). The auxin of the leaf primordium maxima is channeled basipetally (Reinhardt et al. 2003; Benková et al. 2003), specifying in the process the trajectory of procambial strands that will form the leaf traces (Benková et al. 2003), and feeds into the stream of auxin that is transported basipetally through xylem parenchyma of the pre-existing stem below (Smith et al. 2006; Leyser 2011; Bennett et al. 2014).

Leaves are the predominant source of auxin in the shoot. After the early growth stages when they act as auxin sinks, leaf primordia switch to being sources of auxin. Auxin flows basipetally in the leaf, from maxima positioned initially at the leaf tip and subsequently at additional locations along the leaf margin (Scarpella et al. 2006; Barkoulas et al. 2007; Bennett et al. 2014_1). These maxima generate auxin gradients that are responsible for auxin flow canalization and, ultimately, leaf venation (Scarpella et al. 2006; Sawchuk et al. 2007). They are also involved in leaf margin serration (Barkoulas et al. 2007; Zhou et al. 2013; Kasprzewska et al. 2015; Wang et al. 2016_1) and the development of compound leaves (Barkoulas et al. 2008; Runions et al. 2017).

10.2.3 Root Apical Meristem

In roots, patterns of polar auxin transport conform with, and are a direct continuation of, the pattern established early in the embryo and defined by an auxin maximum located at the growing tip (Sabatini et al. 1999; Aida et al. 2004). First documented by studies of the root branching, polar auxin transport in the growing root conforms to the "fountain model" (Benková et al. 2003), with a "reflux loop" (Blilou et al. 2005). Auxin moves acropetally (toward the apex) through the center of the root (procambium), maintaining an auxin maximum at the root tip (Benková et al. 2003; Blilou et al. 2005). This auxin maximum promotes stem cell specification (Aida et al. 2004) and is, thus, responsible, for meristem maintenance (Aida et al. 2004; Blilou et al. 2005; Benjamins and Scheres 2008). Auxin is transported away from the apical maximum, basipetally, through the peripheral layers of the root. Away from the root tip, the peripheral auxin flux is redirected in a radial centripetal direction, to close the loop and rejoin the central acropetal stream of auxin that transits through the procambium. This reflux loop localizes meristem and cell expansion zones in the proximal meristem and regulates final cell size (Blilou et al. 2005). At the root apex, the role of the auxin maximum recapitulates the role of auxin maxima in the establishment of the embryonic root pole and the of lateral root primordia, by specifying the stem cell niche, with the mediation of *PLT* genes, and promoting cell division (Benková et al. 2003; Aida et al. 2004; Beveridge et al. 2007; Overvoorde et al. 2010).

10.2.4 Embryo to Mature Sporophyte

Canalized polar flow of auxin is essential for overall elongation and branching of the plant body, as well as for the patterning of vascular tissues, in terms of both specification and differentiation (Tuominen et al. 1997; Berleth et al. 2000; Björklund et al. 2007; De Rybel et al. 2013; Lucas et al. 2013; Miyashima et al. 2013; Fabregas et al. 2015; Harrison 2016). In all these, pathways of high auxin concentration specify the position and trajectories of vascular initials (procambium, vascular cambium). This role of auxin is expressed as early as the division and specification of the first preprocambial cells in the globular embryo (De Rybel et al. 2013; Wendrich and Weijers 2013) and is maintained throughout a plant's lifespan, during both primary and secondary growth (Sachs 1969, 1981; Sachs and Cohen 1982; Hejnowicz and Kurczyńska 1987; Lev-Yadun and Aloni 1990; Aloni 1995; Berleth 2001; Dengler 2006).

The longitudinal polarity (apical-basal axis) of the embryo and the associated apical-to-basal polarization of auxin transport are maintained and amplified continuously throughout plant development and are responsible for the patterns of polarity in auxin transport in the mature sporophyte. Current understanding of auxin homeostasis in relation to plant development suggests that the initiation and orientation of growth axes during embryonic and postembryonic development result from the combined activity of local auxin sources and polar auxin transport (Aida et al. 2004; Robert et al. 2013; Wabnik et al. 2013; Turchi et al. 2015). The result of these is a general pattern of basipetal polar auxin transport in stems and acropetal auxin flow in roots, maintained throughout the lifespan of the mature sporophyte (Sachs 1991; Berleth and Sachs 2001; Sawchuk and Scarpella 2013). Later, as the plant transitions to secondary growth, the basipetal polar auxin transport of the shoot is canalized through the cambium, where it is responsible for the patterning of secondary tissues (Sachs and Cohen 1982; Björklund et al. 2007). These roles of polar auxin transport in shoot patterning are conserved across seed plants (Sanders and Langdale 2013).

Additionally, Benková et al. (2003) pointed out that local accumulations of auxin and redirection of auxin flow play a fundamental role during processes of organ formation, whether those are roots or leaves. In these, the role of auxin is central in both specification of the site of primordium initiation, by accumulation of an auxin maximum, and in the establishment of a new growth axis, by activation of polar auxin transport as a result of the auxin gradient (generated by the primordium-specifying auxin maximum).

10.3 Polar Auxin Transport in Extant and Extinct Seed-Free Plants

Mechanisms for auxin synthesis have been documented in all embryophytes, as well as charophycean algae, and PIN genes have been identified in nearly all embryophyte lineages (Finet and Jaillais 2012; Bennett 2015). These suggest that the basic molecular machinery for the production and regulation of auxin evolved early in the history of embryophytes and was potentially inherited from charophyte ancestors. In angiosperms, the roles of auxin in vascular tissue development and organogenesis are well documented. However, polar auxin transport and the roles of auxin in development and the organization of sporophytes have been documented to a considerably lesser extent in the seed-free tracheophytes (Cooke et al. 2002). Below we review current data on polar auxin transport in seed-free plants.

10.3.1 Embryogenesis

All seed plants have bipolar body plans, defined by two major poles of growth in mature sporophytes: the shoot and root (Rothwell 1995; Tomescu 2011). These two poles of growth are derived directly from their cotyledonary embryos, which at maturity possess two dormant meristems that generate the shoot and rooting systems, respectively. In contrast, all seed-free plants have unipolar body plans, in which there is a single persistent pole of growth from which all organs originate in the mature sporophyte (Rothwell 1995). This unipolar body plan is derived from the non-cotyledonary embryos of seed-free plants. Although these embryos possess both a shoot and a root pole, growth at the root pole produces only an ephemeral embryonic root that is inactivated early in sporophyte development, and thus all subsequent sporophyte growth is derived from the shoot pole of the embryo (Tomescu 2011); the only potential exception occurs in the rhizomorphic lycophytes (Isoetales), in which the mature sporophyte exhibits bipolar growth derived from an early dichotomy of the unipolar embryo (Rothwell and Erwin 1985; Rothwell 1995).

Among seed plants, polar auxin transport is necessary for normal development of cotyledonary embryos, and treatment with auxin transport inhibitors disrupts embryo formation (Larsson et al. 2008; Liu et al. 1993). For seed-free plants the literature on auxin action in embryogeny is sparse, but experiments performed on embryos of the hydropteridalean (water fern) *Marsilea vestita* provide contrasting results (Poli 2005). Like in angiosperms, application of auxin biosynthesis inhibitors produced abnormal or abortive embryos. However, while application of auxin transport inhibitors (1-n-naphthylphthalamic acid [NPA] and 2,3,5-triiodobenzoic acid [TIBA]) resulted in some abnormal leaf morphology, the treatments did not disrupt embryogenesis. These results suggest that while auxin biosynthesis is necessary for normal embryo development, polar auxin transport may not play an integral

role in these processes in seed-free plants (Poli 2005) and may be related to the structural differences between cotyledonary and non-cotyledonary embryos.

