Chapter 1
The Structure and Function of Xylem in Seed-Free Vascular Plants: An Evolutionary Perspective

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1 Introduction

The evolution of xylem was critical to the success of embryophytic land plants. According to the fossil record, the earliest terrestrial autotrophs were algae followed by bryophytes, simple organisms that lacked the ability to efficiently transport water (Raven 1984; Sperry 2003; Taylor et al. 2009; Pittermann 2010). Like their ancestors, today's non-vascular plants have essentially two options for survival: inhabit perennially wet substrates such as stream sides or exploit poikilohydry, the capacity to recover from near complete desiccation (Niklas 2000; Proctor and Tuba 2002; Sperry 2003). In the absence of xylem, non-vascular plants are subject to the vagaries of

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their habitat and remain small because rehydration is a rapid, metabolically expensive process that supports the rapid recovery of photosynthesis (Proctor and Tuba 2002). Over time, the need for effective spore dispersal combined with increased competition for light selected for progressively taller, homoiohydric vascular plants, peaking with evolution of hydraulically efficient woody xylem across both seed-bearing and extinct seed-free lineages (Niklas 2000; Niklas and Speck 2001; Sperry 2003; Wilson 2013).

Much is known about the structure and function of xylem tissue in trees and shrubs because they are economically important, ecologically dominant, and in the case of angiosperms, highly diverse. However, the xylem in seed-free vascular plants such as ferns and lycopods demands equal scrutiny because (1) ferns and lycophytes have persisted for well over 400 million years—no small feat considering several extinction events, changing climates and the evolution of angiosperm-dominated tropical forests (Rothwell and Stockey 2008; Taylor et al. 2009; Watkins and Cardelus 2012) and (2) many species play critical roles in ecosystem processes and may even control forest growth and regeneration (George and Bazzaz 1999; Watkins and Cardelus 2012). Today, seed-free vascular plants are found on nearly every continent and in a variety of habitats ranging from xeric or salt-water niches up to the tropical forest canopies, which are often dominated by epiphytic ferns (Moran 2008; Watkins and Cardelus 2012). In north temperate regions most ferns are seasonal and terrestrial but in warmer climates, the fern morphospace includes vines, tree ferns, epiphytes, aquatic and floating ferns (Ranker and Haufler 2008; Mehltreter et al. 2010). Boasting an impressive 12,000+ species, the fern lineage is the second most diverse only after the angiosperms (Ranker and Haufler 2008). Despite their long history and ecological breadth, little is known about the vascular attributes of these ancestral plants (Pittermann et al. 2013). The goals of this chapter are to provide an overview of xylem structure and function in modern ferns, and to consider the selection pressures that lead to observed macroevolutionary patterns of xylem structure in extinct and extant seed-free vascular plants. The discussion will be restricted to sporophytic shoots and leaves since these structures are the functional equivalents to stems and leaves in seed plants.

2 Vascular Tissues in Stems and Leaves of Seed-Free Vascular Plants

The fossil record shows that the vascular tissues of seed-free vascular plants appeared prior to those of conifers and angiosperms. Because extant pteridophytes do not produce wood, their primary xylem and phloem tissues are packaged in discrete vascular bundles (meristeles) that are surrounded by an endodermis and often a waxy, circum-endodermal band (see stele and vascular patterns in Figs. 1.1 and 1.2). The shape and arrangement of these bundles differs among genera; even within bundles the xylem can adopt a variety of patterns. In contrast to xylem of woody seed plants, which mechanically supports the canopy as well as transports water, the primary xylem of
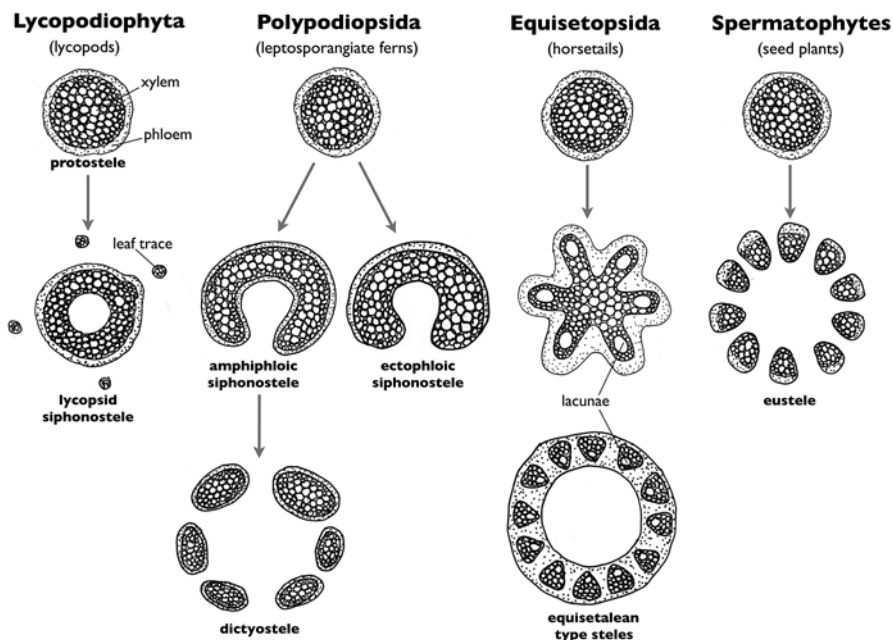
Fig. 1.1 Stem and rhizome stelar patterns in vascular land plants. All fossil and extant vascular arrangements have their origin in the protostele, the simplest steles. Drawings based on illustrations in Beck (2010) and Taylor et al. (2009)

ferns and lycophytes serves solely for the purpose of water transport (Pittermann et al. 2011, 2013). Despite their developmental canalization, extant pteridophyte shoots exhibit a surprising diversity of vascular arrangements, suggesting that evolution may have acted on fern xylem in response to numerous selection pressures.

2.1 Vascular Arrangement

The primary transport tissues are arranged in central zones of roots or stems known as steles. Stelar theory has traditionally pertained to stems and roots (Beck et al. 1982) but in this discussion, the nomenclature is applied to the arrangement of primary tissues in the main axes of fern leaves (fronds) and less frequently, the stems of lycophytes. One or several vascular traces, each containing xylem and phloem, can constitute a stele and these bundles may be variously situated in ground parenchyma or sclerenchyma tissues (Beck 2010; see Figs. 1.1 and 1.2a–h). Of extant and fossil stelar patterns, the protostelic arrangement characteristic of lycopods is considered the most ancestral (Fig. 1.1; Beck et al. 1982; Taylor et al. 2009). In what is thought to be the very first protostele (also termed a haplostele), the xylem is in the centre of the vascular cylinder and is surrounded by the phloem (Beck et al. 1982). In contrast,
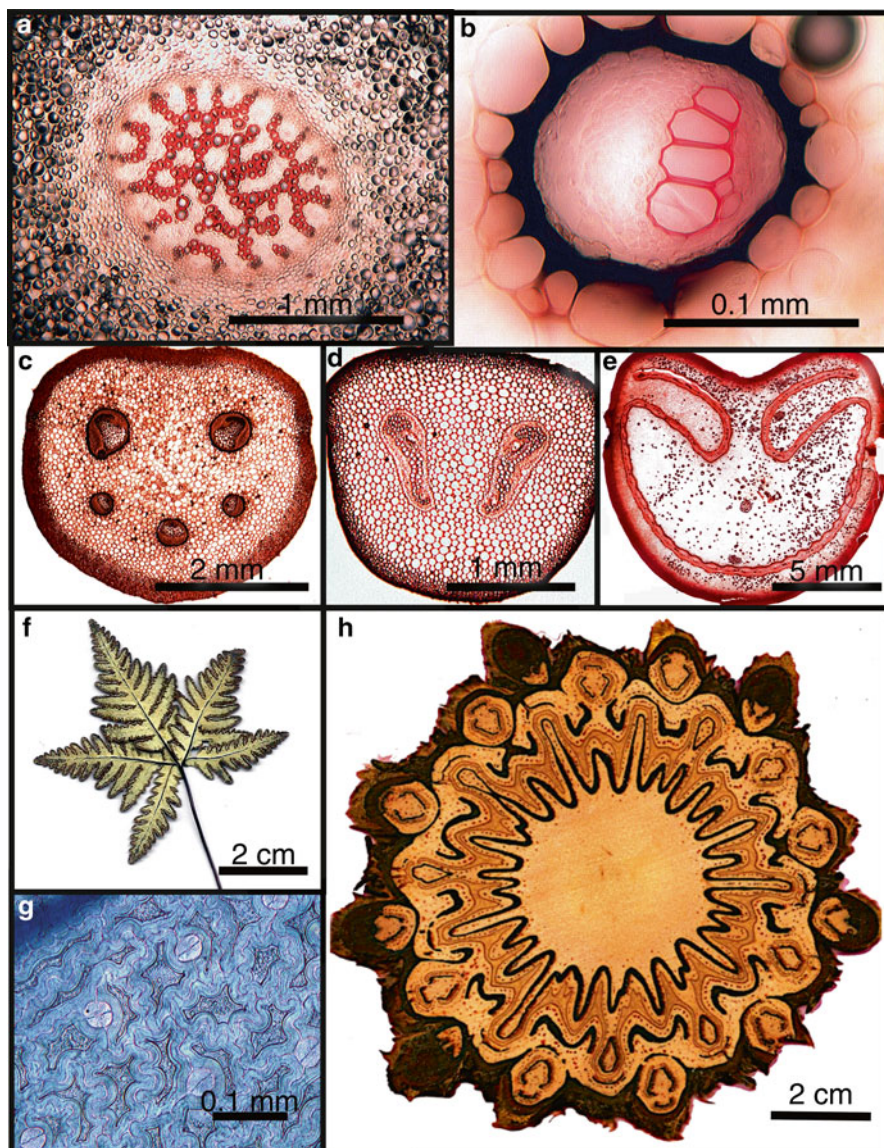
Fig. 1.2 Stelar arrangements and leaf characteristics of lycophytes and ferns. (**a**) The mixed protostele of *Phlegmariurus squarrosus*, an erect lycophyte. Xylem tissue is stained *red* while phloem and parenchyma remain unstained. (**b**) A close-up on a vascular bundle of *Platycerium bifurcatum* (Cav.) C. Chr. showing *red-stained* xylem and clear phloem surrounded by an endodermis with a thick circumendodermal band. (**c**) The dictyostele of *Dryopteris arguta* (Kaulf.) Watt comprising two large vascular bundles and three smaller auxillary ones; the fibrous, peripheral sterome is also stained *red*. (**d**) The dictyostele of *Athyrium filix-femina* (L.) Roth. (**e**) An omega-shaped vascular strand in *Pteris livida* Mett. (**f**) The underside of a frond of *Notholaena standleyi* Maxon, a desiccation-tolerant cheilanthoid fern found in rocky, dry outcrops of the American Southwest; the underside of the leaf is covered with a pale yellow farina that is exposed when the leaf curls while desiccating. (**g**) Stomatal and epidermal patterns on the underside of pinnae in *Pellaea truncata* Goodd., a dry adapted cheilanthoid fern with sclerophyllous leaves. (**h**) Cross section of the trunk of *Dicksonia antarctica* Labill. showing sclerenchyma tissue (*dark brown*) adjacent to a contiguous strand of primary xylem (*dark beige*); leaf traces are evident on the periphery of the trunk. (Panel **h** by Christopher Rico)

the more derived protostele of *Phlegmariurus squarrosus* (G. Forst. Á. Löve and D. Löve; Huperziaceae) exhibits mixed elements of xylem and phloem (see micrograph in Fig. 1.2a; Beck et al. 1982). In the rhizomes and shoots of many lycopods and leptosporangiate ferns, the protostele appears to have given rise to the more derived siphonostele arrangement, which is a hollow or parenchyma-filled cylinder with ectophloic (external) phloem tissue (Fig. 1.1; Beck et al. 1982; Beck 2010). In typical siphonosteles, a diverging leaf trace is evident as an interruption of the cylinder, giving it the resemblance of an arch, but in the absence of leaf traces, the siphonosteles form complete cylinders with phloem that can be on both sides of the xylem (amphiphloic) or simply on the external face (ectophloic) (Fig. 1.1; Beck 2010). The dictyostele is the most dissected and derived of the leptosporangiate fern steles, formed by numerous vascular strands that may or may not come into contact over the length of the rhizome or the frond axis (Figs. 1.1 and 1.2b–d). Unusual among extant steles, Equisetalean stelar arrangements incorporate characteristic air-filled lacunae (Fig. 1.1). Lastly, the primary stem tissues of seed plants form the characteristic eustele, a cylindrical arrangement of numerous vascular bundles (Fig. 1.1).

