New Phytologist

Review

Tansley review

Evolution of development of vascular cambia and secondary growth

Author for correspondence:
Andrew Groover
Tel: +1 530 759 1738
Email: agroover@fs.fed.us

Received: *29 December 2009*
Accepted: *14 February 2010*

Rachel Spicer[1] and Andrew Groover[2]

[1]The Rowland Institute at Harvard, Cambridge, MA, USA; [2]Institute of Forest Genetics, Pacific Southwest Research Station, USDA Forest Service, Davis, CA, USA

Contents

New Phytologist (2010) **186**: 577–592
doi: 10.1111/j.1469-8137.2010.03236.x

Key words: forest trees, genomics, *Populus*, wood anatomy, wood formation.

Summary

Secondary growth from vascular cambia results in radial, woody growth of stems. The innovation of secondary vascular development during plant evolution allowed the production of novel plant forms ranging from massive forest trees to flexible, woody lianas. We present examples of the extensive phylogenetic variation in secondary vascular growth and discuss current knowledge of genes that regulate the development of vascular cambia and woody tissues. From these foundations, we propose strategies for genomics-based research in the evolution of development, which is a next logical step in the study of secondary growth.

I. Introduction

Secondary vascular growth provides a means of radially thickening and strengthening plant axes initiated during primary, or apical growth. Although secondary vascular growth is often equated with the woody growth that distinguishes trees and shrubs from herbs, the process exhibits remarkable variation in nature. The 'typical' woody stem develops a single bifacial (i.e. bidirectional) cambium that produces secondary phloem (inner bark) externally and secondary xylem (wood) internally (Larson, 1994). Although this pattern characterizes most extant forest trees, significant variation exists among taxa, ranging from extinct woody lycopods and horsetails with unifacial cambia (Cichan & Taylor, 1990; Willis & McElwain, 2002), to angiosperms with multiple cambia functioning simultaneously (Carlquist, 2007) or with disjunctive cambia that produce secondary xylem furrowed by wedges of phloem (Pace *et al.*, 2009). Dramatic variation in secondary vascular development can also occur within individual plants in response to environmental conditions and seasonal cues. For instance, some woody plants produce distinctive 'tree rings' of xylem in

climates punctuated by cold or dry seasons, whose widths and physical properties reflect the environmental conditions of that year (e.g. de Kort, 1993). Similarly, mechanical perturbation stimulates the production of specialized secondary xylem called 'reaction wood' (Du & Yamamoto, 2007).

The natural variation found in both the process and the products of secondary vascular growth suggests that it encompasses a plethora of adaptive traits under complex selective pressures. Indeed, the physiological roles of wood are critical to plant survival, as woody tissues provide the mechanical support for complex and sometimes massive body plans that provide an advantage in the competition for light. Wood structure also determines the resistance to water flow from roots to leaves, the capacity for water storage, and resistance to drought- and freeze-induced embolism (Domec *et al.*, 2008; Choat & Pittermann, 2009; Poorter *et al.*, 2009). Secondary phloem serves a crucial role in the efficient long-distance transport of carbohydrates and signaling molecules throughout the stem (Lough & Lucas, 2006). In addition, secondary xylem and phloem both function in carbohydrate storage. The ability to alter secondary vascular growth in response to environmental changes and external stimuli is thus of high adaptive significance, but requires the coordination of complex developmental events to produce appropriate tissues and physiological outcomes. These and related observations suggest that secondary vascular growth involves highly plastic developmental processes, which are reflected in extensive anatomical and functional variation both within individual plants and among taxa, particularly in angiosperms (Carlquist, 2001).

An understanding of the evolutionary histories and developmental mechanisms that underlie secondary vascular growth will ultimately require phylogenetic analysis of anatomical variation and the genes regulating corresponding developmental processes. Anatomical variation in secondary xylem (i.e. wood) is well characterized and is the focus of an extensive classical literature describing wood anatomy for a large number of species (Zobel & Van Buijtenen, 1989; Carlquist, 2001), although unfortunately ontogeny is rarely described. Woody tissues are also often well preserved during fossilization such that wood anatomy has been described for diverse extinct species (Willis & McElwain, 2002; Taylor *et al.*, 2009). By contrast, the majority of research on secondary vascular growth and wood formation using molecular genetic and genomic approaches has been limited to a modest number of forest tree species with economic relevance. Notably, *Populus* has been developed as a model genus complete with full genome sequence (Tuskan *et al.*, 2006), advanced genomic tools, transformation, and molecular techniques to assess gene expression and function. Although important insights are emerging regarding the mechanisms underlying secondary vascular growth in *Populus* (reviewed in Groover *et al.*, 2010), our understanding of development is incomplete and there are a limited number of well-characterized regulatory genes. Importantly, little effort has been given to comparative studies that would provide a comprehensive view of the ancestral regulatory mechanisms or the evolutionary histories of observed phylogenetic variation (Cronk, 2001). This situation is likely to change given advances in sequencing and other genomic technologies that are delivering increasingly powerful tools at lower costs. However, appropriate strategies need to be devised to maximize the effectiveness of comparative studies.