10.3.2 Polar Auxin Transport in the Mature Sporophyte

Among seed-free plants, patterns of polar auxin transport have been documented in axial organs of *Selaginella*, as well as several fossil plants: *Paralycopodites*, an arborescent lycophyte; *Archaeopteris*, which belongs to a group of seed-free lignophytes called progymnosperms; and *Arthropitys*, an arborescent sphenophyte whose closest modern relative is *Equisetum* (Rothwell and Lev-Yadun 2005; Rothwell et al. 2008; Sanders et al. 2011). In *Selaginella*, like in angiosperms, polar auxin transport is basipetal in shoots and is sensitive to the auxin transport inhibitor NPA (Wochok and Sussex 1973; Sanders and Langdale 2013). Polar auxin transport has not been investigated in roots of *Selaginella*, but it has been studied in the rhizophore – an organ unique to *Selaginella* and for which homology is uncertain. Rhizophores are leafless axial organs that develop exogenously from meristems positioned in branching angles of shoots ("angle meristems") and grow downward, producing roots at their tip on reaching the soil (Jernstedt et al. 1994). Studies of *Selaginella* rhizophores indicate that polar auxin transport is acropetal (Wochok and Sussex 1974) and that rhizophore development can be altered by auxin (Cusick 1954; Wochok and Sussex 1976; Jernstedt et al. 1994; Sanders and Langdale 2013). For instance, excised rhizophores cultured on media supplemented with auxin exhibit normal growth and morphology. When no auxin is present in the medium, rhizophores sometimes produce leaves and continue to grow as shoots (Wochok and Sussex 1976; Sanders and Langdale 2013). Similarly, at least in some species, severing the shoot apical meristems above a branching point causes the nearest angle meristems to develop as shoots rather than as rhizophores; if the severed apices are replaced with an auxin source, angle meristems develop as rhizophores (Cusick 1954).

Among fossil plants, polar auxin transport has been inferred based on the position of circular or distorted tracheids ("auxin swirls") in the secondary xylem of woody organs. In modern plants, tracheids elongate and differentiate along polar auxin gradients (Berleth et al. 2000; Aloni 2010), and disruptions in the flow of auxin can produce unusual tracheid geometries. For instance, in shoots circular vessels form above tissue wounds in herbaceous seedlings (Sachs and Cohen 1982) and above axillary buds and branches in woody stems (Hejnowicz and Kurczyńska 1987; Lev-Yadun and Aloni 1990; Lev-Yadun 1996). Importantly, the position of auxin swirls is consistent with auxin flow polarity – in shoots, auxin moves in a basipetal direction from the apex, and thus auxin swirls form on the apical side of disrupting structures (e.g., branches, leaf traces). In woody shoots of extinct *Paralycopodites*, *Archaeopteris*, and *Sphenophyllum*, auxin swirls are found on the apical side of diverging branches and leaf traces, consistent with basipetal polar auxin transport in shoots, similar to extant lignophytes (Rothwell and Lev-Yadun

2005; Rothwell et al. 2008; Decombeix et al. 2010; Sanders et al. 2011). Roots of *Archaeopteris* exhibit distorted tracheids around branch roots, on the side facing the shoot system rather than the root apex, consistent with acropetal polar auxin transport, like in seed plant roots (A.-L. Decombeix, personal communication, 2015). Similarly, in the rooting system of *Paralycopodites* auxin swirls are formed above rootlet vascular traces, on the side facing the shoot (Sanders et al. 2011). Interestingly, the rooting system of *Paralycopodites* represents a shoot homolog highly modified for rooting (Rothwell and Erwin 1985; Rothwell and Tomescu 2017), termed a rhizomorph. Thus, although rhizomorphs lack root homology, they exhibit auxin transport polarity characteristic of roots.

10.3.3 Leaf Development

Auxin has several roles in angiosperm leaf development, including in leaf initiation, vascular (vein) differentiation, and phyllotaxis. Moreover, developing leaves function as the primary source of auxin in shoot apical meristems (Scarpella et al. 2006; Barkoulas et al. 2007). Among seed-free plants, auxin action in leaves has been studied in lycophytes (*Selaginella*), Filicales, and Hydropteridales. With respect to auxin production, polar auxin transport, and vascular differentiation, the data from seed-free plants are consistent with observations of angiosperms. In *Adiantum* and *Osmunda* (Filicales), auxin is produced in leaf pinnules (Steeves and Briggs 1960; White 1971) and is involved in vascular differentiation in *Osmunda* leaves (Steeves and Briggs 1960). Within leaf rachises, basipetal polar auxin transport was demonstrated in *Regnellidium* (Hydropteridales) and *Osmunda* (Filicales), as well as in the young leaves of *Pteris* (Filicales) (Albaum 1938; Steeves and Briggs 1960; Walters and Osborne 1979).

Few studies have addressed auxin action in leaf initiation and phyllotaxis, but experiments on *Selaginella* indicate that the role of auxin in these processes may differ for lycophytes. Application of auxin transport inhibitors to shoot apical meristems of *Selaginella* resulted in abnormal phyllotaxis and vascular development and eventual inactivation of the meristem (Sanders and Langdale 2013). In contrast, in comparable experiments on tomato shoots, leaf production ceased, but the meristem remained active, continuing to grow as a leafless axis (Reinhardt et al. 2000).

10.3.4 Root Development

Data on root initiation and development come primarily from culturing experiments involving auxin treatments, either using seedlings, excised structures, or callus cultures. Some of these studies were not explicitly investigating the effects of auxin on root production and growth but nevertheless provide some evidence for growth responses to auxin in ferns. For instance, when beads of auxin were applied to

cultured apices of *Matteuccia* (Filicales) to investigate changes in stem vasculature, adventitious roots were produced (Ma and Steeves 1992). Cultures of *Pteridium* roots supplemented with auxin showed reduced root elongation and increased lateral root production (Partanen and Partanen 1963). Roots were also induced from callus cultures of *Pteris* and *Nephrolepis* (Filicales) using auxin ratios comparable to those used for root induction from angiosperm callus (Bristow 1962; Byrne and Caponetti 1992).

Other studies were specifically aimed at investigating responses to auxin. In *Selaginella*, like in seed plants, auxin promoted root branching (Sanders and Langdale 2013) despite the fact that lycophyte roots branch apically – a mode of branching that is developmentally very different from root branching in euphyllophytes, which occurs via endogenous origin of branch roots from the pericycle (Esau 1965). In *Ceratopteris* (Filicales), auxin treatments inhibited root growth and, unlike in *Pteridium*, had no effect on branching (Hou et al. 2004). Similarly, auxin was shown to inhibit root growth in *Azolla* (Hydropteridales) (de Vries et al. 2016).

10.4 Similarities and Differences, Knowns and Unknowns

Summarizing the extent of current knowledge, although the literature on auxin in seed-free plant development is sparse as compared to seed plants, representatives from six out of ten major groups of seed-free plants have been studied (Table 10.1). Among extant taxa, polar auxin transport has been investigated in the shoot and rooting systems of *Selaginella* (Selaginellales), as well as in the leaves of *Regnellidium* (Hydropteridales) and *Osmunda* and *Pteris* (Filicales). The directionality of polar auxin transport has also been inferred for extinct woody taxa based on the position of auxin swirls in the secondary xylem of *Paralycopodites* (Isoetales), *Archaeopteris* (progymnosperm), and *Arthropitys* (sphenopsid). Roles for auxin in leaf and root development have further been demonstrated for filicalean (*Pteris*, *Nephrolepis*, *Ceratopteris*, *Pteridium*, *Matteuccia*) and hydropteridalean ferns (*Azolla*), as well as in *Selaginella*. Several general conclusions can be drawn from this survey of polar auxin transport in living and extinct seed-free plants.

- The basic directional patterns of polar auxin transport appear to be shared by all tracheophytes: basipetal transport in negatively gravitropic shoots and acropetal transport in positively gravitropic rooting systems, irrespective of the homology of those rooting systems (e.g., Wochok and Sussex 1974; Sanders et al. 2011; Sanders and Langdale 2013).
- PIN-mediated polar auxin transport regulation of branching is likely conserved across vascular plants (Harrison 2016).
- The role of auxin in vascular differentiation is conserved (e.g., Steeves and Briggs 1960; Rothwell and Lev-Yadun 2005; Rothwell et al. 2008; Lucas et al. 2013); at least in stems and leaves, vascular tissue development is similarly regulated by polar auxin transport in lycophytes and euphyllophytes (Sanders and Langdale 2013).