The three-dimensional arrangement of the vascular network is much less well characterized than the stelar arrangements in transverse sections. The detailed representations of Ogura (1972) and Beck et al. (1982) were probably created from numerous cross sections and careful dissections, but the imaging methods pioneered by Zimmermann and Tomlinson (1974) and Zimmermann and Tyree (2002) improved both efficiency and precision by using sequential photographs of serial stem cross sections to assemble, frame by frame, three-dimensional reconstructions of vessel or vascular bundle arrangements. This cinematographic approach was limited to small pieces of tissue but it provided important insight into the longitudinal and three-dimensional positioning of vascular tissues in both woody and monocot plants (Zimmermann and Tyree 2002). White and Weidlich (1995) used it to document the longitudinal, interdigitated pattern of meristeles in semi-arborescent members of the fern genera *Diplazium* and *Blechnum*, demonstrating that comparable phyllotaxis has evolved convergently in ferns and seed plants. A newer method in which resins and polymers are directly injected into xylem tissue has proven extremely useful for understanding angiosperm vessel networks and has been especially revealing about vessel-to-vessel pit connections (Mauseth and Fujii 1994; Kitin et al. 2004). This approach is also valuable for measurements of conduit length in seed-free vascular plants because tracheids seed-free vascular plants because tracheids are often longer than a few millimetres and frequently resistant to standard solutions of hydrochloric acid and hydrogen peroxide, making tissue macerations problematic (Wheeler et al. 2005; Pittermann et al. 2011).

Newer methods may overcome some of the problems associated with xylem network imaging. Recently, high resolution computed tomography (HRCT) along with straightforward manual frond xylem extractions have revealed interesting differences in the vascular organization of ferns (Brodersen et al. 2012). The HRCT method relies on a synchrotron-generated source of focused X-rays to provide micrometre-range resolution of vascular tissues in three dimensions (Brodersen et al. 2011) and can be used for visualization of water movement and droplet formation within the xylem as well as for reconstruction of tissue arrangements (Brodersen et al. 2010, 2012). Unlike serial sectioning and macerations,

Fig. 1.3 Vascular
arrangements along the
frond primary axis
in *A Osmundastrum
cinnamomeum* (L.) C. Presl,
B Polystichum acrostichoides
(Michx.) Schott, *C Onoclea
sensibilis* L. Open circles
represent the location of
connections between vascular
bundles
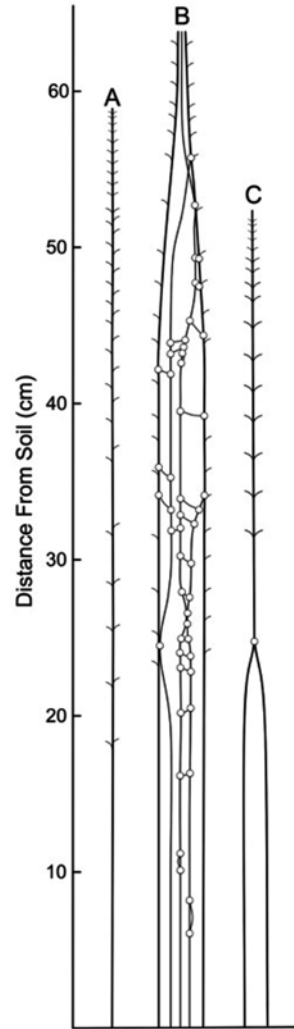
HRCT is non-destructive, but at present, processing time and computing power preclude the imaging of tissues longer than a few centimetres.

Dissections and meristele extractions have revealed that protosteles, such as those in the lycopod *Phlegmariurus squarrosus* and the whisk fern *Psilotum nudum* (L.) P. Beauv. (Psilotaceae) remain invariable along the shoot axis (Pittermann unpublished data), but similar patterns can be seen in *Osmundastrum cinnamomeum* (L.) C. Presl (Osmundaceae) in which a C-shaped stele remains intact over the length of the frond (see xylem map in Fig. 1.3). In more derived pteridophyte clades, the number of vascular bundles as well as their frequency of contact may vary considerably. For example, a 110-cm-long frond of *Pteridium aquilinum* (L.) Kuhn (Dennstaedtiaceae) has anywhere from 23 to 29 vascular bundles at the base of the stipe and between 3 and 6 bundles at the tip of the rachis, with well over 100 bundle

connections throughout the main axis of the frond (Brodersen et al. 2012). This is an intricate network and probably an extreme example of stelar dissection. By contrast, only 4–5 basal bundles fuse to a single strand at the tip of the rachis in the simpler network of *Woodwardia fimbriata* Sm. (Blechnaceae) and *Polystichum acrostichoides* (Michx.) Schott (Dryopteridaceae; Brodersen et al. 2012). There is some variation between the base of the frond (petiole) and the rachis region which gives rise to the pinnae (Fig. 1.3). Lateral veins emerge from the large, main vascular strands in the rachis of all three species but network complexity increases in *P. acrostichoides*, whereas it simplifies in the more derived *Onoclea* (Onocleaceae). How selection drives xylem network variation is currently unclear.

2.2 Xylem and Phloem Ultrastructure

Phloem function is closely coupled with water transport, and Münch's pressure flow hypothesis posits that mass flow of phloem sap depends in part on water influx from adjacent xylem cells (Pickard and Abraham-Shrauner 2009). Recent models of leaf gas exchange show plant productivity to be coupled to the dynamics of xylem and phloem pressure gradients, and phloem has also been implicated in the recovery of hydraulic function after a water deficit (Salleo et al. 2004; Nikinmaa et al. 2014). The rapid wounding response of phloem makes it a difficult tissue to study but the use of aphid stylets, scanning electron microscopy and models has enabled insight into its functional attributes (Gaupels et al. 2008; Mullendore et al. 2010; Jensen et al. 2012). Unfortunately, studies on phloem tissue in seed-free vascular plants are rare. Early studies of *Equisetum* revealed that the phloem tissue comprises sieve elements and perhaps also parenchyma cells (Agashe 1968). In *Blechnum orientale* L. (Blechnaceae), sieve elements have sieve areas with numerous pores and possibly callose tissue but the presence of companion cells is not clearly established (Fotedar and Shah 1975). Clearly, phloem function in early-derived plants deserves more study in contemporary plant biology.

The xylem structure of seed-free vascular plants has received considerably more attention than phloem. The tracheids of ferns may be substantially longer and wider than those of conifers, reaching widths well over 100 µm and lengths exceeding 1 cm (Veres 1990; Pittermann et al. 2011; see conduit diameter distributions in Fig. 1.4). By comparison, conifer tracheids rarely exceed 40 µm in diameter and 2 mm in length, particularly in stems (Pittermann et al. 2006), with narrower, shorter tracheids found in stiffer wood (Panshin and de Zeeuw 1980). In the absence of a mechanical function, fern tracheids serve solely to transport water, and thus exhibit none of the hydraulic constraints imposed by smaller lumina, relatively thicker walls, and short cells evident in conifer tracheids (Pittermann et al. 2006; Sperry et al. 2006). Several fern genera are believed to have vessel elements in their rhizomes (*Astrolepis* (Pteridaceae), *Marsilea* (Marsileaceae), *Woodsia* (Woodsiaceae) and *Pteridium*; Carlquist and Schneider 2007), with silicon injections confirming vessels in excess of 20 cm in length in the petiole of *Pteridium aquilinum* (Wheeler et al. 2005; Pittermann et al. 2011).
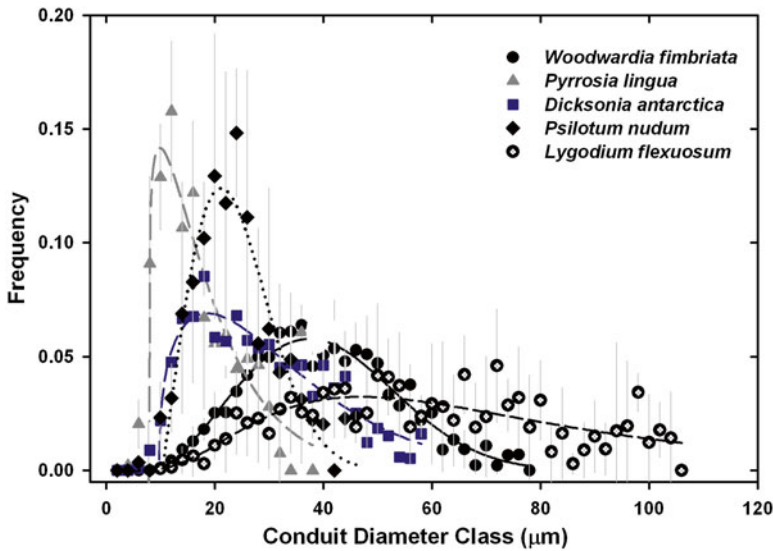
Fig. 1.4 Conduit diameter frequencies in five fern species (reprinted from Pittermann et al. 2011)