The aim of this review is to develop foundations for an emerging field of evolution of development ('evo-devo') for secondary vascular growth. We first draw on an extensive classical literature to provide illustrative examples of variation in the activities of vascular cambia and the anatomy of secondary vascular tissues. This is followed by a synthesis of current knowledge regarding the molecular genetic regulation of secondary vascular growth, and some of the evolutionary insights flowing from this knowledge. We end with a discussion of strategies for evolution of development research on cambial functions and wood formation, including the establishment of new model species and comparative studies that harness the power of genomic technologies.

II. Generalized function of vascular cambia and their developmental and evolutionary origins

As outlined below in Section 1, the basic function of vascular cambia is in thickening plant axes with secondary xylem and phloem. Although often treated as two distinct stages in development, transitions from primary to secondary growth are gradual. Nonwoody herbs and large woody trees can be thought to represent two ends of a continuum, and the degree of woodiness expressed by a given plant can be influenced by environmental conditions. While current evidence suggests that the cambia of extant seed plants are homologous, vascular cambia have arisen independently in other vascular plant lineages, and variation in cambial activity and the anatomy of secondary vascular tissues can be found at various taxonomic levels.

1. Vascular cambia produce secondary xylem and phloem

A practical distinction between primary and secondary growth is that primary growth is responsible for elongation at the tips of plant axes, whereas secondary growth is responsible for the thickening of plant axes. It should be noted that, for the purposes of this review, we focus our discussion of secondary growth on secondary vascular growth in stems from vascular cambia, and do not address other types of secondary growth, such as production of cork from phellogen, or secondary thickening in monocots. The vascular cambia found in extant gymnosperms and angiosperms produce secondary xylem to the inside (centripetally) and

secondary phloem to the outside (centrifugally), and are therefore described as 'bifacial'. The vascular cambium is composed of meristematic cells called 'initials' that are perpetually regenerated: when an initial divides it produces a mother cell and another initial (Fig. 1) (Esau, 1977; Larson, 1994). Phloem and xylem mother cells typically divide one or more times before differentiating into mature cell types (Bannan, 1968). Although the dividing initials are thought to exist as a single layer of cells, the initials and mother cells are collectively referred to as the 'cambial zone', as it is often impossible to identify which cells represent the true initials based on cell morphology. Two types of initials exist – fusiform and ray – which together produce all cell types that make up secondary xylem and phloem. Fusiform initials are elongated axially and produce all longitudinally oriented cells, whereas ray initials are roughly isodiametric, arranged in groups called 'rays', and produce all radially oriented cells.

Fusiform initials can divide either periclinally or anticlinally (Fig. 1). Periclinal divisions predominate and occur parallel to the surface of the axis, producing new xylem and phloem. The relative rate of periclinal divisions need not be equal across the cambial zone, and it is common for periclinal divisions to occur more frequently on the xylem side such that more xylem cells are produced than phloem cells (Esau & Cheadle, 1955). As more xylem is deposited and the diameter of the stem increases, the cambium expands circumferentially by adding new initials through anticlinal divisions, which occur perpendicular to the surface of the stem. A record of these divisions is preserved in the form of radial cell files. Anticlinal divisions are indicated where two xylem cell files emanate from a single file. In addition, cambial initials are frequently lost from the cambium and replaced through anticlinal divisions (Bannan, 1951, 1965), consistent with the maintenance of a stem cell niche in plant apical meristems (Sharma *et al.*, 2003). Evidence that positional information is involved in specifying cell fates for cambial initials and their derivatives (Wilson, 1978; Sundberg & Uggla, 1998) suggests that the vascular cambium, like other plant meristems, relies on spatial signaling and position (as opposed to lineage) to determine patterning and cell differentiation (Kidner *et al.*, 2000).