Table 10.1 Summary of literature on polar auxin transport (PAT) and auxin action in seed-free plant development

Taxon	Available data	Extant/ fossil	References
Lycopodiales	Not documented	–	
Selaginellales	Stem PAT Rhizophore PAT Leaf primordia & development	Extant	Wochok and Sussex (1973, 1974), Sanders and Langdale (2013)
Isoetales	Stem PAT Rhizomorph PAT	Fossil	Sanders et al. (2011)
Psilotum	Not documented	–	
Ophioglossales	Not documented	–	
Marattiales	Not documented	–	
Filicales	Leaf PAT Leaf development Root initiation and branching	Extant	Steeves and Briggs (1960), Bristow (1962), Partanen and Partanen (1963), White (1971), Walters and Osborne (1979), Byrne and Caponetti 1992, Ma and Steeves 1992, Hou et al. 2004
Hydropteridales	Leaf PAT Root branching Embryogeny	Extant	Walters and Osborne 1979, Poli 2005, de Vries et al. 2016
Sphenopsids	Stem PAT	Fossil	Rothwell et al. (2008)
Progymnosperms	Stem PAT Root PAT	Fossil	Rothwell and Lev-Yadun (2005), A.-L. Decombeix, "personal communication"

- Like in seed plants, leaves are a source of auxin in ferns, as suggested by basipetal polar auxin transport in the leaf rachises of *Osmunda* and *Regnellidium* (Steeves and Briggs 1960; Walters and Osborne 1979) and in young leaves of *Pteris* sporelings (Albaum 1938).
- Auxin-associated mechanisms that regulate apical meristematic growth in shoots are different between lycophytes and seed plants (Sanders and Langdale 2013). Specifically, the role of auxin in leaf initiation and meristem maintenance appears to be different in lycophytes and seed plants: whereas in *Selaginella* disruption of polar auxin transport leads to abnormal phyllotaxis, abnormal leaf vascular development, and loss of meristematic activity (Sanders and Langdale 2013), in tomato, the same treatment shuts down leaf production, but meristematic activity is maintained (Reinhardt et al. 2000).

- In ferns (e.g., *Pteris*, *Nephrolepis*), ratios of auxin and other hormones similar to those used for the same purpose in seed plants can induce root or shoot formation in callus culture (Bristow 1962; Byrne and Caponetti 1992).
- There is conflicting evidence for the role of auxin in promoting root initiation and root branching in seed-free plants. In some ferns (*Pteridium*, *Matteuccia*), auxin promotes lateral root production and adventitious root formation (Partanen and Partanen 1963; Ma and Steeves 1992). High auxin concentrations also induce root formation in *Pteris* and *Nephrolepis* callus (Bristow 1962; Byrne and Caponetti 1992), and auxin promotes root branching in *Selaginella* (Sanders and Langdale 2013). In contrast, auxin inhibits root growth in *Ceratopteris* and *Azolla*, leads to reduced root elongation in *Pteridium*, and also appears to have no effect on root branching of *Ceratopteris* (Partanen and Partanen 1963; Hou et al. 2004; de Vries et al. 2016).

Accordingly, auxin plays fundamental and broadly similar roles in tracheophyte development, but there remain significant gaps in our understanding of the variation that exists between and within major seed-free lineages. For instance, while in-depth studies of auxin transport have been conducted in *Selaginella*, comparable studies of other lycophytes are lacking. Consequently, it is unclear whether the differences in the response of *Selaginella* shoot apices to auxin inhibition, as compared to similar experiments in angiosperms, are characteristic of all lycophytes. Auxin production and transport have also never been investigated in *Psilotum*, which is the only extant tracheophyte exhibiting a simple body plan that lacks roots and leaves, like the early land plants (Stewart and Rothwell 1993; Tomescu 2011). Similarly, very little is known about auxin transport in *Equisetum*.

Deeper understanding of these developmental and physiological processes, and how such processes are reflected in anatomy, has important implications for understanding morphological evolution among plants. Owing to the central role of auxin in root and leaf development, auxin is particularly relevant to questions on the origins of these organs. This raises the possibility that changes in auxin signaling underlie major transitions in the body plans of land plants (Cooke et al. 2004; Finet and Jaillais 2012; Bennett 2015), such as the transition from the simple body plans of early tracheophytes to complex morphologies characterized by leaves and roots. Because roots and leaves evolved independently multiple times among tracheophytes (Boyce 2005a, 2005b, 2010; Langdale 2008; Sanders et al. 2009; Tomescu 2009; Harrison 2017), understanding how development differs between major lineages is imperative in refining the evolution of development and morphological evolution in plants.

The long and complex evolutionary history of plants, as well as the spectrum of morphological diversity documented in the fossil record, means that answers to these questions must come from reciprocal illumination, in which morpho-anatomical features of extant plants are used to understand the development and morphology of extinct plants, and where patterns observed in the fossil record form the basis of evolutionary hypotheses for explaining traits of morphology and anatomy in extant plants. This has been done extensively in vertebrate macroevolution

(e.g., Zhu and Ahlberg 2004; Shubin 2008; Nakamura et al. 2016), but comparable examples for plants are exceptionally rare (Tomescu et al. 2017; Rothwell and Tomescu 2017).

For studying the evolution of development in plants, recognition of anatomical fingerprints of developmental processes that can be identified in fossils (Rothwell et al. 2014) is central to a reciprocal illumination approach. Auxin is particularly interesting in the context of developmental fingerprints because (1) auxin has a demonstrated role in patterning vascular tissues (e.g., Sachs 1969, 1981; Aloni 1995; Mattsson et al. 1999; Berleth et al. 2000; Dengler 2006; Dettmer et al. 2009; Sawchuk and Scarpella 2013; Lucas et al. 2013) and (2) because tracheary elements of the xylem have some of the highest preservation potential of all plant cells. To illustrate this point, we present two examples in which a reciprocal illumination approach utilizing developmental fingerprints of polar auxin transport can be used to better understand the evolution of leaves and rooting systems among lycophytes and to formulate testable hypotheses for future studies.

10.5 Is Auxin Involved in the Evolution of Lycophyte Leaves?

Lycophyte leaves, sometimes referred to as microphylls (Tomescu 2009), are morphologically simple and typically contain a single vein. Although there is abundant evidence that lycophyte leaves evolved independently from those of euphyllophytes (Kenrick and Crane 1997; Boyce and Knoll 2002; Friedman et al. 2004; Langdale 2008; Boyce 2010; Harrison 2017), their evolutionary origins are unresolved (Kenrick and Crane 1997; Tomescu 2009). Two prevailing hypotheses posit that lycophyte leaves evolved through modification of pre-existing structures (e.g., Stewart 1964; Stewart and Rothwell 1993; Kenrick and Crane 1997). (1) The enation hypothesis (Bower 1935; Banks 1968; Gensel 1975) proposes that leaves arose through the vascularization of enations – lateral flap-like appendages lacking vasculature and regular phyllotaxis, which are seen in many extinct basal tracheophytes, as well as in extant *Psilotum*. (2) The sterilization hypothesis (Kenrick and Crane 1997) proposes that leaves evolved through the sterilization of sporangia. [A third hypothesis – associated with Zimmermann's (1938, 1952) telome theory – proposed lycophyte leaf evolution by reduction of more complex, branched structures; Stewart and Rothwell (1993)]. There is some evidence supporting each of the two prevailing hypotheses, from developmental genetics and the fossil record.

Studies on comparative gene expression reveal that Class III HD-Zip genes are expressed in both leaf primordia and sporangium primordia in *Selaginella*. This has been interpreted as support for the origin of lycophyte leaves through the sterilization of sporangia (Vasco et al. 2016), with an implicit assumption of homology of leaves and sporangia. However, it is also possible that the shared expression of HD-Zip III genes in sporangia and leaf primordia is not directly relevant to

leaf-sporangium homology (Rothwell and Tomescu 2017). HD-Zip III genes also have a role in the vascular tissue development in all tracheophytes (Floyd and Bowman 2006, 2010; Prigge and Clark 2006), and this role could have evolved after duplication of the ancestral HD-Zip III, which regulated sporangium development. Therefore, it is possible that HD-Zip III expression patterns in lycophyte leaves 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 support of the enation hypothesis, the basal lycophyte *Asteroxylon* possesses leaflike appendages exhibiting regular phyllotaxis, but which have a vascular strand that terminates at the base of the leaf and does not extend into the lamina (Lyon 1964). The partial vascularization of these appendages in *Asteroxylon* is thought to reflect an intermediate stage in the evolution of leaves (Banks 1968; Gensel 1975). However, *Asteroxylon* is coeval with and even younger than lycophytes possessing fully vascularized leaves, such as *Sengelia* (Matsunaga and Tomescu 2016), which raises the question of whether *Asteroxylon* is truly an evolutionary intermediate or simply possessed an unusual morphology.