Conduits must be perforated by inter-conduit pit membranes to allow water to flow from one conduit to another. As in other vascular attributes, the pit membrane structure in seed-free vascular plants differs substantially from that of conifers and angiosperms. Angiosperms possess what is known as a homogenous pit membrane, which is a partially digested primary wall protected by an overarching, secondary wall border (Choat and Pittermann 2009). These membranes typically occupy radial vessel walls and may be grouped near vessel-to-vessel junctions or located sporadically along the full length of the vessel wall. Conifers, on the other hand, possess a torus–margo pit membrane in which water moves through the porous margo region while the torus, a thickening in the centre of the membrane, serves to protect conduits from the spread of air throughout the xylem (Choat and Pittermann 2009; see Chaps. 2 and 3). The vast majority of ferns and lycopods possess homogenous pit membranes that span the length of the tracheid in a scalariform arrangement and are located on nearly all the tracheid walls (see micrographs in Fig. 1.5a, b). Ranging in thickness from 150 to 350 ηm (Brodersen et al. 2014), pteridophyte pit membranes appear less variable than those of angiosperms, in which range from 70 to 1,892 ηm (Jansen et al. 2009). Similar pit membrane arrangements have been observed in seed-free vascular plants since the Early Devonian (Kenrick and Crane 1997; Taylor et al. 2009). Interestingly, torus–margo pit membranes are present in ferns, but only in the anomalous secondary growth in rhizomes in the genus *Botrychium* (Ophioglossaceae; Morrow and Dute 1998; Rothwell and Karrfalt 2008). The presence of conifer-like pit membranes in *Botrychium* as well as bordered pits in *Psilotum* indicates that ferns have the genetic potential to evolve xylem that is functionally comparable to higher plants, but either selection acted on attributes of fern physiology or life history that override the importance of pit membranes, or the genetic or developmental capacity to capitalize on these traits is absent.
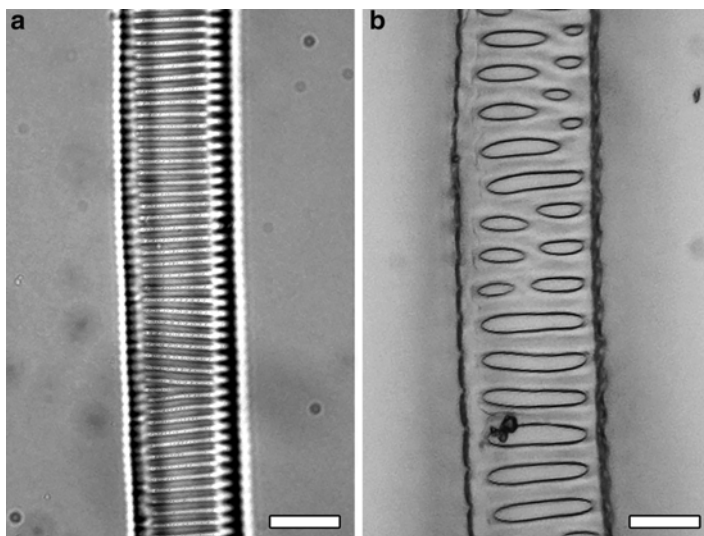
Fig. 1.5 Tracheids of (**a**), *Selaginella pallescens* (C. Presl) Spring (air-seed pressure = 3.8 MPa) and (**b**), *Phlebodium aureum* (L.) J. Sm. (air-seed pressure = 2.12 MPa). Tracheid walls are perforated by scalariform (*S. pallescens*) and reticulate (*P. aureum*) pit membranes. Scale bar = 5 μm

3 The Xylem of Seed-Free Vascular Plants in the Fossil Record

The fossil record opens a window into the fascinating morphological diversity of early land plants, and also into some unusual excursions in plant structure and function. Many of these botanical experiments have failed but others have persisted into the modern flora for reasons that may have little to do with physiology. Aside from physiological attributes, ecological, reproductive and other life history traits certainly have played a role in taxon persistence. In fact, the disappearance of variously woody progymnosperms, sphenopsids and isoëtopsids demonstrates that having secondary xylem is no guarantee against extinction.

3.1 Xylem in Early Land Plants

Fossil spore and possibly micro-fossil records suggest that vascular plants made their appearance in the Silurian (Niklas and Smocovitis 1983; Kenrick and Crane 1997; Taylor et al. 2009), with the Lower Devonian giving rise to a diversity of plants with transport tissues (Niklas 1985). Of these proto-vascular plants, the Rhyniophytes (so-named for the Early Devonian Rhynie Chert locality) are probably the best characterized. Genera such as *Rhynia*, *Aglaophyton* and *Cooksonia* are rhizomatous, with dichotomizing, stomatal-bearing shoots up to 18 cm tall,
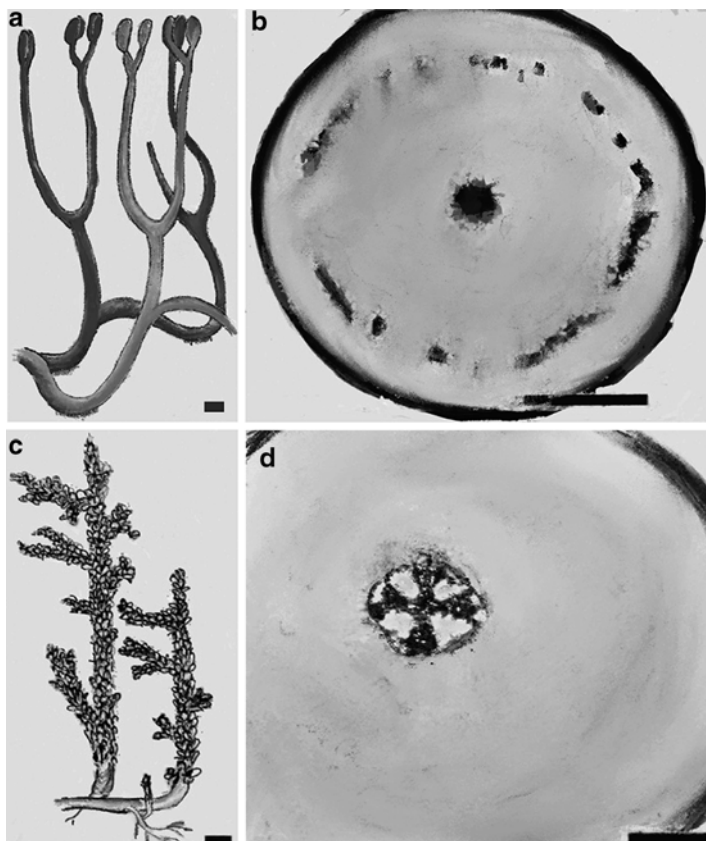
Fig. 1.6 Reconstructions and stem cross sections *Aglaophyton major* (**a** and **b**) and *Asteroxylon mackiei* (**c** and **d**). Scale bars equal 1 cm in the plant reconstructions and 1 mm in the cross sections. Figure adapted from Boyce et al. (2003)

possessing variously developed central conducting strands (Edwards 1986; Taylor et al. 2009; see illustrations of *Aglaophyton* in Fig. 1.6a, b). The vascular tissues of *Rhynia gwynne-vaughanii* and *Aglaophyton major* are described as hybrids between the hydroids of bryophytes and the xylem of more derived plants (Edwards 1986; Kenrick and Crane 1991) but chemical analysis of fossil material suggests that the vascular tissue was not lignified (Boyce et al. 2003). Bearing a close resemblance to bryophytes, *A. major* is categorized as a pro-tracheophyte (see Boyce et al. 2003; Fig. 1.6a, b), with *R. gwynne-vaughanii* falling closest to the next most-derived group, the tracheophytes (Kenrick and Crane 1997; Boyce et al. 2003). *Asteroxylon mackiei*, a more derived eutracheophyte from the Lower Devonian Rhynie Chert, has well-developed lignified primary xylem, the presence of which is consistent with the need to hydrate enations (small flaps of photosynthetic tissue) that cover its stems and increase its evaporative surface area (Boyce et al. 2003; Taylor et al. 2009; see illustrations of *Asteroxylon* in Fig. 1.6c, d).

The conduit structure of early land plants may have been more diverse than that of extant seed-free vascular plants. Moss hydroids are unlignified and, aside from plasmodesmatal-derived pores, they lack additional structural features (Kenrick and Crane 1997; Beck 2010). By comparison, conduits of more derived rhyniophytes show greater complexity and reinforcement against collapse. In *Rhynia*, for example, the transport cells have spongy helical wall thickenings (Kenrick and Crane 1991). In Early Devonian *Gosslingia* and *A. mackiei*, the tracheid walls comprise a two-layered cell wall with a chemically resistant inner layer (Kenrick and Crane 1991; Taylor et al. 2009). The P-type tracheids of *Psilophyton*, a Lower- to Mid-Devonian eutracheophyte, are probably the most structurally robust with what appears to be a higher wall fraction and degradation-resistant cell layer (Kenrick and Crane 1991, 1997). Taken together, the fossil record during the early Devonian trends toward an increase in xylem content and tracheid size, as well as tracheid reinforcement. Wall perforations resembling pit membranes become evident in the eutracheophytes as hydraulic demand must meet increasing plant size.

The Cladoxylopsids, early euphyllous fern-like plants with various degrees of xylem organization, make their appearance in the Middle to Upper Devonian, along with the more derived Rhachophytales, Stauropteridales and Zygopterids (extinct taxa with large, frond-like leaves; Taylor et al. 2009). Collectively, these groups appear to be related to ferns but it is unclear whether they are ancestral or sister taxa. Many of these plants have xylem arrangements that not featured in today's flora (Rothwell and Stockey 2008). For example, the star-shaped protostele of the Cladoxylopsids is defined as an actinostele and is unlike any extant stelar arrangement (Rothwell and Stockey 2008; Taylor et al. 2009; Beck 2010). Some specimens such as *Pseudosporochnus* may have been small and arborescent, but others such as *Eospermatopteris* and *Pietzschia schulleri* reached heights in excess of 8 m (Taylor et al. 2009). In Cladoxylopsids such as the Iriopteridales, the main axes measured over 5 cm in diameter, with cross sections showing dissected cross-shaped or bifurcating star-shaped protosteles that supported leaves with a single vascular trace (Taylor et al. 2009).

3.2 Secondary Xylem in Seed-Free Vascular Plants

Increased competition for light and other resources selected for taller plants with more developed canopies in the early evolution of eutracheophytes. Higher rates of transpiration necessitated increased hydraulic demand that was satisfied by greater xylem fraction and wider (and presumably longer) conduits. Fossils show that tracheid diameter increased by an order of magnitude during the Devonian from the 8 μm tracheids of the primitive *Cooksonia* to the 140 μm metaxylem conduits of *Stenomylon primaevum*, an early Carboniferous seed fern (Niklas 1985). Although early vascular plants such as *Rhynia* and *Asteroxylon* were herbaceous, the recent identification of true secondary xylem in putative relatives of *Psilophyton* and *Armoricaphyton* suggests that this trait evolved much earlier than originally thought (Gerrienne et al. 2011; Strullu-Derrien et al. 2014). It appears that *Psilophyton* had a bifacial vascular cambium in which cell initials divide both radially and tangentially.
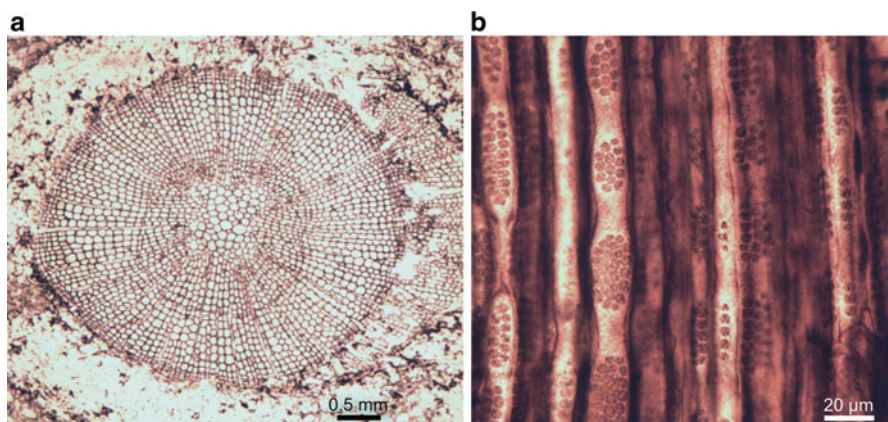
Fig. 1.7 Cross section of a root (**a**) and (**b**) a longitudinal section of stem tissue belonging to *Callixylon-Archaeopteris*, a Devonian progymnosperm. Clustered pit membranes with cruciform apertures are evident in panel **b**. (Material courtesy of Dr. Diane Erwin, UC Berkeley Museum of Natural History)

Secondary xylem, whether unifacial (only tangential cell divisions) or bifacial, is now believed to have evolved on at least five separate occasions, and by the Late Devonian, secondary vascular tissues were present in many dominant seed-free vascular plant lineages including the Lepidodendrales, Sphenopsids, pro-gymnosperms, the Zygopterids and Devonian–Carboniferous seed ferns (Barghoorn 1964; Beck 1970; Cichan 1985a, b; Sperry 2003; Taylor et al. 2009). Unifacial vascular cambia were present in arborescent lycopods such as the Lepidodendrales. Indirect analysis of xylem development suggests that cambial initials divided centrifugally with no anticlinal (radial) division, such that any increase in trunk circumference was accommodated by progressively larger fusiform initials (Cichan 1985a). Similar patterns of development probably occurred in the Carboniferous horsetail relatives including *Sphenophyllum* and *Arthropitys* species (Cichan 1985b).