Striking variation can be found among extant species in the development of vascular cambia, the extent of their activity, and the secondary vascular tissues derived from them. The origin of a vascular cambium is often presented

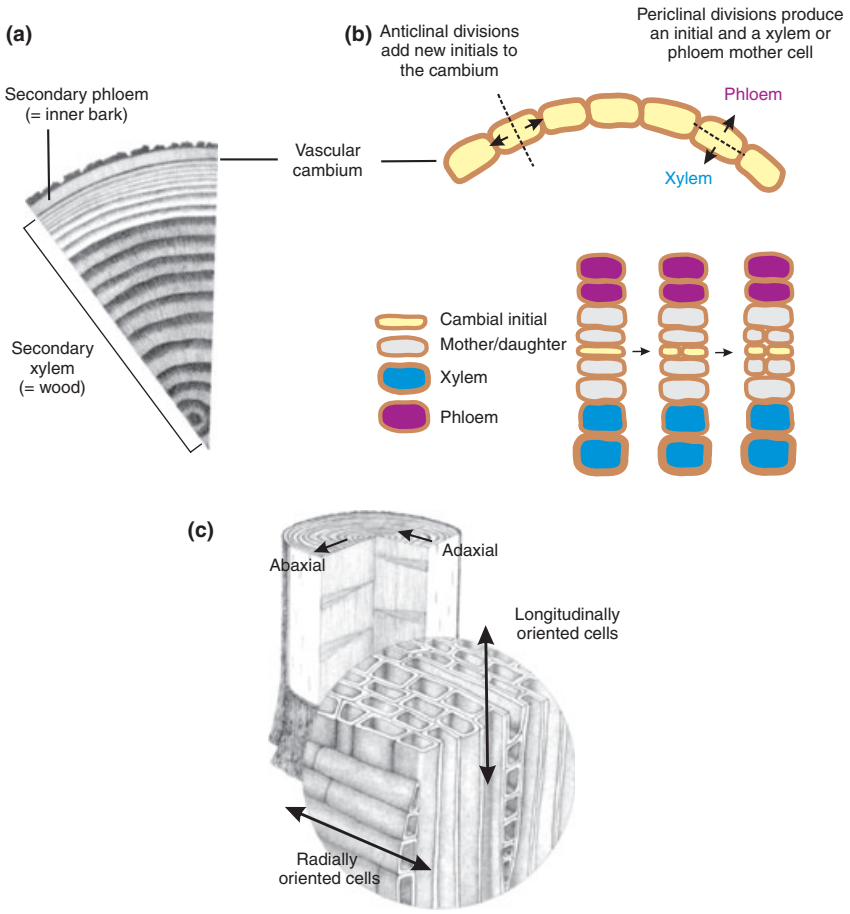

Fig. 1 Orientation of cells and tissues within a woody stem. (a) The vascular cambium produces secondary xylem to the inside and secondary phloem to the outside of the stem. (b) Cambial initials divide anticlinally to produce new initials and increase the circumference of the cambium. The same initials also divide periclinally to produce xylem and phloem mother cells, always leaving behind another initial. Both types of divisions are preserved in radial files of xylem cells, with anticlinal divisions indicated by the appearance of a new file. (c) Fusiform initials produce all of the longitudinally oriented cells in the stem. Ray initials produce all of the radially oriented cells. The vascular cambium produces phloem abaxially and xylem adaxially. (Adapted, with permission, from *Understanding Wood* by Bruce Hoadley, published by The Taunton Press.)