Clues to the nature of lateral appendages in *Asteroxylon* may come from studies of auxin in extant seed-free plants. Experiments on *Coleus* shoot apices demonstrated that excising leaf primordia, thereby terminating the basipetal flux of auxin from the leaf, results in arrested development of the leaf trace (Wangermann 1967). Similarly, treatment of *Selaginella* shoot apices with auxin transport inhibitors results in abnormal phyllotaxis and incomplete development of leaf traces (Sanders and Langdale 2013). In this context, the morphology of *Asteroxylon* could be addressed in terms of auxin physiology. We can hypothesize that the incomplete vascularization of lateral appendages in *Asteroxylon* is the result of a weak or transient flux of auxin from the primordia of these appendages. In extant plants, vascular tissues of leaf traces mature acropetally, from the stem stele toward the leaf base and toward the leaf tip (opposite the transport direction of auxin) (Esau 1965; Dengler 2001). If this were also the case in *Asteroxylon*, a transient auxin flux that ceases before differentiation of vascular tissues along the entire length of lateral appendages could result in a vascular trace that is differentiated into conducting tissues only over part of its entire potential length, at the base.

A closer look at well-preserved *Asteroxylon* specimens from the Rhynie chert has shown that the vascular trace departing from the stele toward the base of the lateral appendage continues into the appendage as a strand of narrower, elongated cells (Hueber 1992; Edwards 1994) – cell patterning consistent with procambial identity and, thus, polar auxin transport. These strands support a developmental model in which basipetal polar auxin transport ceases prior to differentiation of tracheary elements from procambium inside lateral appendages, resulting in the differentiation of those procambial cells as a strand of elongated parenchyma rather than as vascular tissue. This developmental hypothesis could be tested using leaf excision and auxin inhibition experiments in lycopodialean lycophytes (e.g., *Lycopodium*, *Huperzia*), which are morphologically much more similar to *Asteroxylon* than *Selaginella*. Developmental transitions between enations and

leaves could potentially be further explored in *Psilotum*, which, while not a lycophyte, is possibly the only extant seed-free plant bearing enations (Stewart and Rothwell 1993; Tomescu 2011).

Presently, there is not enough data supporting either of the hypotheses for leaf evolution in lycophytes. However, we note that the two morphological characters separating leaves from enations are regular arrangement (phyllotaxis) and vascularization, both of which are regulated at least in part by auxin. In proposing the sterilization hypothesis, Kenrick and Crane (1997) pointed out that the enations of basal lycophytes lacked regular phyllotactic patterns, which rendered the enation hypothesis an unsatisfying explanation for the origin of leaves among lycophytes. While this is indeed true, experiments on tomato plants suggest that regular phyllotaxis and formation of leaf vasculature are developmentally related, at least in seed plants – laser ablation of the incipient midvein, which caused an accumulation of auxin in the leaf primordium, resulted in irregular positioning of subsequent leaf primordia (Deb et al. 2015). If this developmental relationship between phyllotaxis and the formation of leaf vasculature is borne out in future studies, we can speculate that regular phyllotaxis and leaf vascularization may be evolutionarily coupled and the two characters might be expected to emerge concurrently in evolutionary time without a morphological intermediate. However, these relationships would need to be explicitly tested for lycophytes, since numerous studies hint at subtle differences in lycophyte and euphyllophyte development. For instance, a study of *Lycopodium* by Gola et al. (2007) found evidence for uncoupling between leaf phyllotaxis and stem vasculature. This suggests limited developmental interaction between stem and leaf vasculature, unlike such correlations documented in the euphyllophytes (e.g., Wardlaw 1944, 1946; Meicenheimer 1986; Ma and Steeves 1992; Kwiatkowska 1992).

10.6 Polar Auxin Transport and Lycophyte Rooting Structures

The lycophytes (Lycophytina Kenrick and Crane 1997) comprise six major lineages. Three of these are represented in modern floras by clubmosses (Lycopodiales), *Selaginella* (Selaginellales), and *Isoetes* (Isoetales), but the majority of lycophyte diversity is extinct and known only from their long and extensive fossil record, which stretches at least 400 million years, into the Early Devonian (Gensel and Andrews 1984; Gensel 2008). Extinct lineages include Zosterophyllopsida (Kenrick and Crane 1997), a monophyletic group of stem lycophytes (hereafter zosterophylls); Drepanophycales, which combine characters seen in zosterophylls with characters of crown group lycophytes (Gensel and Andrews 1984; Matsunaga and Tomescu 2017); the poorly understood Protolepidodendrales, a clade of heterosporous ligulate lycophytes; and the rhizomorphic lycophytes (Lepidodendrales,

Pleuromeiales, and Isoetales) – a derived clade containing many arborescent taxa (Pigg and Rothwell 1983; Pigg 2001).

The body plans of living and extinct lycophytes encompass a significant amount of morphological diversity, particularly with respect to rooting systems. Starting with the most basal lineages, zosterophylls had simple body plans lacking true leaves and roots. Instead, they possessed photosynthetic stems and in some cases specialized axes with inferred downward growth for rooting (Walton 1964; Gensel et al. 2001; Hao et al. 2007). Drepanophycalean lycophytes exhibit the earliest examples of roots in the fossil record (Schweitzer and Giesen 1980; Rayner 1984; Li and Edwards 1995; Matsunaga and Tomescu 2016, 2017). Among them, *Drepanophycus* bore fine dichotomously branching roots on rhizomatous portions of the shoot system (Schweitzer 1980; Rayner 1984; Li et al. 2000). Similarly, rhizomatous stems of *Asteroxylon* produced downward-growing branching axes, which are interpreted as having exogenous origin (Kidston and Lang 1920), in contrast to true roots, which originate endogenously (Raven and Edwards 2001; Kenrick and Strullu-Derrien 2014). Another drepanophycalean, *Sengelia*, exhibits a more complex rooting system, consisting of downward-growing stems bearing lateral branching structures interpreted as roots (Matsunaga and Tomescu 2016, 2017).

Two different types of rooting structures are seen among the extant Lycopodiales. Nearly all species produce adventitious roots in a mode similar to that of most seed-free euphyllophytes (e.g., *Lycopodium*, *Huperzia*; Gifford and Foster 1989). However, *Phylloglossum* has a body plan comprised of a single reduced shoot and a bulbous, downward-growing stem called a tuber that functions in nutrient storage and perennation. Roots are borne laterally at the junction between the shoot and tuber and have been described as arising exogenously (Bower 1885). In Selaginellales (*Selaginella*), roots are borne at the apex of rhizophores, which are themselves rooting organs of uncertain homology (Jernstedt et al. 1994).

Living and extinct rhizomorphic lycophytes exhibit a body plan wherein the rooting system is comprised of an organ termed a "rhizomorph." Studies of comparative anatomy and the fossil record have demonstrated the rhizomorph as a shoot homolog, with appendages termed "rootlets" corresponding to highly modified leaves (Rothwell and Erwin 1985; Rothwell and Tomescu 2017). Rhizomorphs are either cormose, forming a bulbous or lobed base of the plant, with numerous radiating rootlets seen in taxa like *Isoetes*, *Pleuromeia*, and *Chaloneria* (Stewart and Rothwell 1993), or are elongated and often highly branched as in Lepidodendrales (e.g., Frankenberg and Eggert 1969). In some cormose lycophytes, the rootlets lack the rootlike morphology of other members of the clade, and the corm instead bears laminar leaves that grow straight down into the sediment (e.g., Cúneo and Andreis 1983; Jasper and Guerra-Sommer 1999), further illustrating their shoot homology. To date the rooting structures of protolepidodendraleans are unknown.

When considering the rooting structures of lycophytes, one salient pattern emerges despite their seemingly wide morphological disparity – in every major lineage we can recognize rooting organs derived from stems or undifferentiated axes. These include the rooting axes of some zosterophylls, the root-bearing stems of *Sengelia* and the rootlike axes of *Asteroxylon* (Drepanophycales), the tuber of

Phylloglossum, the *Selaginella* rhizophore (the homology of which is unresolved), and the isoetalean rhizomorph. Importantly, all these organs exhibit either positive gravitropism or downward growth orientation indicative of positive gravitropic responses (Kidston and Lang 1920; Bower 1885; Edwards 1994; Matsunaga and Tomescu 2017; Matsunaga et al. 2017). This is very different from what we see in euphyllophytes, among which rooting systems are almost universally comprised only of roots; when stems do form part of the rooting system, they are typically rhizomatous and lack positive gravitropism. As discussed above, studies of extant plants demonstrate that auxin has important roles in tropic responses. Moreover, polar auxin transport is acropetal in roots of all plants for which it has been studied. This raises the question of whether aspects of auxin physiology that characterize euphyllophyte roots are shared by the diverse types of rooting structures of lycophytes.