Prior to the discovery of true secondary xylem in *Psilophyton*, the progymnosperms were thought to represent the earliest known class of plants with true wood derived from a bifacial cambium (Gerrienne et al. 2011). This group of plants possessed the unusual combination of coniferous, pycnoxylic wood with a canopy comprising free-sporing foliage, superficially similar to those of ferns (Beck 1970; Taylor et al. 2009). Growth rings, ray parenchyma and clustered, bordered pits with offset, cruciform apertures were present in both root and trunk xylem of the Archaeopteridales, of which the spore-bearing Devonian/Carboniferous *Archaeopteris-Callixylon* is best studied (Meyer-Berthaud et al. 1999; see micrographs of *Callixylon* xylem in Fig. 1.7a, b). Although it was seed-free, *Archaeopteris* was crowned "the Earth's first tree" because it demonstrates several developmental attributes of more derived plants, including perennial lateral branches (Meyer-Berthaud et al. 1999, 2000).

3.3 Fern Xylem in the Fossil Record

Ferns are functionally defined as megaphyllous plants with spores on the underside of their leaves (Rothwell and Stockey 2008, but see also Smith et al. 2006 for a more thorough treatment), but as *Archaeopteris* and *Psilophyton* demonstrate, specimens from the fossil record can certainly stretch this definition. For clarity, we define ferns as vascular, spore-bearing, megaphyllous plants with only primary growth. The fossil record shows that modern ferns, including the Psilotaceae, Ophioglossaceae, Equisetaceae, Marattiaceae and the leptosporangiate ferns have their origins in the Devonian (Rothwell and Stockey 2008). Although some extinct taxa exhibit stelar patterns that are unlike those of modern ferns today, the vasculature of the true ferns has remained relatively conserved over deep time.

Three major diversification events characterize the evolution of the leptosporangiate ferns, the first of these occurring during the climatically mild Carboniferous period (Rothwell and Stockey 2008). Fossilized petioles (stipes) of Botryopteridaceae and Kaplanopteridaceae fronds show variable tripartite and C-shaped steles surrounded by a thick, fibrous sterome layer (Rothwell and Stockey 2008). Similarly, fossil leaf scars of Marattialean tree ferns (representatives of which are extant in today's tropics) indicate a variety of stelar arrangements including C-shapes as well as more complex, possibly dictyostelic patterns. Stelar arrangements can vary along the length of the frond, but it is difficult to comment on the overall arrangement of the vascular strands with limited fossil material.

The Mesozoic gave rise to the second significant radiation of ferns, and included the Gleicheniales and Schizaeales, descendants of which are found in today's fern flora. Features of other Mesozoic taxa fall within the range of trait variation found in extant plants, as discovered in the Cretaceous remains of *Osmundastrum cinnamomeum*, in which the stipe stelar arrangement (a C-shape) resembles that of modern representatives (Serbet and Rothwell 1999). The steles in the petioles of modern leptosporangiates range from highly dissected, as in the Marattiaceae, to variously C-shaped; given the conserved anatomy of *Osmunda* over deep time, it may be reasonable to suppose that stelar arrangements in the other leptosporangiates with modern representatives are similarly conserved.

The third well-characterized fern radiation probably has origins in the upper Jurassic, with the diversification of the Eupolypod I and II clades (Schneider et al. 2004; Schuettpelz and Pryer 2009), even though most of the fossil diversity is apparent during the Cretaceous. Many taxa are now extinct but the fossil record suggests conserved dictyostelic vascular architecture in the petioles. For example, two large vascular strands resembling those of modern taxa are evident in Eocene specimens of Blechnaceae and Athyriaceae (Rothwell and Stockey 2008; Fig. 1.2c, d). Interestingly, this pattern is prevalent in the most recently derived fern clades, the Eupolypods I and II, a large fraction of which are epiphytic (Schneider et al. 2004; Schuettpelz and Pryer 2009). Characterizing and understanding the biotic and abiotic filters that selected for various vascular attributes over deep time will be a multidisciplinary undertaking requiring expertise in paleobotany, paleoecology, and plant structure and function.

4 Xylem Function in Modern Pteridophytes

Water transport in ferns and other seed-free vascular plants presents an interesting conundrum because in the absence of traits such a bifacial cambium, vessels and torus–margo pit membranes, one might suppose that seed-free vascular plants are at a competitive disadvantage relative to conifers and angiosperms (Pittermann et al. 2011, 2013). However, spore-bearing lineages have thrived since the Devonian, and their physiology certainly warrants a closer look. Indeed, the mechanisms by which ferns and lycophytes compensate for the absence or loss of these features makes for an interesting discussion because it bears not only on the limits of their so-called morphospace but also their evolutionary trajectory over time.

4.1 Hydraulic Efficiency in Ferns and Lycophytes

Of all the "missing" traits, the absence of a cambial layer in modern ferns and lyco-phytes is perhaps the most costly because it severely limits transport capacity and prohibits lateral branching. Consequently, ferns cannot develop complex canopies or occupy the massive ecological footprints of woody plants (Rowe et al. 2004; Rowe and Speck 2005). Ferns can, however, be dominant elements in tropical and subtropical systems in places such as Hawaii, where the tree fern *Sphaeropteris cooperi* (Hook. ex F. Muell.) R. M. Tryon (Cyatheaceae) has become invasive (Robinson et al. 2010). Native tree ferns are ecologically important in the South American and South Pacific floras where they frequently emerge after disturbances (Coomes et al. 2005; Robinson et al. 2010). In arborescent genera, such as *Dicksonia* and *Cyathea*, fibrous overlapping leaf traces form a several centimetre-thick sheath around the trunk and fibrous roots can also serve a supportive function (Sharpe and Mehltreter 2010; Fig. 1.2h). A second, less conspicuous constraint arising from the absence of wood is an inability to increase canopy size and complexity with age— the tree fern trunk simply elevates the canopy but the number of fronds remains rela-tively constant. There is little if any room for developmental flexibility when only a limited amount of primary xylem serves the canopy.

The absence of vessels in the vast majority of ferns precludes the leaps in hydrau-lic efficiency achieved by flowering plants (Brodribb and Feild 2010; Feild et al. 2011). Vessels evolved several times across several lineages including angiosperms, ferns, and possibly in several extinct taxa but for the vast majority of ferns, which are tropical, the xylem remains tracheid based (Calkin et al. 1985; Sperry 2003; Pittermann 2010; Watkins et al. 2010; Watkins and Cardelus 2012). *Pteridium aqui-linum*, an aggressive weed, is a conspicuous exception: vessels have been identified in fronds on several occasions, making this species one of the most hydraulically efficient ferns amongst those surveyed (Wheeler et al. 2005; Pittermann et al. 2011; Brodersen et al. 2012). Unlike most pteridophytes, *P. aquilinum* is tolerant of both low light and full sun environments and can support transpiration and photosynthe-sis rates up to 0.5 mol m^{-2} s^{-1} and 15 µmol CO_2 m^{-2} s^{-1}, respectively, which are on

par with those of angiosperms (Page 2002; Marrs and Watt 2006; Pittermann et al. 2011; Brodersen et al. 2012). Over 60 species of pteridophytes have managed to invade both intact and disturbed ecosystems, often outcompeting and even smothering native angiosperms and conifers (Robinson et al. 2010). The reasons behind the ferns' competitive edge are complex but a physiological approach may help explain their rapid rates of spread, as well as the mechanism by which these species push their xylem function and overall physiology beyond the norm. It is possible that vessels may be present in a greater number of fern species than we currently know.

Given that the vast majority of ferns have xylem comprised exclusively of tracheids, the absence of conifer type torus–margo pit membranes seems to add yet another handicap that pteridophytes failed to solve during the course of evolution. Torus–margo pit membranes are found primarily in conifer xylem and are about 60 times more permeable than the average angiosperm homogenous pit membrane (Pittermann et al. 2006; Sperry et al. 2006). This is because water moves from tracheid to tracheid through the highly porous margo region of the pit rather than the dense network of microfibrils and hydrogels characteristic of homogenous pit membranes (Sperry et al. 2006; Domec et al. 2008; Pittermann et al. 2010). Functionally, the increased permeability of torus–margo pit membranes compensates for xylem that consists of otherwise short, single-celled, high-resistance tracheids (Pittermann et al. 2006). If torus–margo pit membranes were replaced with an average angiosperm-type homogenous pit membrane, the tracheid-based vascular system would be 38× less efficient (Pittermann et al. 2005; Sperry et al. 2006).

If woody plants represent paragons of hydraulic optimization, it seems remarkable that ferns manage to transport any water all with what appears to be the least desirable combination of xylem traits. And yet, fronds of *Angiopteris evecta* (G. Forst.) Hoffm. (Marattiaceae) and *Woodwardia fimbriata* can exceed 1.5 m in length with little more than a few strands of primary xylem! The notion that ferns are constrained by primitive, inefficient xylem prevails because the bulk of published data has been generated on tropical taxa, which experience neither high evapotranspirative stress nor a high demand for water. For example, a survey of hydraulic function in tropical terrestrial and epiphytic ferns revealed K_s to be no higher than 8 kg MPa^{-1} m^{-1} s^{-1} (Watkins et al. 2010). These values are corroborated by gas exchange and leaf hydraulic data, which show that ferns barely achieve 20 % of the physiological capacity of angiosperms (Brodribb et al. 2007). However, fern habitats and ecological niches are nearly as varied as those of angiosperms, so when non-tropical taxa are considered, the picture of fern hydraulics changes dramatically. The K_s has consistently been found to be higher than 20 kg MPa^{-1} m^{-1} s^{-1} in the stipes of the seasonally deciduous *P. aquilinum*, the perennial *W. fimbriata* and the stems of climbing *Lygodium japonicum* (Thunb.) Sw. (Lygodiaceae; Pittermann et al. 2011, 2013; Brodersen et al. 2012). Figures 1.8 and 1.9 show numerous species that have K_s in excess of 10 kg MPa^{-1} m^{-1} s^{-1}, exceeding the hydraulic efficiency of temperate ring- and diffuse-porous species by at least a factor of 2 (McCulloh et al. 2010; Lens et al. 2011; see Chaps. 2 and 4). Temperate ferns should be sampled more intensively because these values may be the norm rather than the exception for seasonally deciduous species.
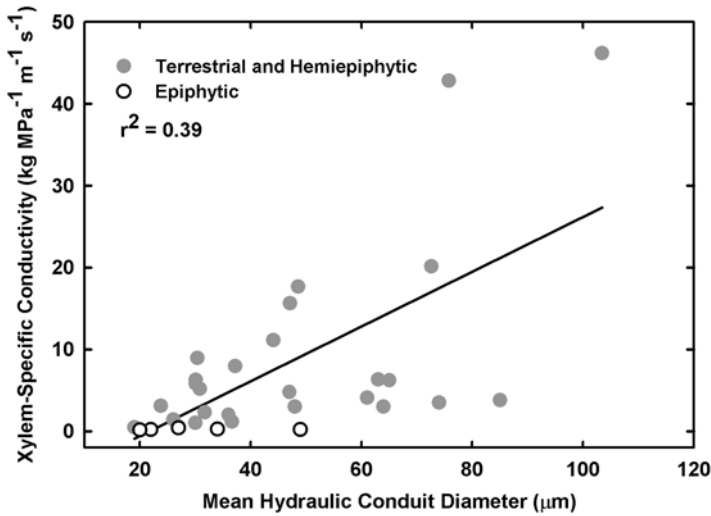
Fig. 1.8 Xylem-specific conductivity as a function of mean hydraulic conduit diameter in terrestrial, hemiepiphytic, and epiphytic ferns from tropical and temperate habitats. Data from Watkins et al. (2010) and Pittermann et al. (2011, 2013)