as a simple developmental progression in which fascicular cambia (cambia within individual vascular bundles) eventually become united with interfascicular cambia that arise *de novo* from parenchyma between bundles, but this paradigm may be more the exception than the rule (Beck, 2005). In fact, many woody plants form a continuous ring of procambium and primary tissues as adjacent leaf traces merge with one another (Esau, 1943). The first appearance of secondary vascular tissues in the shoot is linked to phyllotaxy, with older leaf traces producing secondary xylem first, adjacent to younger traces that are still forming primary xylem (Larson & Isebrand, 1974; Larson, 1975, 1976). The transition between primary and secondary growth in the root has been far less extensively studied, but here the cambium initiates adjacent to primary phloem (Eames & MacDaniels, 1947). While secondary vascular tissues are truly lacking in some angiosperm taxa (e.g. monocots), many angiosperms described as 'herbaceous' do in fact undergo secondary growth, which may be limited to vascular bundles or develop from a continuous cambium, or occur only in the root. Indeed, the terms 'herbaceous' and 'woody', while practical, do not acknowledge the vast anatomical variation and degrees of woodiness among plants variously assigned to these classes (discussed in Carlquist, 2009), and do not reflect phylogenetic relationships. For example, herbaceous and woody plants can be found scattered across diverse angiosperm taxa (Groover, 2005), and the anatomy and physiological properties of secondary xylem produced by cambia can vary tremendously even among closely related angiosperm taxa, as discussed below in Section 2. The extent of cambial activity and degree of woodiness expressed by a plant can also be affected by environmental conditions and life history. For example, *Arabidopsis thaliana* genotypes used for developmental genetics research (e.g. Landsberg *erecta*) do not undergo secondary growth under laboratory conditions that minimize generation times, but can form a complete cambium and produce secondary xylem and phloem if flowering is delayed by either physical or genetic means (Brugiere *et al.*, 2003; Espinosa-Ruiz *et al.*, 2004; Melzer *et al.*, 2008).

2. Vascular cambia have evolved multiple times, but the cambia of extant seed plants probably share a common evolutionary origin

Although secondary vascular growth in extant taxa is limited to seed plants (Fig. 2), independent origins of vascular cambia can be found in the arborescent lycopods and sphenopsids (Cichan & Taylor, 1982; Cichan, 1985, 1986) that dominated the coal swamps of the Carboniferous. Although some were large trees reaching 10–30 m in height, their stems were mostly cortex and the vascular cambium was unifacial, producing secondary xylem to the inside but no tissue externally (the smaller *Sphenophyllum* is an exception, and represents the only bifacial cambium outside of the progymnosperm/seed plant lineage (Eggert & Gaunt, 1973)). In addition, an extensive fossil record shows no evidence of anticlinal divisions and suggests that the

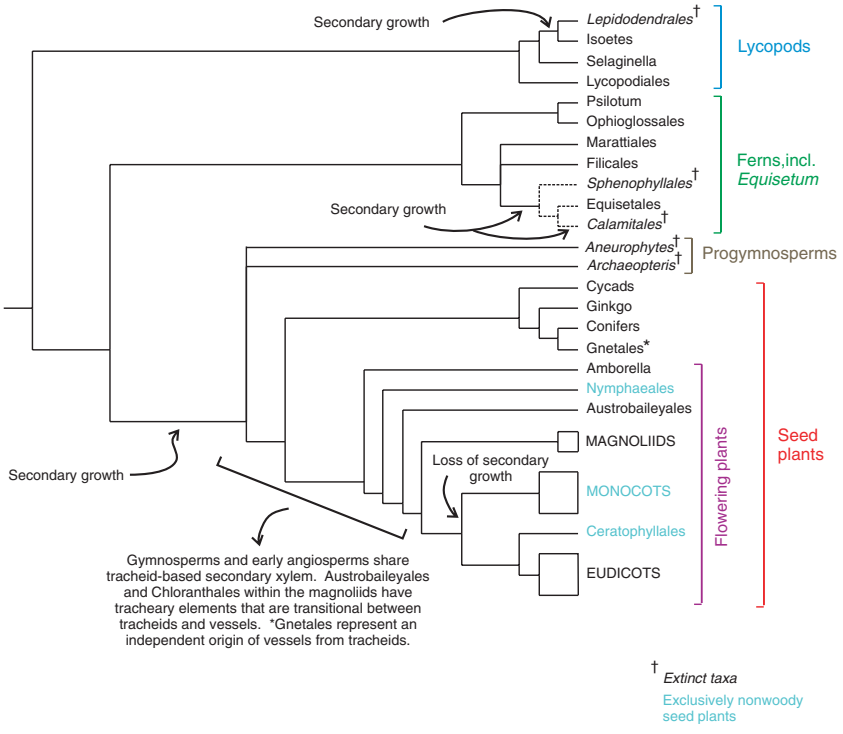

Fig. 2 A phylogeny of vascular plants illustrating multiple origins of secondary growth via a vascular cambium. The cambia of arborescent lycopods and Calamitales were unifacial, producing only secondary xylem. The cambium of Sphenophyllales was bifacial and produced both secondary xylem and phloem, suggesting an origin distinct from the Calamitales (although note that relationships among these groups are not clear). The bifacial cambium of extant seed plants is thought to be homologous with that of the spore-bearing progymnosperms (Friedman *et al.*, 2004; Judd *et al.*, 2008; Rothwell & Karrfalt, 2008), implying that this origin of secondary growth predates the seed. The earliest extant angiosperms share both a bifacial cambium and tracheid-based wood structure with the gymnosperms. Vessels (open-ended tracheary elements) arose independently in the Gnetales and angiosperms, with ferns also displaying some transitional forms.