To date patterns of auxin transport have been studied in only two lycophytes: *Selaginella* (Wochok and Sussex 1973, 1974; Sanders and Langdale 2013) and *Paralycopodites* (Sanders et al. 2011), an extinct arborescent lepidodendralean. Despite such sparse taxonomic sampling, these data on lycophytes are intriguing. In *Selaginella* rhizophores and in the rhizomorph of *Paralycopodites*, polar auxin transport is acropetal like in the roots of seed plants. Rhizomorphs have been demonstrated as shoot homologs (Rothwell and Erwin 1985), and while the homology of the rhizophore is unresolved, their exogenous development and capacity to produce leaves under certain experimental conditions (Cusick 1954; Wochok and Sussex 1976; Sanders and Langdale 2013) argue strongly against their interpretation as roots. The downward growth of rhizophores and the presence of acropetal polar auxin transport are the primary features that have been used to argue for root homology (Wochok and Sussex 1974). However, in the context of the auxin transport pattern documented in the *Paralycopodites* rhizomorph, and alongside it, the acropetal polar auxin transport of the *Selaginella* rhizophore suggests that rather than being a defining feature of roots, acropetal polar auxin transport is related to a more general growth habit characterized by downward growth and rooting function. Under this hypothesis we would also expect the rooting structures of other lycophytes to exhibit acropetal polar auxin transport, regardless of their homology. While this is relatively easy to ascertain experimentally for extant taxa like *Phylloglossum* and *Isoetes*, testing this hypothesis for fossil lycophytes presents a significant challenge. This is where anatomical fingerprints of development are essential for understanding the evolution of development in deep time.

10.7 Anatomical Fingerprints of Polar Auxin Transport

We have already briefly mentioned one example of an anatomical fingerprint for polar auxin transport, which has been applied to studies of plant development in the fossil record: auxin swirls in the secondary xylem of woody plants. Auxin swirls form in the secondary xylem of stems and roots in positions upstream (along the

polar flow of auxin) of obstructions such as branches, leaf traces, and root traces (Lev-Yadun and Aloni 1990). Consequently, when looking at fossilized wood, if the direction of the apex is known and structures like branches or vascular traces are present in the secondary xylem, the polarity of auxin transport can be inferred. Auxin swirls have been documented in fossilized secondary xylem of seed plants, as well as arborescent lycophytes, sphenopsids, and progymnosperms, in which secondary growth evolved independently, thus demonstrating shared mechanisms in the developmental regulation of secondary growth in shoots and rooting systems (Rothwell and Lev-Yadun 2005; Rothwell et al. 2008). This anatomical fingerprint was used to infer the direction of polar auxin transport in the rhizomorph of the lepidodendralean lycophyte *Paralycopodites* (Sanders et al. 2011). However, because auxin swirls only form in secondary xylem, they cannot be used to infer the polarity of auxin transport in herbaceous basal lycophytes. Testing this hypothesis requires different anatomical fingerprints.

In a recent anatomical study of *Selaginella* branching (Matsunaga et al. 2017), we observed an anatomical feature that may serve as a fingerprint for auxin flow redirection at branching points and which is relevant to herbaceous basal lycophytes that produce rooting axes through branching of the aboveground axial system (e.g., some Drepanophycales and zosterophylls). Because tracheids elongate and mature along auxin gradients (Sachs 1981; Sachs and Cohen 1982; Lev-Yadun and Aloni 1990), their orientation can be used as a marker of the pathways of polar auxin transport. In *Selaginella*, the shape and position of tracheids reveals changes in the polarity of auxin flow from basipetal in shoots to acropetal in the rhizophore. Observations in cleared *Selaginella* shoots show that in shoots without rhizophores, at branching points tracheids from each branch converge smoothly and run parallel to one another below the bifurcation. In contrast, at branching points with attached rhizophores, some tracheids curve from each branch into the rhizophore base, consistent with an auxin flux that enters the rhizophore from the shoot. This pattern was observed in all nine *Selaginella* species studied, regardless of stele type (Matsunaga et al. 2017). Importantly, it was also observed in incipient and young rhizophores that had not yet produced roots, indicating that these tracheids did not form later in development as a consequence of water transport and distribution into the shoot system after the establishment of roots.

Curved tracheids like those documented in *Selaginella* could be searched for in fossil specimens that exhibit exogenously produced rooting structures, potentially providing evidence for developmental processes related to polar auxin transport in basal lycophytes. However, the utility of this anatomical feature for understanding development in early vascular plants is yet to be tested and requires exceptional preservation of anatomy. Nevertheless, both examples of anatomical signatures of polar auxin transport discussed here, auxin swirls and curved tracheids, demonstrate that some aspects of auxin action are recorded in anatomy. This is certainly promising, but more data are sorely needed on auxin physiology in lycophytes and other seed-free plants.

10.8 Conclusions and Future Directions

In this chapter we reviewed current knowledge of polar auxin transport in seed-free plant development and suggested areas where this knowledge can be used to better understand development in the fossil record. We exemplify a reciprocal illumination approach to address questions on morphological evolution, in proposing developmental models that provide testable hypotheses for understanding leaf evolution and rooting systems among lycophytes. However, despite significant advances in methods for studying development and physiology over the last few decades, our understanding of seed-free plant development lags far behind that of seed plants, and there remains a long road ahead toward a comprehensive understanding of auxin action across all tracheophyte lineages. In 2002, Cooke and collaborators undertook an exhaustive review of the literature on auxin in plant development. Now, more than 15 years later, considering the exciting and promising advances in our understanding of auxin in seed plant development, it is striking how little comparable progress has been made in seed-free plants – only a handful of studies, since, have gathered new data relevant to these questions (e.g., Hou et al. 2004; Sanders and Langdale 2013; de Vries et al. 2016).

Further progress toward understanding the role of auxin in the evolution of development and morphology in plants must come from continued study of seed-free lineages. Many major aspects of morphological evolution and homology across tracheophyte body plans are still unresolved (Rothwell and Tomescu 2017), and we are still far from a detailed understanding of the genetic regulation of development in all vascular plant lineages (Tomescu 2011). However, even just understanding the different roles of polar auxin transport throughout development, from the embryo to the mature sporophyte, in all the major tracheophyte lineages would help us make great strides toward these goals. For this to happen, work on seed-free plants must continue to be conducted and funded, and non-angiosperm model systems need to be developed for understanding developmental genetics. Incremental but necessary progress can also be made through basic research aimed at replicating results of older studies of auxin physiology, with treatments designed to tease apart different aspects of auxin action (e.g., controlling for auxin diffusion or testing responses to auxin inhibition), or by empirically confirming in each major lineage the presence of general patterns of polar auxin transport documented from seed plants.

While there is still a lot we do not know about developmental mechanisms in seed-free plants, a perspective centered on the deep evolutionary history and fossil record of plants has important potential for progress toward better understanding the evolution of plant form. This is not a novel idea – two great plant morphologists of the twentieth century, Frederick Orpen Bower and Wilson Nichols Stewart, long advocated such a view (Bower 1935; Stewart 1964). In this context, anatomical fingerprints of developmental processes are essential both for testing current evolutionary hypotheses and for observing patterns in living and extinct taxa that lead to the formation of novel hypotheses. Discovery and application of anatomical fingerprints requires not only an understanding of molecular and physiological processes

but also of how these processes are reflected phenotypically in terms of anatomy and morphology. Because the evolutionary history of plants stretches hundreds of millions of years and includes numerous extinct lineages, inclusion of fossils in studies of phylogeny and evo-devo is imperative for obtaining increased resolution. For some evolutionary questions, particularly those pertaining to deep nodes in plant phylogeny, the problem of missing taxa cannot be overcome by sampling only extant representatives (Rothwell and Nixon 2006). Progress in many aspects of plant evolutionary biology must therefore come from studies that incorporate fossils, and doing so requires the continued study of plant anatomy and morphology, whether it is for building rigorous phylogenetic matrices or for understanding the biology of extinct organisms. The approaches and perspectives advocated here, as well as others that integrate data from the fossil record with analytical and methodological innovations (e.g., Bateman et al. 2006; Smith et al. 2009; Escapa and Catalano 2013; Rudall et al. 2013; Bomfleur et al. 2014, 2017; Hetherington and Dolan 2016; Hetherington et al. 2016; Fujinami et al. 2017; Wilson et al. 2017), provide an exciting frontier in plant evolutionary biology and move us closer toward understanding the processes that have generated the immense diversity of plant form through time.