Three attributes may explain potential high specific conductivities in ferns. First, fern tracheids can explore a surprisingly broad anatomical and functional morphospace because shoot biomechanics are largely dependent on the sterome. Indeed, their central position in the stipe-rachis ensures that they occupy a mechanically neutral zone that is subject to neither the tension nor the compressive stresses imposed on the hypodermal sterome by external loads (Niklas 1992). Hence, species' hydraulic conduit diameters range from 20 to 100 µm, exceeding the tracheid and vessel sizes of several tropical and temperate woody taxa (see fern tracheid dimensions in Figs. 1.4 and 1.10; Veres 1990; McCulloh et al. 2010). Hydraulic efficiency of conduits scales to the fourth power of the lumen diameter (Tyree et al. 1994), so even a modest number of large tracheids can disproportionately contribute to the overall transport capacity to the xylem. A second important consideration is conduit arrangement within the fern vascular bundle. Despite devoting less than 30 % of frond cross-sectional area to xylem tissue, hydraulic efficiency is achieved in part by the close packing of conduits within the vascular bundles, much like tracheids in conifer xylem (see fern xylem structure in Fig. 1.2b–d). In this way, fern xylem combines the best of both worlds: water moves through wide, long, angiosperm-sized conduits that are tightly packed like those of conifers (Pittermann et al. 2013). Lastly, pit membrane attributes also contribute to high K_s in seed-free vascular plants (see pit membranes in Fig. 1.5a). Several studies have shown that the permeability of pteridophyte pit membranes to water is surprisingly similar to
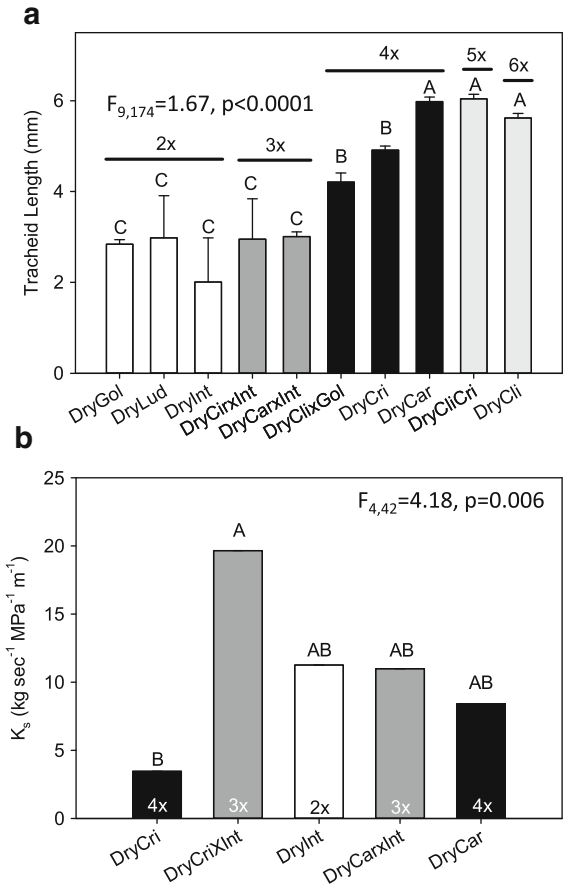
Fig. 1.9 (**a**) Variation in fern stipe tracheid lengths across taxa of hybrid origin and/or ploidy levels. DryGol: fertile diploid *Dryopteris goldiana*; DryLud: fertile diploid *Dryopteris ludoviciana* (Kunze) Small; DryInt: fertile diploid *Dryopteris intermedia* (Muhl. ex Willd.) A. Gray; DryCri×Int (also recognized as×*bootii*): Sterile triploid hybrid between *Dryopteris cristata* (L.) A. Gray and *D. intermedia*; DryCar×Int (also recognized as×*triploidea*): Sterile triploid hybrid between *Dryopteris carthusiana* (Vill.) H.P. Fuchs and *D. intermedia*; DryCli×Gol: fertile tetraploid hybrid between *Dryopteris clintoniana* (D.C. Eaton) Dowell and *D. goldiana*; DryCri: *D. cristata*, DryCar: *D. carthusiana*, both fertile tetraploids; DryCli×Cri: Sterile hexaploid hybrid between *D. clintoniana* and *D. cristata*; DryCli: Fertile heptaploid *D. clintoniana*. Data were taken from a mixture of herbarium specimens maintained at the Cooley Herbarium at Colgate University and from fresh material. In an attempt to control for environment, all taxa were collected or listed on their herbarium label as having been collected in New York state. (**b**) Xylem specific hydraulic conductivity (K_s) of the stipes of DryCri: *D. cristata*; DryCri×Int (also recognized as×*bootii*): Sterile triploid hybrid between *D. cristata* and *D. intermedia*; DryInt: *D. intermedia*, DryCar×Int (also recognized as ×*triploidea*): Sterile triploid hybrid between *D. carthusiana* and *D. intermedia*; DryCar: *D. carthusiana*. Ploidy levels are given at the bottom of each bar. Data were taken from species collected in the field at the Huyck Preserve in Upstate New York. Conductivity was determined by the balance method

Fig. 1.10 The average diameter of individual tracheids in the stipes of epiphytic, hemiepiphytic (all primary), terrestrial root climbing (see Canestraro et al. 2014), and terrestrial fern species. These data represent multiple species from the tropical lowland forest at La Selva Biological Station in Costa Rica. Stipes were hand-sectioned and a minimum of 30 individual tracheids were measured per stipe section
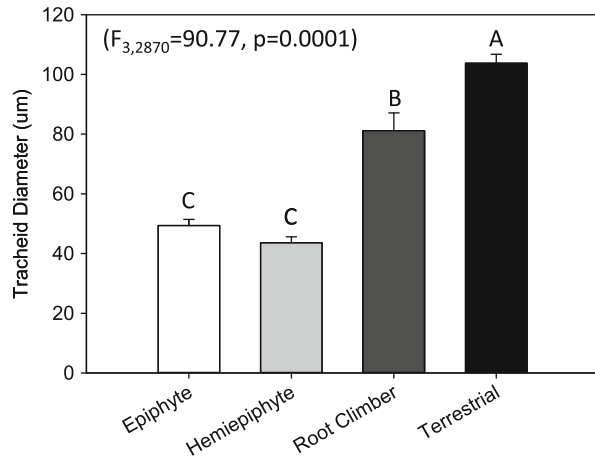
conifers and vesselless angiosperms. The area-standardized pit resistance (r_{pit}) of ferns and lycophytes ranges from 1.99 to 283 ± 130 MPa s m^{-1}, with an average of 54.6 MPa s m^{-1} (Schulte et al. 1987; Wheeler et al. 2005; Brodersen et al. 2014), whereas r_{pit} in conifers and vesselless angiosperms is estimated at 5.7 and 16 MPa s m^{-1}, respectively (Pittermann et al. 2005; Hacke et al. 2007). By contrast, the average r_{pit} of angiosperm vessels is 336 MPa s m^{-1} (Wheeler et al. 2005; Hacke et al. 2006). These values suggest that, similar to conifers and vesselless angiosperms, highly permeable pit membranes compensate in part for the higher resistance imposed by a tracheid-based vascular system. Neither ferns nor vesselless angiosperms show the predicted 38× loss in hydraulic efficiency associated with non-conifer tracheids because convergent evolution acted to reduce membrane permeability across these distant lineages.

Ferns may be capable of evolving hydraulically efficient xylem, but it is primary xylem nonetheless, and it imposes a fixed limit on physiological performance (Pittermann et al. 2013). Indeed, the vast majority of ferns are found in the understory of moist tropical forests where low light levels combined with high humidity reduce the need for highly efficient xylem. Filmy ferns (Hymenophyllaceae) are an extreme example: their xylem tissue is reduced to less than a dozen conduits in stipe cross section because the pinnae are usually only a single cell thick and lack both a cuticle and stomata (Proctor and Tuba 2002; Proctor 2012). In the understory, there is simply no need for selection to act on increased leaf venation or hydraulic efficiency. Consequently, the data indicate a tight scaling between functional xylem area and the distal frond area (Limm and Dawson 2010; Pittermann et al. 2011). This may have profound implications for leaf gas exchange. Recent studies suggest that stomatal closure in seed-free vascular plants is initiated at higher water potentials than in angiosperms (Brodribb and Holbrook 2004; Brodribb and McAdam 2011), an adaptive response to impending drought stress because ferns lack the hydraulic redundancy that high vein densities provide in angiosperms. High-density

reticulate venation offers alternative pathways to water transport should localized embolism occur (Scoffoni et al. 2011). It is easy to argue based on current data that limited productivity in ferns is the penalty imposed by primary xylem, but without seeing a parallel evolutionary trajectory of photosynthetic enzyme function, the reasons behind the physiological canalization of ferns may not be fully understood.

Taken together, the evolution of large tracheids and, in some taxa, vessels, coupled with selection for close conduit packing and permeable pit membranes, appears to offset the hydraulic constraints that are inherent to ancestral, tracheid-based xylem tissue. Yet despite their physiological limitations, it is impressive that seed-free vascular plants managed to not merely persist but even thrive in habitats that are dominated by conifers and angiosperms.

4.2   Hydraulic Failure by Cavitation

All vascular plants contend with low water potentials that can arise from any combination of water deficit or high transpiration rates. Low water potentials render the xylem susceptible to cavitation, that is the entry and subsequent expansion of air within the water column that can lead to an air-vapour embolism. Air enters water-filled conduits through pit membranes because they are mechanically the weakest and most permeable regions of the conduit wall. Embolized conduits cannot transport water, so selection has acted on xylem to keep an appropriate safety margin that protects against cavitation while maintaining efficient rates of water transport. Many studies have examined how xylem structure and function balances cavitation safety with hydraulic efficiency in woody plants (see, for instance the Chap. 7) but only a handful of papers have addressed these issues in seed-free vascular plants.