growth of these plants was determinate, with a cambium capable of producing a limited amount of secondary xylem but ultimately differentiating into a band of parenchyma. Most extant relatives (e.g. *Lycopodium* and *Equisetum*) lack secondary growth entirely. Similarly, although no modern ferns produce secondary growth (but see Rothwell & Karrfalt, 2008), evidence of a vascular cambium has been found in several extinct pre-fern lineages, most notably in *Rhacophyton* (Cichan & Taylor, 1990; Taylor *et al.*, 2009).

The bifacial vascular cambia of extant seed plants may share a common evolutionary origin that predates the divergence of angiosperms and gymnosperms (Fig. 2), and in fact may predate the seed. Cycads and all known (including extinct) gymnosperms have bifacial cambia, as did the spore-bearing progymnosperms (Cichan, 1986; Stewart & Rothwell, 1993; Ryberg *et al.*, 2007). Within angiosperms, results from molecular phylogenetic analysis and character state reconstructions support the idea that a woody habit is ancestral for both basal angiosperms and early-diverging eudicots (Zanis *et al.*, 2002; Kim *et al.*, 2004). Determination of whether vascular cambia had a single or multiple origins during seed plant evolution will ultimately require comparison of the underlying genetic regulatory mechanisms across taxa. Even then, large gaps in the fossil record are likely to make this determination difficult, at least for early branching lineages. For instance, a diverse group of progymnosperms ('progymno' because they produced spores and not seeds) produced secondary xylem and phloem from a bifacial cambium (Cichan & Taylor, 1990), but it is not known for certain if this cambium is homologous with that of extant seed plants. Current phylogenies suggest homology (Friedman *et al.*, 2004; Judd *et al.*, 2008) and this is at present the accepted view, so for the purposes of further discussion we assume homology as well. While production of secondary growth from a bifacial vascular cambium may be the ancestral state, the extent and type of cambial activity clearly have been modified in different seed plant lineages, and seem especially variable within the angiosperms (discussed in Carlquist, 2009).

III. Variation in secondary vascular growth in angiosperms

Angiosperms show extensive variation in secondary vascular growth. Some angiosperm taxa have lost secondary vascular growth entirely, while in other lineages there have been changes in the extent of cambial activity and woody growth, including the appearance of secondarily woody species that have only recently acquired a woody habit. More extreme variation is illustrated by cambial variants (also termed 'anomalous secondary growth'), including plants with furrowed xylem and successive cambia. The general anatomical features of some of these variants have arisen independently multiple times in unrelated taxa (i.e. through convergent evolutionary events), suggesting that relatively simple evolutionary steps might produce these anatomical novelties.

1. Cambial activity varies among angiosperm taxa

Within the angiosperms, vascular cambia have been entirely lost in at least three lineages, including the monocots (Fig. 3). While monocots as a group lack secondary vascular growth, there are conspicuous arborescent monocots (e.g. *Aloe*, *Agave*, *Yucca*, *Dracaena* and *Cordyline*) that develop a novel cambium from the parenchyma of the cortex or pericycle. Termed the 'secondary thickening meristem' (Rudall, 1991), this cambium produces ground tissue and entire vascular bundles to the inside of the stem and cortical parenchyma to the outside (Cheadle, 1936; Tomlinson & Zimmermann, 1967; Fisher, 1973). Information as to genetic mechanisms regulating monocot cambia is entirely lacking, but, given the dramatic differences in ontogeny and function, monocot cambia are assumed to not be homologous with the vascular cambium of seed plants.