References

Aida M, Beis D, Heidstra R et al (2004) The *PLETHORA* genes mediate patterning of the *Arabidopsis* stem cell niche. Cell 119:109–120

Albaum HG (1938) Inhibition due to growth hormones in fern prothallia and sporophytes. Am J Bot 25:124–133

Aloni R (1995) The induction of vascular tissues by auxin and cytokinin. In: Davies PJ (ed) Plant hormones: physiology, biochemistry, and molecular biology, 2nd edn. Kluwer Academic Publishers, Dordrecht, pp 531–546

Aloni R (2010) The induction of vascular tissues by auxin. In: Davies PJ (ed) Plant hormones. Springer, Dordrecht, pp 485–506

Banks HP (1968) The early history of land plants. In: Drake ET (ed) Evolution and environment. Yale University Press, New Haven, pp 73–107

Barkoulas M, Galinha C, Grigg SP et al (2007) From genes to shape: regulatory interactions in leaf development. Curr Opin Plant Biol 10:660–666

Barkoulas M, Hay A, Kougioumoutzi E et al (2008) A developmental framework for dissected leaf formation in the *Arabidopsis* relative *Cardamine hirsuta*. Nat Genet 40:1136–1141

Bateman RM, Hilton J, Rudall PJ (2006) Morphological and molecular phylogenetic context of the angiosperms: contrasting the 'top-down' and 'bottom-up' approaches used to infer the likely characteristics of the first flowers. J Exp Bot 57:3471–3503

Benjamins R, Scheres B (2008) Auxin: the looping star in plant development. Annu Rev Plant Biol 59:443–465

Benková E, Michniewicz M, Sauer M et al (2003) Local, efflux-dependent auxin gradients as a common module for plant organ formation. Cell 115:591–602

Bennett T (2015) PIN proteins and the evolution of plant development. Trends Plant Sci 20:498–507

Bennett T, Hines G, Leyser O (2014) Canalization: what the flux? Trends Genet 30:41–48

Berleth T (2001) Top-down and inside-out: directionality of signaling in vascular and embryo development. J Plant Growth Regul 20:14–21

Berleth T, Sachs T (2001) Plant morphogenesis: long-distance coordination and local patterning. Curr Opin Plant Biol 4:57–62

Berleth T, Mattsson J, Hardtke CS (2000) Vascular continuity and auxin signals. Trends Plant Sci 5:387–393

Beveridge CA, Mathesius U, Rose RJ et al (2007) Common regulatory themes in meristem development and whole-plant homeostasis. Curr Opin Plant Biol 10:44–51

Björklund S, Antti H, Uddestrand I et al (2007) Cross-talk between gibberellin and auxin in development of *Populus* wood: gibberellin stimulates polar auxin transport and has a common transcriptome with auxin. Plant J 52:499–511

Blilou I, Xu J, Wildwater M et al (2005) The PIN auxin efflux facilitator network controls growth and patterning in *Arabidopsis* roots. Nature 433:39–44

Bomfleur B, McLoughlin S, Vajda V (2014) Fossilized nuclei and chromosomes reveal 180 million years of genomic stasis in royal ferns. Science 343:1376–1377

Bomfleur B, Grimm GW, McLoughlin S (2017) The fossil Osmundales (Royal Ferns) – a phylogenetic network analysis, revised taxonomy, and evolutionary classification of anatomically preserved trunks and rhizomes. PeerJ 5:e3433

Bower FO (1885) On the development and morphology of *Phylloglossum drummondii*. Phil Trans R Soc Lond 176:665–678

Bower FO (1935) Primitive land plants, also known as the Archegoniatae. Macmillan, London

Boyce CK (2005a) Patterns of segregation and convergence in the evolution of fern and seed plant leaf morphologies. Paleobiology 31:117–140

Boyce CK (2005b) The evolutionary history of roots and leaves. In: Holbrook NM, Zwieniecki MA (eds) Vascular transport in plants. Elsevier, Amsterdam, pp 479–499

Boyce CK (2010) The evolution of plant development in a paleontological context. Curr Opin Plant Biol 13:102–107

Boyce CK, Knoll AH (2002) Evolution of developmental potential and the multiple independent origins of leaves in Paleozoic vascular plants. Paleobiology 28:70–100

Bristow MJ (1962) The controlled in vitro differentiation of callus derived from a fern, *Pteris cretica* L., into gametophytic or sporophytic tissues. Dev Biol 4:361–375

Byrne TE, Caponetti JD (1992) Morphogenesis in three cultivars of Boston fern. III. Callus production and plantlet differentiation from cell suspensions. Am Fern J 82:12–22

Campbell L, Turner S (2017) Regulation of vascular cell division. J Exp Bot 68:27–43

Cooke TJ, Poli D, Sztein AE et al (2002) Evolutionary patterns in auxin action. Plant Mol Biol 49:319–338

Cooke TJ, Poli D, Cohen JD (2004) Did auxin play a crucial role in the evolution of novel body plans during the late Silurian – early Devonian radiation of land plants? In: Hemsley AR, Poole I (eds) The evolution of plant physiology. From whole plants to ecosystems. Elsevier, Amsterdam, pp 85–107

Cúneo NR, Andreis RR (1983) Estudio de un bosque de licofitas en la Formación Nueva Lubecka, Pérmico de Chubut, Argentina. Implicancias paleoclimáticas y paleogeográficas. Ameghiniana 20:132–140

Cusick F (1954) Experimental and analytical studies of pteridophytes. XXV. Morphogenesis in *Selaginella willdenowii* Baker. II. Angle-meristems and angle-shoots. Ann Bot 18:171–181

De Rybel B, Moller B, Yoshida S et al (2013) A bHLH complex controls embryonic vascular tissue establishment and indeterminate growth in *Arabidopsis*. Dev Cell 24:426–437

de Vries J, Fischer AM, Roettger M et al (2016) Cytokinin-induced promotion of root meristem size in the fern *Azolla* supports a shoot-like origin of euphyllophyte roots. New Phytol 209:705–720

Deb Y, Marti D, Frenz M et al (2015) Phyllotaxis involves auxin drainage through leaf primordia. Development 142:1992–2001

Decombeix A-L, Taylor EL, Taylor TN (2010) Epicormic shoots in a Permian gymnosperm from Antarctica. Int J Plant Sci 171:772–782

Dengler NG (2001) Regulation of vascular development. J Plant Growth Regul 20:1–13

Dengler NG (2006) The shoot apical meristem and development of vascular architecture. Can J Bot 84:1660–1671
Dettmer J, Elo A, Helariutta Y (2009) Hormone interactions during vascular development. Plant Mol Biol 69:347–360
Durbak A, Yao H, McSteen P (2012) Hormone signaling in plant development. Curr Opin Plant Biol 15:92–96
Edwards D (1994) Toward an understanding of pattern and process in the growth of early vascular plants. In: Ingram DS, Hudson A (eds) Shape and form in plants and fungi. Academic Press & Linnean Society, London, pp 39–59
Esau K (1965) Plant anatomy, 2nd edn. Wiley & Sons, New York
Escapa IH, Catalano SA (2013) Phylogenetic analysis of Araucariaceae: integrating molecules, morphology, and fossils. Int J Plant Sci 174:1153–1170
Fabregas N, Formosa-Jordan P, Confraria A et al (2015) Auxin influx carriers control vascular patterning and xylem differentiation in *Arabidopsis thaliana*. PLoS Genet 11:e1005183
Finet C, Jaillais Y (2012) AUXOLOGY: when auxin meets plant evo-devo. Dev Biol 369:19–31
Floyd SK, Bowman JL (2006) Distinct developmental mechanisms reflect the independent origins of leaves in vascular plants. Curr Biol 16:1911–1917
Floyd SK, Bowman JL (2010) Gene expression patterns in seed plant shoot meristems and leaves: homoplasy or homology? J Plant Res 123:43–55
Frankenberg JM, Eggert DA (1969) Petrified *Stigmaria* from North America: part I. *Stigmaria ficoides*, the underground portions of Lepidodendraceae. Palaeontographica B 128:1–47
Friedman WE, Moore RC, Purugganan MD (2004) The evolution of plant development. Am J Bot 91:1726–1741
Friml J, Vieten A, Sauer M et al (2003) Efflux-dependent auxin gradients establish the apical-basal axis of *Arabidopsis*. Nature 426:147–153
Fujinami R, Yamada T, Nakajima A et al (2017) Root apical meristem diversity in extant lycophytes and implications for root origins. New Phytol 215:1210–1220
Gensel PG (1975) A new species of *Sawdonia* with notes on the origin of microphylls and lateral sporangia. Am J Bot 136:50–62
Gensel PG (2008) The earliest land plants. Annu Rev Ecol Evol Syst 39:459–477
Gensel PG, Andrews HN (1984) Plant life in the Devonian. Praeger, New York
Gensel PG, Kotyk ME, Basinger JF (2001) Morphology of above- and belowground structures in early Devonian (Pragian–Emsian) plants. In: Gensel PG, Edwards D (eds) Plants invade the land. Columbia University Press, New York, pp 83–102
Gifford EM, Foster AS (1989) Morphology and evolution of vascular plants, 3rd edn. Freeman, New York
Gola EM, Jernstedt JA, Zagorska-Marek B (2007) Vascular architecture in shoots of early divergent vascular plants, *Lycopodium clavatum* and *Lycopodium annotinum*. New Phytol 174:774–786
Hao S, Xue J, Liu Z et al (2007) *Zosterophyllum* Penhallow around the Silurian-Devonian boundary of northeastern Yunnan, China. Int J Plant Sci 168:477–489
Harrison CJ (2016) Auxin transport in the evolution of branching forms. New Phytol 215:545. https://doi.org/10.1111/nph.14333
Harrison CJ (2017) Development and genetics in the evolution of land plant body plans. Philos Trans R Soc B 372:20150490
Hejnowicz Z, Kurczyńska EU (1987) Occurrence of circular vessels above axillary buds in stems of woody plants. Acta Soc Bot Pol 56:415–419
Hetherington AJ, Dolan L (2016) The evolution of lycopsid rooting structures: conservatism and disparity. New Phytol 215:538–544
Hetherington AJ, Dubrovski JG, Dolan L (2016) Unique cellular organisation in the oldest root meristem. Curr Biol 26:1629–1633
Hou G, Hill JP, Blancaflor EB (2004) Developmental anatomy and auxin response of lateral root formation in *Ceratopteris richardii*. J Exp Bot 55:685–693