Despite the general perception of seed-free vascular plants as water-loving understory plants, ferns occupy a wide enough range of habitats and niches to expect that xylem would be subject to adaptive selection. For example, roots are commonly reduced in epiphytic ferns, which helps explain why epiphytes may experience mid-day water potentials lower than −4 MPa as compared to co-occurring terrestrial ferns that stay above −2 MPa (Watkins et al. 2010). Patterns of water use may also vary with life history strategies. A recent study showed that sun-exposed individuals of the perennial *W. fimbriata* retain mid-day water potentials above −0.5 MPa, unlike the seasonally deciduous *P. aquilinum*, in which water potentials frequently dropped below −1.5 MPa (Brodersen et al. 2012). In moist, temperate habitats, the minimum seasonal water potentials varied from −0.2 to −1 MPa in canyon-dwelling *Woodwardia*, *Dryopteris* and *Polystichum* exposed to different light levels (Lo Gullo et al. 2010). Water potentials of lycopods, tree ferns and desiccation-tolerant ferns have yet to be measured, and much more work is needed to characterize fully the water relations of seed-free vascular plants in situ.

Although few studies document fern water potentials in the field, the cavitation response of ferns has received significantly more attention. Evaluating species

cavitation resistance involves generating a response curve that shows the percent loss of hydraulic conductivity due to cavitation against progressively decreasing segment water potential. This can be done in any number of ways, including measurement on whole leaves during controlled dehydration (Brodribb and Holbrook 2004), measurement on stipes with the centrifuge method in which water potentials are simulated by spinning at high RPM such that air is pulled in through pit membranes (Alder et al. 1997; Wheeler et al. 2005; Pittermann et al. 2011), and axial air injection whereby air is gradually introduced into one end of a cut stipe, thereby pushing air through pit membranes (Watkins et al. 2010). Several parameters can be derived from this curve, the most widely used being the average water potential at which species experience a 50 % loss of hydraulic conductivity (P_{50}). Early estimates of fern vulnerability to cavitation were performed using whole fronds of tropical species from lowland Costa Rica; these studies generated P_{50} values ranging from about −1.4 to −2 MPa, suggesting that ferns are more sensitive to water deficit than angiosperms (Brodribb and Holbrook 2004). However, in a broad survey of tropical epiphytic and terrestrial fern taxa, Watkins et al. (2010) applied the axial air-injection method to show that P_{50} values can vary from a maximum of −0.4 MPa in terrestrial pteridophytes to a minimum of −4.1 MPa in the cavitation-resistant, epiphytic *Elaphoglossum herminieri* (Bory & Fée) T. Moore. Taken together, P_{50} values in the epiphytes were below −2.5 MPa and significantly lower than what was measured in the terrestrial ferns, an adaptive response consistent with expectations. A broader range of values was reported by Pittermann et al. (2011) who used the centrifuge method to show that several species were unusually cavitation resistant including the perennial *Dryopteris arguta* (Kaulf.) Maxon, *W. fimbriata* and *Pteris cretica* L., which showed P_{50} values ranging from −4 to below −6 MPa, not unlike the P_{50} range experienced by woody plants (Pittermann et al. 2013). The functional significance of such seemingly resistant xylem is unclear because it's not known whether these species experience such low water potentials in situ. One possibility is that low P_{50} values may endow these plants with a significant safety margin to buffer the xylem against excessive cavitation when seasonal water potentials reach their minimum. In these Mediterranean climate-adapted perennials, a several MPa buffer may mean the difference between mere survival versus growth and reproduction. Aside from the Watkins et al. (2010) study, there are few field data with which this question can be explored and certainly no long-term field measures to demonstrate a definitive connection between seasonal water potential and spore production.

   The costs of cavitation resistance are well understood in woody plants but much less is known about this in ferns. In cavitation resistant conifer xylem, reduced tracheid diameters and thicker tracheid walls lead to lower specific conductivities and higher wood density because narrower, more reinforced tracheids are necessary to withstand the hoop and bending stresses imposed by the water column during drought (Pittermann et al. 2006). This is quantified by the conduit double-wall thickness (t) to lumen diameter (b) ratio $(t/b)^2$ whereby higher values are associated with denser, more cavitation-resistant xylem (Hacke et al. 2001). Similar costs are

evident in angiosperm xylem in which narrower, shorter vessels are mechanically reinforced by thick-walled fibres (Jacobsen et al. 2005). Taken together, xylem structure and function in woody plants is guided by a reasonably predictive safety versus efficiency framework in which cavitation-resistant species with a low P_{50} exhibit reduced transport efficiency and greater xylem construction costs (Hacke et al. 2001; Pittermann 2010). However, no such costs have been identified with cavitation resistance in ferns; neither hydraulic efficiency nor the $(t/b)^2$ metric vary predictably across the broad range of P_{50} values sustained by fern xylem (Watkins et al. 2010; Pittermann et al. 2011). Given that ferns are not equally cavitation resistant, what might drive the observed variation in P_{50}? Brodersen et al. (2012) suggested that vascular organization may control the spread of air but possibly at the cost of hydraulic efficiency. For example, *P. aquilinum* has many vascular bundles that frequently fuse and divide along the length of the frond, a pattern that may reduce hydraulic resistance by creating multiple pathways for water transport, but by the same token, also providing multiple opportunities for network-wide embolism by air-seeding. The P_{50} of *P. aquilinum* ranges from −1.23 to −2.23 MPa, while K_s can exceed 40 kg MPa^{-1} m^{-1} s^{-1} (Pittermann et al. 2011; Brodersen et al. 2012). The opposite pattern was observed in the more cavitation-resistant *W. fimbriata* (P_{50} = −1.11 to −5.21 MPa), in which two large and three smaller vascular bundles were infrequently bridged by small, lateral bundles (Brodersen et al. 2012). The K_s of this species ranges from 10 to 42 kg MPa^{-1} m^{-1} s^{-1} (Pittermann et al. 2011; Brodersen et al. 2012). The anatomical attributes of the vascular bridges were not evaluated, but wider tracheids were consistently observed in the large vascular strands leading Brodersen et al. (2012) to speculate that these perform the majority of water transport, with the smaller bundles acting as a hydraulic reserve should the large bundles fail by embolism. Manipulative experiments indicate that in *W. fimbriata*, the small vascular strands are indeed more resistant to embolism (Brodersen et al. 2012). Whether similar patterns are found in other species remains to be seen.

The safety-efficiency trade-off is also in large part determined by pit membranes, the function of which has been well-studied in both seed-bearing and seed-free vascular plants. The pit membranes of ferns are similar enough to the homogenous pit membranes of angiosperms to allow for some comparisons to be drawn. Typically, thicker pit membranes are associated with higher air-seed pressures, meaning that lower water potentials (or greater air pressures) are required to draw (or push) air across the largest pore present in a pit membrane (Choat et al. 2005; Brodersen et al. 2014). Air-seed pressures are determined experimentally by injecting air into single conduits with glass capillaries and watching for air bubbles at the distal cut end of a stem segment, which is immersed in water (Zwieniecki et al. 2001). In contrast to angiosperms, thicker pit membranes in seed-free vascular plants are only weakly associated with greater air-seed pressures, suggesting that membranes may not be the most direct target of selection (Jansen et al. 2009; Lens et al. 2011; Brodersen et al. 2014). Instead, air-seed pressures were more consistently related to conduit diameter, such that smaller conduits with smaller pit membranes and overall lower pit membrane areas required higher air injection

pressures to penetrate the pit membrane (Brodersen et al. 2014; Fig. 1.5b). Smaller pit areas reduce the probability that a large membrane pore will be present acting as the so-called weak link, that allows air to propagate from one conduit to another (Wheeler et al. 2005; Jansen et al. 2009). Functionally, this implies that species with narrow conduits should exhibit low specific conductivities but with the benefit of greater cavitation resistance, while the opposite should be true in taxa with large, hydraulically-efficient conduits (Brodersen et al. 2014). While this was found to be true across tropical terrestrial ferns (Watkins et al. 2010), epiphytic taxa showed no relationship between tracheid diameter, cavitation resistance and K_s despite the clear differences in conduit size (Figs. 1.8 and 1.10; Watkins et al. 2010).

It is not known whether all seed-free vascular plants recover from embolism but desiccation-tolerant plants may hold some clues. Many species of ferns and lycophytes have retained the ability to lose over 95 % of their water yet fully regain their photosynthetic and metabolic activity within hours or days after re-watering (Proctor and Tuba 2002). Poikilohydry is found in small plants such as bryophytes, seed-free vascular plants, and even herbaceous angiosperms, but it is for the most part considered an ancestral trait (Proctor and Tuba 2002; Sperry 2003). However, selection favoured the reversal from homoiohydry to poikilohydry in the filmy fern genus *Hymenophyllum* insofar as members of this genus exhibit highly reduced xylem tissue as well as pinnae that are only one cell thick with no stomata (Proctor 2012). These delicate ferns measure less than 20 cm in height, so desiccation and recovery represent a more adaptive strategy than homoiohydry. How the xylem refills in poikilohydric ferns is unclear but desiccation-tolerant taxa such as *Selaginella lepidophylla* (Hook. & Grev.) Spring and numerous cheilanthoid ferns typically have narrow xylem conduits so capillarity may be sufficient to drive hydraulic recovery. Metabolically assisted refilling along the lines of Secchi and Zwieniecki (2011) cannot as yet be ruled out. Given that *S. lepidophylla* has a weakly developed to non-existent root system and still recovers from complete desiccation, root pressure is unlikely to play a role in embolism reversal.

4.3 Ecological Fern Hydraulics: The Role of Polyploidy in Fern Physiology and Hydraulics

No review of fern physiology would be complete without discussion of the role that polyploidy and introgression play in shaping niche partitioning. Polyploidy is a common phylogenetic occurrence across ferns (Manton 1950; Wood et al. 2009). Ecologically, polyploidy in ferns is important as many of our modern temperate landscapes are dominated by polyploid and/or hybrid ferns. In a recent community survey of *Dryopteris* species in a mesic 20 ha plot at the Huyck Preserve in New York, 80 % of species encountered were either polyploids or hybrids, and 85 % of the individuals encountered fell in this category (Watkins unpublished data). Polyploids, especially those of recent origin, can be constrained during

reproduction for lack of compatible cytotypes (Levin 1975). In theory, this could result in reduced matings relative to parents. Yet, polyploids persist and often come to dominate in some cases (but see Buggs and Pannell 2007). One potential mechanism of this maintenance is ecophysiological niche differentiation across ploidy levels. If polyploids are more physiologically capable (e.g. greater hydraulic conductance, higher photosynthetic rates and increased spore production) than diploids, the former could attain an equal or greater ecological footing (DeBodt et al. 2005; Fawcett et al. 2009; Coate et al. 2011; Testo et al. 2014). Whereas some studies have shown this relationship (e.g. Kao and Parker 2010; Warner and Edwards 1993; Li et al. 1996, 2009), others have found no differences or the opposite pattern (e.g. Baack and Stanton 2005). Our understanding of how physiological differences relate to ploidy is limited. One of the most thorough efforts to date has been Sessa's (2012, 2014) work on the genus *Dryopteris*. In a series of studies the authors found that allopolyploids were frequently transgressive in field ranges, yet they failed to find any relationship of carbon relations with ploidy and no evidence of introgression in physiological characters in allopolyploid hybrids.