Within dicot lineages, cambial activity and degrees of woodiness can vary dramatically, even among closely related species. Interestingly, some herbaceous species can evolve a woody habit relatively rapidly after island colonization. Numerous examples of such 'secondary woodiness' have been demonstrated in members of at least eight orders spread throughout the eudicots (Fig. 3) (Carlquist, 1969, 1992, 1995; Bohle *et al.*, 1996; Ballard & Sytsma, 2000; Lee *et al.*, 2005; Lens *et al.*, 2005; Baldwin, 2007). The wood of secondarily woody plants often differs from that of primarily woody plants in a predictable way, reflecting a form of juvenilism in which characteristics of primary xylem are carried forward into secondary xylem (Carlquist, 2009). This in turn is a form of heterochrony, in which changes in the timing (e.g. onset, offset, duration and rate) of developmental events in different species produce variation in anatomy and morphology. Heterochrony in xylem development has had a profound effect on the diversification of angiosperm growth forms (Olson, 2007; Carlquist, 2009; Olson *et al.*, 2009).

2. Stem shape and anatomy can be altered by varying xylem and phloem production around the circumference of the cambium

In most woody plants, a relatively constant ratio of xylem to phloem production is maintained about the circumference of the cambium such that the stem is roughly cylindrical, but simple shifts in the relative rates of xylem and phloem production can dramatically alter both the internal and the external structure of the stem. If xylem production is accelerated in certain regions of the cambium, a buttressed, fluted or flattened stem shape can result (e.g. Basson &