Hueber FM (1992) Thoughts on the early lycopsids and zosterophylls. Ann Mo Bot Gard 79:474–499

Jasper A, Guerra-Sommer M (1999) Licófitas arborescentes in situ como elementos importantes na definição de modelos deposicionais (Formação Rio Bonito - Bacia do Paraná - Brasil). Pesq Geoci 26:49–58

Jernstedt JA, Cutter EG, Lu P (1994) Independence of organogenesis and cell pattern in developing angle shoots of *Selaginella martensii*. Ann Bot 74:343–355

Jones AM (1998) Auxin transport: down and out and up again. Science 282:2201–2202

Kasprzewska A, Carter R, Swarup R et al (2015) Auxin influx importers modulate serration along the leaf margin. Plant J 83:705–718

Kenrick P, Crane PR (1997) The origin and early diversification of land plants. Smithsonian Institution Press, Washington

Kenrick P, Strullu-Derrien C (2014) The origin and early evolution of roots. Plant Physiol 166:570–580

Kidston R, Lang WH (1920) On old red sandstone plants showing structure, from the Rhynie Chert Bed, Aberdeenshire. Part III. *Asteroxylon mackiei*, Kidston and Lang. Trans R Soc Edinb 52:643–680

Kierzkowski D, Nakayama N, Routier-Kierzkowska A-L et al (2012) Elastic domains regulate growth and organogenesis in the plant shoot apical meristem. Science 335:1096–1099

Kwiatkowska D (1992) The relationships between the primary vascular system and phyllotactic patterns of *Anagallis arvensis* (Primulaceae). Am J Bot 79:904–913

Lacombe B, Achard P (2016) Long-distance transport of phytohormones through the plant vascular system. Curr Opin Plant Biol 34:1–8

Langdale JA (2008) Evolution of developmental mechanisms in plants. Curr Opin Genet Dev 18:368–373

Larsson E, Sitbon F, Ljung K et al (2008) Inhibited polar auxin transport results in aberrant embryo development in Norway spruce. New Phytol 177:356–366

Lev-Yadun S (1996) Circular vessels in the secondary xylem of *Arabidopsis thaliana* (Brassicaceae). IAWA J 17:31–35

Lev-Yadun S, Aloni R (1990) Vascular differentiation in branch junctions of trees: circular patterns and functional significance. Trees 4:49–54

Leyser O (2011) Auxin, self-organisation, and the colonial nature of plants. Curr Biol 21:R331–R337

Li C-S, Edwards D (1995) A re-investigation of Halle's *Drepanophycus spinaeformis* Goepp. from the Lower Devonian of Yunnan Province, southern China. Bot J Linn Soc 118:163–192

Li C-S, Hueber FM, Hotton CL (2000) A neotype for *Drepanophycus spinaeformis* Göppert 1852. Can J Bot 78:889–902

Liu C-m, Xu Z-h, Chua N-h (1993) Auxin polar transport is essential for the establishment of bilateral symmetry during early plant embryogenesis. Plant Cell 5:621–630

Lucas WJ, Groover A, Lichtenberger R et al (2013) The plant vascular system: evolution, development and functions. J Integr Plant Biol 55:294–388

Lyon AG (1964) Probable fertile region of *Asteroxylon mackiei* K. and L. Nature 203:1082–1083

Ma Y, Steeves TA (1992) Auxin effects on vascular differentiation in ostrich fern. Ann Bot 70:277–282

Matsunaga KKS, Tomescu AMF (2016) Root evolution at the base of the lycophyte clade: insights from an early Devonian lycophyte. Ann Bot 117:585–598

Matsunaga KKS, Tomescu AMF (2017) An organismal concept for *Sengelia radicans* gen. et sp. nov. – morphology and natural history of an early Devonian lycophyte. Ann Bot 119:1097–1113

Matsunaga KKS, Cullen NP, Tomescu AMF (2017) Vascularization of the *Selaginella* rhizophore: anatomical fingerprints of polar auxin transport with implications for the deep fossil record. New Phytol 216:419–428

Mattsson J, Sung ZR, Berleth T (1999) Responses of plant vascular systems to auxin transport inhibition. Development 126:2979–2991

Meicenheimer RD (1986) Role of parenchyma in *Linum usitatissimum* leaf trace patterns. Am J Bot 73:1649–1664
Miyashima S, Sebastian J, Lee J-Y et al (2013) Stem cell function during plant vascular development. EMBO J 32:178–193
Moller B, Weijers D (2009) Auxin control of embryo patterning. Cold Spring Harb Perspect Biol 1:a001545
Nakamura T, Gehrke AR, Lemberg J et al (2016) Digits and fin rays share common developmental histories. Nature 537:225–228
Overvoorde P, Fukaki H, Beeckman T (2010) Auxin control of root development. Cold Spring Harb Perspect Biol 2:a001537
Partanen JN, Partanen CR (1963) Observations on the culture of roots of the bracken fern. Can J Bot 41:1657–1661
Pigg KB (2001) Isoetalean lycopsid evolution: from the Devonian to the present. Am Fern J 91:99–114
Pigg KB, Rothwell GW (1983) Megagametophyte development in the Chaloneriaceae fam. Nov., permineralized Paleozoic Isoetales (Lycopsida). Bot Gaz 144:295–302
Poli D (2005) The role of auxin on the evolution of embryo development and axis formation in land plants. PhD Dissertation, University of Maryland
Prigge MJ, Clark SE (2006) Evolution of the class III HD-Zip gene family in land plants. Evol Dev 8:350–361
Raven JA, Edwards D (2001) Roots: evolutionary origins and biogeochemical significance. J Exp Bot 52:381–401
Rayner RJ (1984) New finds of *Drepanophycus spinaeformis* Goppert from the lower Devonian of Scotland. Trans R Soc Edinb Earth Sci 75:353–363
Reinhardt D (2005) Phyllotaxis – a new chapter in an old story about beauty and magic numbers. Curr Opin Plant Biol 8:487–493
Reinhardt D, Mandel T, Kuhlemeier C (2000) Auxin regulates the initiation and radial position of plant lateral organs. Plant Cell 12:507–518
Reinhardt D, Pesce E-R, Stieger P et al (2003) Regulation of phyllotaxis by polar auxin transport. Nature 426:255–260
Robert HS, Grones P, Stepanova AN et al (2013) Local auxin sources orient the apical-basal axis in *Arabidopsis* embryos. Curr Biol 23:2506–2512
Rothwell GW (1995) The fossil history of branching: implications for the phylogeny of land plants. In: Hoch PC, Stephenson AG (eds) Experimental and molecular approaches to plant biosystematics. Missouri Botanical Garden, St. Louis, pp 71–86
Rothwell GW, Erwin DM (1985) The rhizomorph apex of *Paurodendron*: implications for homologies among the rooting organs of Lycopsida. Am J Bot 72:86–98
Rothwell GW, Lev-Yadun S (2005) Evidence of polar auxin flow in 375 million-year-old fossil wood. Am J Bot 92:903–906
Rothwell GW, Nixon KC (2006) How does the inclusion of fossil data change our conclusions about the phylogenetic history of euphyllophytes? Int J Plant Sci 167:737–749
Rothwell GW, Tomescu AMF (2017) Structural fingerprints of development at the intersection of evolutionary developmental biology and the fossil record. In: Nuño de la Rosa L, Müller GB (eds) Evolutionary developmental biology – a reference guide. Springer International Publishing, Cham (Switzerland) https://doi.org/10.1007/978-3-319-33038-9_169-1
Rothwell GW, Sanders H, Wyatt SE et al (2008) A fossil record for growth regulation: the role of auxin in wood evolution. Ann Mo Bot Gard 95:121–134
Rothwell GW, Wyatt SE, Tomescu AMF (2014) Plant evolution at the interface of paleontology and developmental biology: an organism-centered paradigm. Am J Bot 101:899–913
Rudall PJ, Hilton J, Bateman RM (2013) Several developmental and morphogenetic factors govern the evolution of stomatal patterning in land plants. New Phytol 200:598–614
Runions A, Tsiantis M, Prusinkiewicz P (2017) A common developmental program can produce diverse leaf shapes. New Phytol 216:401–418