Some authors have speculated that xylem attributes vary with plant ploidal levels. Stebbins (1971) observed that cell size increases with ploidy, and Pockman and Sperry (1997) suggested that ploidy-driven differences in vessel size may drive population distributions of *Larrea tridentata* in the American southwest. Hence, a priori predictions are that the xylem of polyploid ferns will comprise wider and longer conduits that are more likely to lead to higher transport rates. Ferns may be the ideal system in which to study the hydraulic consequences of polyploidy, and recent studies by Watkins (unpublished data) have examined xylem structure and function in the wood fern *Dryopteris*, a common genus in temperate North American forests. The genus comprises 11 species that include six diploid taxa and five allopolyploid taxa that vary in ploidy. In a survey of tracheid lengths from herbarium sheets of ten taxa (6–9 individuals each), there was a general increase in length with ploidal level (see tracheid length in Fig. 1.9a). Diploid species and triploid hybrids produced similar tracheid lengths while tetraploids, pentaploids and hexaploids were significantly longer (Fig. 1.9a). In an effort to evaluate the association of hydraulics with ploidal level and/or parent:hybrid relationships, Watkins surveyed xylem conductance in five taxa in northern regions of New York state. *Dryopteris cristata* (4n) hybridizes with *D. intermedia* (2n) to produce the 3n *D.×bootii*. *Dryopteris intermedia* also hybridizes with 4n *Dryopteris carthusiana* to produce another sterile hybrid, 3n *D.×triploidea*. K_s was evaluated across parents and hybrids and *D.×bootii* was significantly higher and thus transgressive in K_s relative to either parent (see K_s in Fig. 1.9b), *Dryopteris×triploidea* had similar values of K_s to both parents. Curiously, *Dryopteris×bootii* tends to be quite rare and frequently limited to wet swampy sites; whereas *D.×triploidea* can be abundant in some forests and in this upstate New York site was more common than 4n *D. carthusiana*. The mechanism controlling the distributions of hybrids and diploid/polyploidy ferns is unknown, yet the data presented here suggest that greater exploration of the role of ploidy and hybrid origin is warranted.

4.4 Comparative Hydraulics of Epiphytic and Terrestrial Ferns

Our understanding of fern ecology has grown significantly in recent years (Ranker and Haufler 2008; Mehltreter et al. 2010). Of particular interest in the study of fern biology are the functional differences between epiphytic and terrestrial ferns (Hietz and Briones 1998; Watkins and Cardelus 2012, 2009; Watkins et al. 2007a, b, c). Like many other lineages, ferns experienced a major radiation following the Cretaceous. A significant proportion of the diversity of this radiation is of epiphytic ferns that rapidly entered the novel angiosperm-dominated canopy (Watkins and Cardelus 2012 and references therein). Comparative studies of both gametophytes and sporophytes of epiphytic and terrestrial ferns have revealed unique suites of functional characters associated with each group. Watkins et al. (2007a, b) have shown that the gametophytes of epiphytic ferns are significantly longer lived, are less likely to recruit from the gametophyte stage into the sporophyte stage and are more stress tolerant than terrestrial species (Watkins et al. 2007a; Pittermann et al. 2013). Studies on comparative sporophyte hydraulics have shown that epiphytic ferns have significantly reduced leaf and xylem K_s and increased P_{50} values relative to terrestrial species. Watkins et al. (2010) and Watkins and Cardelus (2012) have suggested that increased P_{50} values may have come at the expense of hydraulic conductivity, and that the reduced stipe lengths commonly observed in epiphytic ferns may be a direct response to reduce path lengths to water movement. A survey of total vascular area in the stipes of several tropical species of terrestrial, epiphytic, root climbing and hemiepiphytic ferns shows that terrestrial species produce more total vascular area than other growth forms and that epiphytic species produce the least area. This pattern holds when total leaf area is taken into account. These data suggest that significant and unappreciated gross differences exist in the vascular system of different fern growth forms.

Diameter measurements of hundreds of individual tracheids from more than two dozen species reveal a fundamental difference in the frequency of tracheid size classes (see Fig. 1.10). Epiphytic and hemiepiphytic species have similar tracheid diameter distributions with a broad normal distribution with many small diameter tracheids (Watkins, unpublished data). On the opposite end of the spectrum, terrestrial species can be characterized by having relatively few tracheids with small diameters and large proportion of conduits with large diameters. On average, terrestrial species produced individual tracheids that were twice as large in diameter compared to epiphytic species (Fig. 1.10). Whereas pit membrane attributes play a critical role in cavitation resistance, reduced pit area, and thus small-diameter tracheids, have been shown to limit the spread of air from one conduit to another (Brodersen et al. 2014). The epiphytic habitat in the canopy is frequently drought stressed, and such conditions could select for reduced tracheid diameters in an effort to avoid cavitation. The four hemiepiphytic species surveyed were all primary, as defined by Canestraro et al. (2014), establishing first as epiphytes and then connecting and never losing connection to the forest floor. These four species had similar

diameter distributions as epiphytic taxa, yet all fronds sampled were from mature individuals that were rooted in the terrestrial soil matrix.

Earlier isotopic and nutrient analyses suggest that both water and nutrient relations in hemiepiphytic ferns are more similar to terrestrial than epiphytic species (Watkins et al. 2007c). Based on phylogenetic evidence, hemiepiphytic species can arise from terrestrial, epiphytic or terrestrial root-climbing ancestors. Terrestrial root-climbers are outwardly similar to hemiepiphytes (in fact this group is frequently misidentified as hemiepiphytes) but never lose terrestrial connections (Canestraro et al. 2014). These species were more similar to terrestrial species than hemiepiphytic taxa. Interestingly, Canestraro et al. (2014) have recently shown that photosynthetic rates can vary between terrestrial and climbing stages of some root-climbers, yet not in others. There is a need to understand better the changes in functional anatomy and ecophysiology across taxa that change life forms. Such information will be critical in our quest to understand the evolution of epiphytic ferns and the radiation of lineages into novel habitats. Our sampling is small and the phylogeny of the surveyed species too poorly understood to make detailed categorizations. However, it is possible that hemiepiphytic species, even when derived from terrestrial ancestors, are canalized in tracheid anatomy. The selective pressures induced by drought may overwhelm pressures for increased growth and reproduction. Terrestrial root-climbers may gain the same benefit as hemiepiphytes (i.e. increased height that results in exploration of higher light levels, increased spore dispersal distances) without the added complexities of an epiphytic stage. Clearly more work needs to be done on the comparative xylem anatomy of these taxa.

5   Evolutionary Patterns in Fern Xylem Structure

Vascular arrangements in fern and lycophytes have fascinated botanists since the nineteenth century and even today, variation in stelar patterns is useful for taxonomic identification (Bower 1923; Ogura 1972). An interest in developmental processes rather than functional attributes motivated earlier studies, but the desire to document stelar structure in a broader evolutionary context (including fossil taxa) was the common thread of Bower's and Ogura's discourses. Beck et al. (1982) contributed a seminal paper that not only explored the known vascular arrangements of stems and rhizomes across extinct and living land plants but also formalized stelar nomenclature. When it comes to understanding the evolutionary and functional significance of stelar structures, modern phylogenetic approaches allow us to build on these contributions with greater resolution and thus extend the realm of possibilities; we are now able to map structural and physiological attributes onto the fern phylogeny to document whether stelar patterns originated independently in response to common selective pressures, whether the observed arrangements appear randomly or whether directional selection favoured specific phenotypes over others. These evolutionary patterns are not mutually exclusive and separate clades may reveal different trends.

Here we have documented stelar patterns in frond petioles (stipes) from 182 fern species. Cross sections were obtained from a variety of sources, including new hand-sections from fresh or herbarium material, published sources such as Ogura (1972) and Bower (1923), and online repositories (e.g. plantsystematics.org). From these data, we identified a consensus stele type for each currently recognized family and mapped this across the family-level phylogeny (see Fig. 1.11). Formal reconstructions have yet to be conducted, but several key patterns emerge from this phylogenetic analysis.

First, it is evident that some of the early diverging families, such as Psilotaceae and Equisetaceae, possess single vascular strands (protosteles) in their highly reduced leaves, which are developmentally homologous with the fronds of higher pteridophytes. The familiar *Psilotum nudum* stems also have protosteles but the vascular arrangement in equisetalean stems appears to have undergone some degree of duplication, as multiple strands of primary xylem and associated lacunae encircle the stem cylinder. Plants are modular organisms, so duplication or extension of the protostele may have also occurred in the Ophioglossaceae and Marattiaceae, leading to polycyclic steles in the latter (Schneider et al. 2002). Iteration of vascular strands is consistent with the greater complexity of these two lineages: *Ophioglossum* develops one lateral megaphyll while the polycyclic Marattiales develop multiple fronds that may be up to five-pinnate. The Marattiales have some of the largest fronds among the pteridophytes.

C-shaped vascular strands with variously curling ends are present in the Osmundaceae, Gleicheniaceae, Dipteridaceae and Matoniaceae (Fig. 1.11). Superficially these resemble an incomplete solenostele. As previously mentioned, the Hymenophyllaceae have adopted a poikilohydric strategy and thus exhibit highly reduced xylem. A return to a central, protostelic vascular strand is evident in the schizaeoid clade, which includes the climbing Lygodiaceae, as well as the Anemiaceae and Schizaeaceae. A cylindrical vascular bundle may be adaptive in the Lygodiaceae because torsional and compressive stresses are more equally distributed than in an asymmetrical stele. As in the Hymenophyllaceae, minimized xylem is evident in the aquatic ferns belonging to Marsileaceae and Salviniaceae that require little or no water transport.

The mostly arborescent families comprising the tree fern clade (Thyrsopteridaceae, Loxsomataceae, Culcitaceae, Plagiogyriaceae, Cibotiaceae, Cyatheaceae, Dicksoniaceae and Metaxyaceae) are generally characterized by large fronds, and this is reflected in their complex, often highly divided stelar structures that may resemble folded ribbons. As in the Marattiaceaeles, the fronds are frequently highly divided so the many iterative bundles of these distantly related lineages may represent convergent evolution.