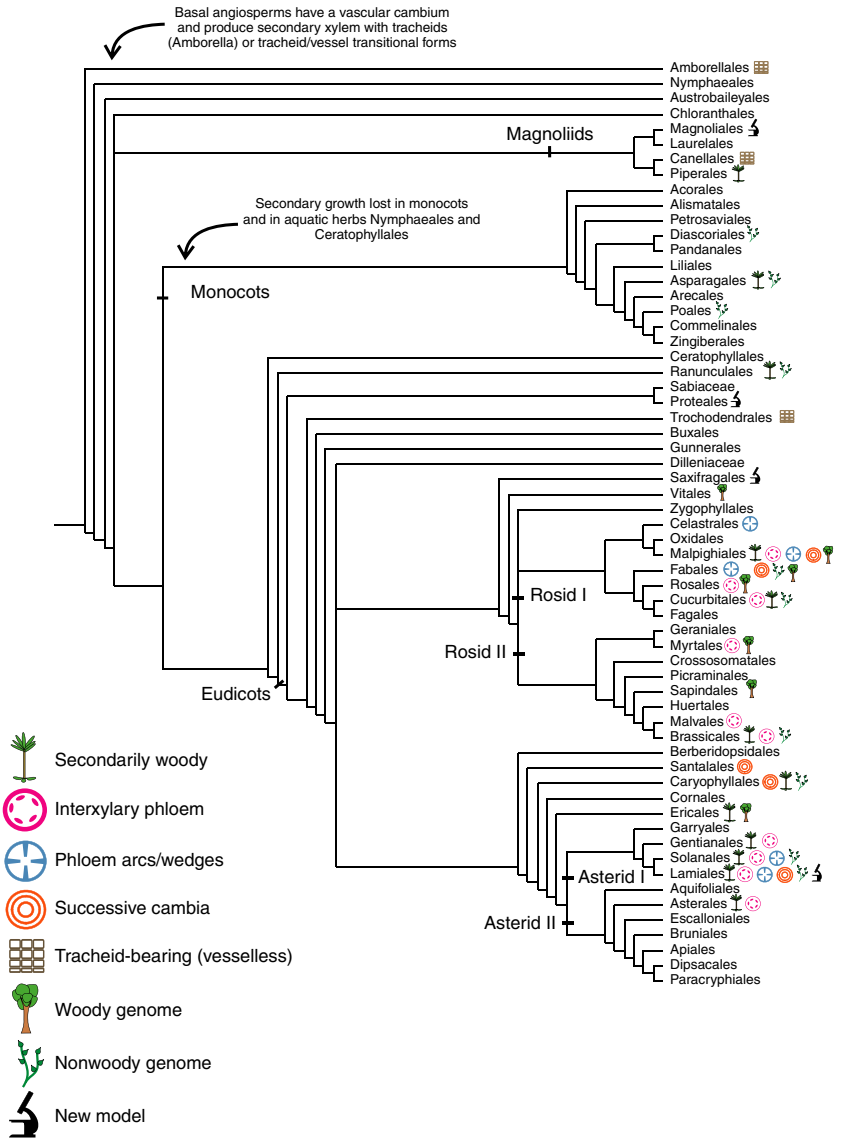

Fig. 3 A phylogeny of the angiosperms (APGIII, Stevens 2008; http://www.mobot.org/MOBOT/Research/APweb/welcome.html) illustrating the distribution of various characters relating to secondary growth. In all cases, orders are annotated with a particular character if they show strong tendencies toward that trait or contain conspicuous species containing the character. The vascular cambium has been lost in at least three angiosperm orders, including the monocots. Secondarily woody plants, in which woody habit has been recently acquired, are dispersed among various orders. Also indicated is the appearance of certain 'cambial variants', including arcs or wedges of phloem developing within furrows of xylem, and successive cambia, in which multiple concentric cambia develop and may function simultaneously. Numerous orders include plants with interxylary phloem, in which secondary phloem is formed normally but phloem can also be found embedded within secondary xylem. Vesselless angiosperms are also indicated. Orders in which woody or nonwoody genomes have been sequences are also indicated, as are suggested new models for wood and cambium evo-devo studies. See text for discussion. The topology was adapted from P. F. Stevens (2001 onwards; http://www.mobot.org/MOBOT/Research/APweb/welcome.html). Angiosperm Phylogeny Website. Version 9, June 2008.

Bierhorst, 1967). A flattened stem shape is typical of many lianas, and probably has mechanical advantages for a climbing habit.

By contrast, some stems maintain a cylindrical shape even though the ratio of xylem to phloem production may vary around the circumference of the cambium. In some lianas of the tribe Bignonieae (Lamiales), xylem production inwards is slowed and phloem production outwards is accelerated in four sectors of the cambium (Fig. 4). In the variant cambial regions, the increased rate of phloem production matches that of xylem in adjacent regions such that, when viewed in cross-section, the xylem is furrowed by arcs of phloem (Fig. 4a) but the stem is still cylindrical in shape (Coleman *et al.*, 2008; Pace *et al.*, 2009; Wang *et al.*, 2009). In more extreme variants of Bignonieae, there are sharp breaks in the relative production of xylem vs phloem about the circumference of the stem (i.e. the cambium becomes disjunct in its activity), with four regions of cambium ceasing anticlinal divisions and producing abundant secondary phloem. The result is the formation of four wedges of phloem extending into secondary xylem (Fig. 4b,c). This process can be reiterated, resulting in multiple, regularly interspersed wedges (Fig. 4d,e). While the mechanisms underlying the developmental processes of this variant are unknown, the variant cambial sectors are associated with leaf traces (Dobbins, 1981), suggesting the influence of leaf-derived signals affecting cambial activity.

The phloem arcs and wedges seen in members of Bignonieae are presumed to be of adaptive significance. The embedding of phloem wedges within more rigid xylem tissues, for example, may provide more flexibility which is