Sabatini S, Beis D, Wolkenfelt H et al (1999) An auxin-dependent distal organizer of pattern and polarity in the *Arabidopsis* root. Cell 99:463–472
Sachs T (1969) Polarity and the induction of organized vascular tissues. Ann Bot 33:263–275
Sachs T (1981) The control of the patterned differentiation of vascular tissues. Adv Bot Res 9:151–262
Sachs T (1991) Pattern formation in plant tissues. Cambridge University Press, Cambridge
Sachs T, Cohen D (1982) Circular vessels and the control of vascular differentiation in plants. Differentiation 21:22–26
Sanders HL, Langdale JA (2013) Conserved transport mechanisms but distinct auxin responses govern shoot patterning in *Selaginella kraussiana*. New Phytol 198:419–428
Sanders H, Rothwell GW, Wyatt SE (2009) Key morphological alterations in the evolution of leaves. Int J Plant Sci 170:860–868
Sanders H, Rothwell GW, Wyatt SE (2011) Parallel evolution of auxin regulation in rooting systems. Pl Syst Evol 291:221–225
Sawchuk MG, Scarpella E (2013) Polarity, continuity, and alignment in plant vascular strands. J Integr Plant Biol 55:824–834
Sawchuk MG, Head P, Donner TJ et al (2007) Time-lapse imaging of *Arabidopsis* leaf development shows dynamic patterns of procambium formation. New Phytol 176:560–571
Scarpella E, Meijer AH (2004) Pattern formation in the vascular system of monocot and dicot plant species. New Phytol 164:209–242
Scarpella E, Marcos D, Friml J et al (2006) Control of leaf vascular patterning by polar auxin transport. Genes Dev 20:1015–1027
Schweitzer H-J, Giesen P (1980) Uber *Taeniophyton inopinatum*, *Protolycopodites devonicus* und *Cladoxylon scoparium* aus dem Mitteldevon von Wuppertal. Palaeontographica B 173:1–25
Schweitzer H-J (1980) Über Drepanophycus spinaeformis Göppert. Bonner Paläobotan Mitt (Selbstverl d Paläobotan Abt d Inst für Paläontologie d Univ Bonn) 7:1–29
Shubin NH (2008) Your inner fish. Vintage Books, New York
Smith RP, Guyomarc'h S, Mandel T et al (2006) A plausible model of phyllotaxis. Proc Natl Acad Sci U S A 103:1301–1306
Smith SY, Collinson ME, Rudall PJ et al (2009) Virtual taphonomy using synchrotron tomographic microscopy reveals cryptic features and internal structure of modern and fossil plants. Proc Natl Acad Sci U S A 106:12013–12018
Steeves TA, Briggs WR (1960) Morphogenetic studies on *Osmunda cinnamomea* L. The auxin relationships of expanding fronds. J Exp Bot 11:45–67
Stewart WN (1964) An upward look in plant morphology. Phytomorphology 14:120–134
Stewart WN, Rothwell GW (1993) Paleobotany and the evolution of plants, 2nd edn. Cambridge University Press, Cambridge
Tomescu AMF (2009) Megaphylls, microphylls and the evolution of leaf development. Trends Plant Sci 14:5–12
Tomescu AMF (2011) The sporophytes of seed-free vascular plants – major vegetative developmental features and molecular genetic pathways. In: Fernandez H, Kumar A, Revilla MA (eds) Working with ferns. Issues and applications. Springer, New York, pp 67–94
Tomescu AMF, Escapa IH, Rothwell GW et al (2017) Developmental programmes in the evolution of *Equisetum* reproductive morphology: a hierarchical modularity hypothesis. Ann Bot 119:489–505
Tuominen H, Puech L, Fink S et al (1997) A radial concentration gradient of indole-3-acetic acid is related to secondary xylem development in hybrid aspen. Plant Physiol 115:577–585
Turchi L, Baima S, Morelli G et al (2015) Interplay of HD-ZIP II and III transcription factors in auxin-regulated plant development. J Exp Bot 66:5043–5053
Vasco A, Smalls TL, Graham SW et al (2016) Challenging the paradigms of leaf evolution: class III HD-Zips in ferns and lycophytes. New Phytol 212:745–758
Wabnik K, Robert HS, Smith RS et al (2013) Modeling framework for the establishment of the apical–basal embryonic axis in plants. Curr Biol 23:2513–2518

Walters J, Osborne DJ (1979) Ethylene and auxin-induced cell growth in relation to auxin transport and metabolism and ethylene production in the semi-aquatic plant, *Regnellidium diphyllum*. Planta 146:309–317

Walton J (1964) On the morphology of *Zosterophyllum* and some other early Devonian plants. Phytomorphology 14:155–160

Wang Q, Hasson A, Rossmann S et al (2016) *Divide et impera*: boundaries shape the plant body and initiate new meristems. New Phytol 209:485–498

Wangermann E (1967) The effect of the leaf on differentiation of primary xylem in the internode of *Coleus blumei* Benth. New Phytol 66:747–754

Wardlaw CW (1944) Experimental and analytical studies of pteridophytes. IV. Stelar morphology: experimental observations on the relation between leaf development and stelar morphology in species of *Dryopteris* and *Onoclea*. Ann Bot 8:387–399

Wardlaw CW (1946) Experimental and analytical studies of pteridophytes. VII. Stelar morphology: the effect of defoliation on the stele of *Osmunda* and *Todea*. Ann Bot 9:97–107

Wendrich JR, Weijers D (2013) The *Arabidopsis* embryo as a miniature morphogenesis model. New Phytol 199:14–25

White RA (1971) Experimental and developmental studies of the fern sporophyte. Bot Rev 37:509–540

Wilson JP, Montanez IP, White JD et al (2017) Dynamic Carboniferous tropical forests: new views of plant function and potential for physiological forcing of climate. New Phytol 215:1333. https://doi.org/10.1111/nph.14700

Wochok ZS, Sussex IM (1973) Morphogenesis in *Selaginella*. Auxin transport in the stem. Plant Physiol 51:646–650

Wochok ZS, Sussex IM (1974) Morphogenesis in *Selaginella*. II. Auxin transport in the root (rhizophore). Plant Physiol 53:738–741

Wochok ZS, Sussex IM (1976) Redetermination of cultured root tips to leafy shoots in *Selaginella willdenowii*. Plant Sci Lett 6:185–192

Zhou C, Han L, Fu C et al (2013) The trans-acting short interfering RNA3 pathway and NO APICAL MERISTEM antagonistically regulate leaf margin development and lateral organ separation, as revealed by analysis of an *argonaute7/lobed leaflet1* mutant in *Medicago truncatula*. Plant Cell 25:4845–4862

Zhu M, Ahlberg PE (2004) The origin of the internal nostril of tetrapods. Nature 432:94–97

Zimmermann W (1938) Die Telometheorie. Biologe 7:385–391

Zimmermann W (1952) Main results of the "Telome theory". Palaeobotanist 1:456–470