The steles of the early-diverging polypod families (Saccolomataceae, Cystodiaceae, Lindsaeaceae, Lonchitidaceae, Dennstaedtiaceae and Pteridaceae) are described generally as an omega shape, with some variation in the Cystodiaceae and Lonchitidaceae. Several species with large, multi-pinnate leaves belong to the Dennstaedtiaceae and Pteridaceae, so folded vascular ribbons are also present in the petioles (Fig. 1.2e; Vasco et al. 2013). Fronds of some Pteridaceae such as the genus
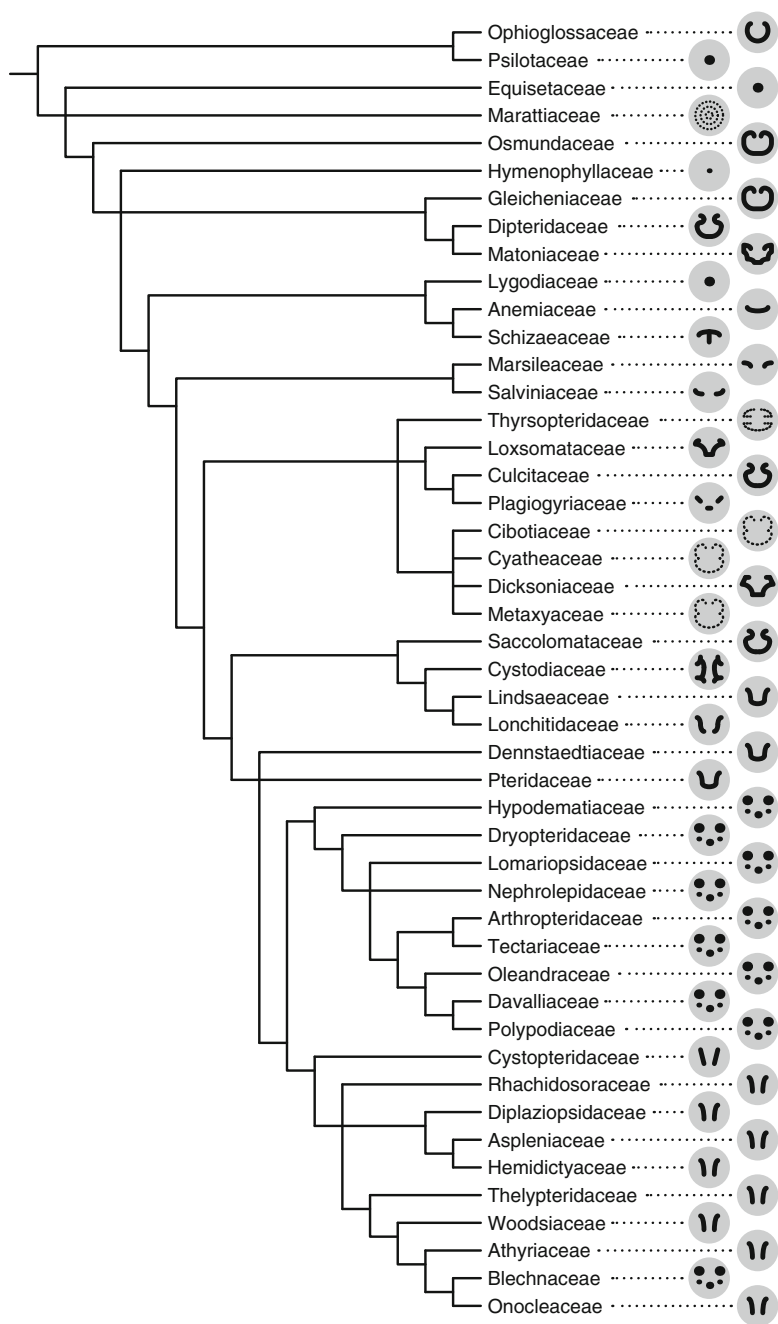
Fig. 1.11 Mapping petiole stelar types onto the fern phylogeny. The phylogenetic relationships and classification presented outside of polypod ferns are based on Smith et al. (2006). Those within eupolypods I follow Liu et al. (2014) and those within eupolypods II follow Rothfels et al. (2012). Other relationships shown reflect those in Schuettpelz and Pryer (2009), with the classification based on Christenhusz et al. (2011). Stelar types represent a consensus for each family from available cross sections. See text for details

Pteris, can exceed 2 m in length but unlike the highly divided dictyosteles of similarly sized tree fern fronds, *Pteris* petiolar steles are usually single stranded. The recently discovered hybrid *Pteris* × *caridadiae* is an interesting exception because its vascular strand is interrupted despite maintaining the overall omega shape of its parent plants (Testo et al. 2015).

Distinct vascular patterns arise in the two most recently derived clades on the fern phylogeny: the eupolypods I (Hypodematiaceae, Dryopteridaceae, Lomariopsidaceae, Nephrolepidaceae, Arthropteridaceae, Tectariaceae, Oleandraceae, Davalliaceae, Polypodiaceae) and eupolypods II (Cystopteridaceae, Rhachidosoraceae, Diplaziopsidaceae, Aspleniaceae, Hemidictyaceae, Thelypteridaceae, Woodsiaceae, Athyriaceae, Blechnaceae, Onocleaceae). The steles in these groups are dictyostelic as they always comprise at least two major vascular bundles. Several smaller strands accompany the two large bundles in the eupolypod I lineage and in the Blechnaceae of the eupolypod II clade (Figs. 1.2c, d and 1.11). So reliable are these patterns that they are frequently used for the purpose of identification.

Understanding how selection acted on stelar patterns in ferns requires consideration of paleoclimate change as well as biome assembly in tropical and temperate regions. Recent phylogenetic approaches have revealed compelling trends in fern diversity that largely point to the post-Cretaceous speciation and radiation of epiphytic taxa. Global climatic shifts may have contributed to this pattern: recent studies of adaptive radiations in monocot, succulent and arid-adapted conifer lineages are consistent with Cenozoic aridification (Edwards et al. 2010; Arakaki et al. 2011; Pittermann et al. 2012). However, equally relevant are several analyses that suggest diversification of fern epiphytes occurred in response to the expansion of complex, angiosperm-dominated forests (Schneider et al. 2004; Schuettpelz and Pryer 2009; Watkins and Cardelus 2012). Release from competition on the crowded forest floor and an increase in light availability may have driven the move to the canopy, but selection for increased cavitation resistance (greater P_{50}), thicker leaf cuticles, and desiccation tolerance in both sporophytes and gametophytes may have allowed epiphytic ferns to gain traction in what is generally a dryer and more climatically variable habitat than in the understory (Watkins et al. 2007a; Dubuisson et al. 2009; Schuettpelz and Pryer 2009; Watkins and Cardelus 2012). Although the speciation of epiphytes stands out in the post-Cretaceous evolutionary trajectory of ferns, the concurrent diversification of eupolypod I terrestrial ferns also warrants a closer look; the formation of temperate forests and drier biomes may have selected for specific physiological attributes associated with greater drought tolerance in non-tropical taxa.

The exclusive presence of dictyosteles in the petioles of more derived fern lineages suggests that xylem structure was under strong selection for drought resistance during the Cenozoic. Sectored dictyosteles in the eupolypods I and II comprise at least two vascular strands, sometimes more (Figs. 1.3 and 1.11). Wider conduits occupy the larger strands, so a broad range of tracheid sizes would ensure that alternative pathways for water movement are available should transport in one of the large bundles be hampered by embolism (Brodersen et al. 2012). In contrast, the mostly integrated C- or omega-shaped stelar arrangements in the early-derived fern groups may increase the probability of air rapidly spreading throughout the

xylem due to close contact between conduits. Selection for more numerous narrower tracheids in epiphytic taxa would drive increased cavitation resistance due to greater conduit redundancy but also because reduced pit area will lower the likelihood of air-seeding (Wheeler et al. 2005; Christman et al. 2009; Brodersen et al. 2014). A broader sampling across the fern phylogeny is needed to gain a more nuanced perspective on the evolution of xylem structure and function in ferns, particularly those in the eupolypod I and II lineages.

Other vascular attributes may factor into the success of eupolypod ferns. In a phylogenetically extended survey, Hernandez-Hernandez et al. (2012) indicated an increased presence of the circumendodermal band in more derived ferns. Resembling a thick casparian strip, this band encapsulates the xylem and phloem tissues (Fig. 1.2b). A number of hypotheses have been proposed to explain the presence of this structure. For example, this waxy layer may increase frond structural rigidity (Hernandez-Hernandez et al. 2012), an important trait in many epiphytes in which fronds are displayed at steep angles and vulnerable to damage by falling canopy debris. Epiphytic ferns may also be capable of foliar water absorption, so biomechanical needs may trump xylem-mediated water transport in humid habitats. Foliar absorption is a critical water-use strategy for plants in the redwood forest understory, especially for *P. munitum* (Kaulf.) C. Presl which derives almost all of its water from the frequent fog events that blanket the redwood forests of northern California (Limm et al. 2009; Limm and Dawson 2010). The aggregate of vascular and leaf traits that underpin the physiology of eupolypod ferns is a topic of much interest and sure to reveal novel physiological and structural solutions to life in the canopy.

6 Concluding Remarks

There are many possibilities for research on the physiology, ecology, evolution and paleobotany of seed-free vascular plants. At the organismal scale, it is not unreasonable to propose that the absence of critical features such as bifacial vascular cambia and lateral branching restricted the phenotypic diversity of ferns and lycopods. Indeed, the body plan of seed-free tracheophytes couldn't be more different than that of eutracheophytes. Though incomplete, the fossil record shows this to be true; with an arborescent canopy, ancient seed-free plants such as the progymnosperms explored a broader morphospace than modern pteridophytes (Meyer-Berthaud et al. 1999, 2000). However, the limits and possibilities of phenotypic variation in ferns have not been considered to the degree that they have in woody plants. For example, Niklas' adaptive walks through the plant kingdom mimic evolutionary processes, and provide multiple morphological solutions that balance conflicting needs of reducing surface area whilst enhancing mechanical stability, light capture, and spore dispersal (Niklas 1992, 1999). The model relies on the predictable but spatially variable bifurcation of branches such that model outputs result in highly variable yet structurally conserved phenotypes, many of which closely resemble today's angiosperm and conifer flora (Niklas 1999). The branching assumptions would

require adjustment to reflect growth patterns of modern ferns but the model does provide a solid framework for coupling vascular anatomy with frond patterns in the context of basic physiology and reproduction.

The interplay of form and function should not only be limited to extant taxa. Michael Cichan's work with woody Carboniferous plants was prescient; not only was he able to infer developmental patterns of unifacial vascular cambia from fossils, but he applied the standard Hagen–Poiseuille model of fluid flow to model water transport in several groups of lianoid and arborescent plants (Cichan 1985a, b, 1986). Wilson and colleagues took this approach one step further by modelling both transport efficiency and cavitation resistance in a range of taxa including *Asteroxylon mackiei* (see illustration in Fig. 1.6) and *Medullosa*, a Carboniferous seed fern (Wilson et al. 2008; Wilson and Knoll 2010; Wilson and Fischer 2010). Their models suggest that unusually wide and long conduits coupled with permeable membranes may have supported high conductivities in the woody *Medullosa*, but by the same token, this suite of traits likely rendered these plants vulnerable to cavitation. Judging by the anatomical similarity of their xylem, especially the bordered pit structure, the progymnosperms such as *Archaeopteris-Callixylon* (see micrographs in Fig. 1.7) may have had similar vascular physiology. New methods such as X-ray synchrotron tomography have recently revealed the xylem and even the inter-conduit pit structure of an early Devonian woody plant, showing conduits in excess of 70 µm in diameter (Strullu-Derrien et al. 2014). The suggestion that this ancient plant was vulnerable to embolism agrees with the opinions of Wilson (2013), who proposed that high transport efficiency evolved many times over the past 400 million years whilst cavitation resistance lagged behind. Testing this hypothesis may mostly be a matter of locating appropriate fossil material since technologies that improve specimen visualization are developing quite rapidly.

Seed-free vascular plants are highly diverse with respect to their ecology and morphology so there is much to be learned about the ecophysiological attributes that support this variation. There is little doubt that the dictyosteles of recently derived eupolypod ferns were under strong selective pressures during the Cenozoic but a thorough sampling regime will be necessary to uncover the tempo and evolutionary trajectory of traits such as xylem area, tracheid size, vascular connectivity, cavitation resistance and hydraulic efficiency. Additional measures of allometry, capacitance, stomatal density and leaf venation patterns will greatly enhance our understanding of how co-ordinated hydraulic function evolved in ferns and lycophytes. An alternative and equally interesting possibility is that morphological diversity is not the product of natural selection per se, but rather neutral state transitions that confer neither a cost nor benefit to the plant. Fern sporophytes are incredibly diverse with respect to size and shape, and given the co-occurrence of different frond structures in similar habitats (Watkins et al. 2010; Vasco et al. 2013), the physiological consequence of this variation may be subtle or nil. Instead, selection for mechanical stability, spore dispersal, longevity, gametophyte biology, edaphic type or other aspects of life history strategy may govern leaf shape (Creese et al. 2011; Watkins et al. 2007a, b, c; Pittermann et al. 2013). The physiological attributes

of extant ferns and lycophytes should be well understood if we are to make any reasonable inferences about the drivers of sporophyte evolution over deep time, but the vasculature is but one element of a complex suite of traits that contribute to the success of seed-free vascular plants.

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