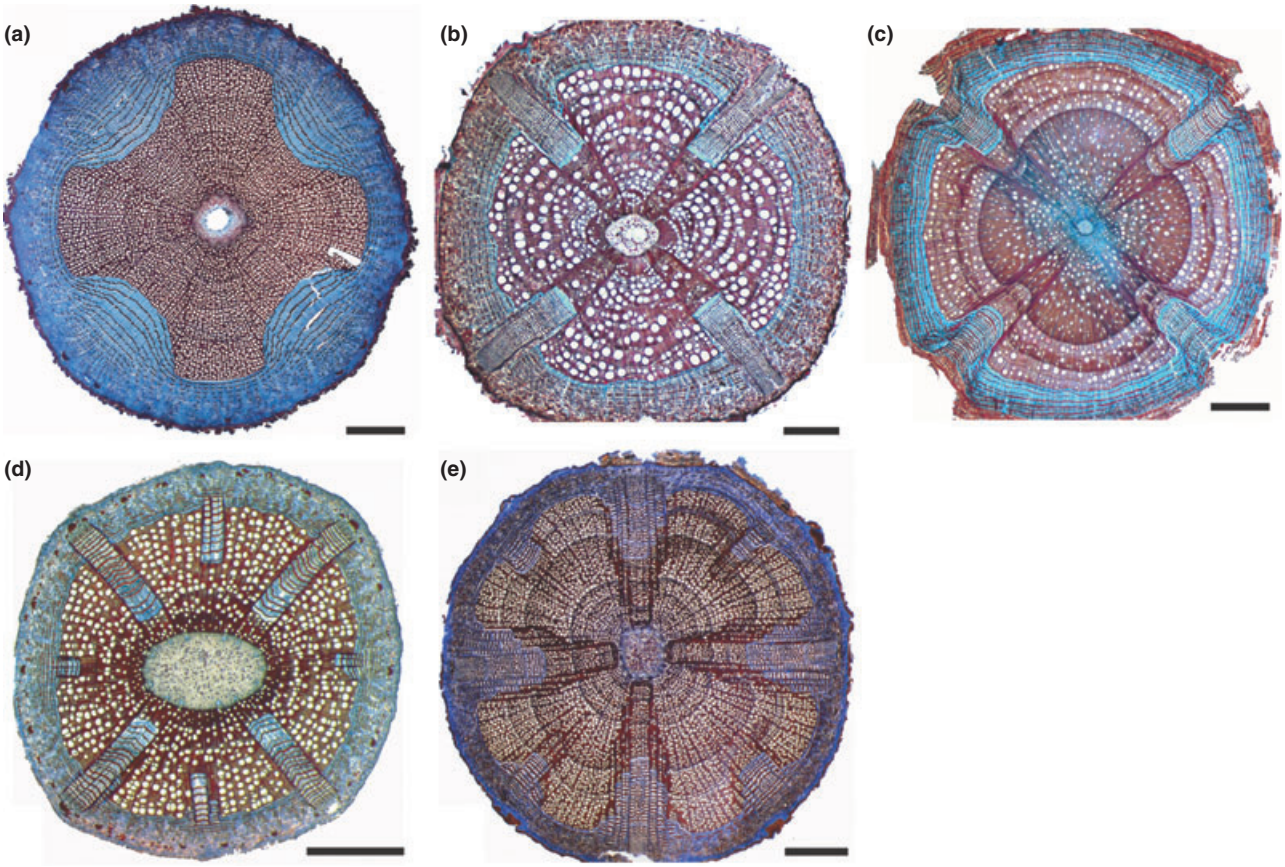

Fig. 4 Transverse sections from stems of Bignonieae species, showing different degrees of xylem furrowed by arcs or wedges of phloem. In *Perianthomega vellozoi* (a), four equidistant arcs of phloem are formed through a relative increase in phloem vs xylem production by the cambium in the variant regions. More dramatic anatomies are characterized by formation of four phloem wedges in (b) *Tanaecium pyramidatum*, and (c) *Fridericia chica*, where the variant cambial sections become disjunct, cease anticlinal divisions, and exclusively produce secondary phloem. This process can be reiterated, leading to formation of additional wedges as seen in (d) *Bignonia binata* and (e) *Adenocalymma divaricatum*. The figure is adapted and reproduced with permission from Pace *et al*. (2009).

advantageous to the climbing habit of the lianas (Carlquist, 1975, 2001; Pace *et al*., 2009). The abundant parenchyma associated with phloem wedges may also allow lianas to recover from wounding associated with twisting (Fisher & Ewers, 1989). Stems with xylem furrowed by arcs or wedges of phloem can be found in at least five angiosperm orders (Fig. 3).

3. Some stems can have multiple, successive vascular cambia

Members of 75+ genera form multiple vascular cambia in succession, each of which can independently produce secondary xylem and phloem. Successive cambia develop in some cycads, in *Gnetum* and *Welwitschia* (Gnetales), and within 14 orders spread throughout the eudicots (Fig. 3), including Caryophyllales, in which the trait is exceptionally common (Carlquist, 2001). Cross-sections of stems from species with successive cambia may exhibit alternating rings or segments of secondary xylem and phloem within a background of conjunctive tissue (Fig. 5). Although there are multiple interpretations of the ontogeny of this condition, some probably attributable to species differences, a few generalizations can be made. The first vascular cambium forms normally (i.e. between primary xylem and phloem) and produces secondary xylem and phloem. A second cambium then develops from parenchyma within the stem cortex and produces conjunctive tissue to the inside (and, less frequently reported, to the outside). Whether this cambium goes on to produce secondary xylem and phloem directly, with new cambia arising outside the oldest phloem (Esau & Cheadle, 1969; Bailey, 1980), or whether it continues to function as a 'master cambium', producing conjunctive tissue as well as new vascular cambia to the inside of the stem (Carlquist, 2007), has been debated and may vary among species. However, the end result is repeating increments of secondary xylem and phloem amidst conjunctive tissue.

Successive cambia are almost certainly of adaptive significance. Stems with successive cambia have large amounts of

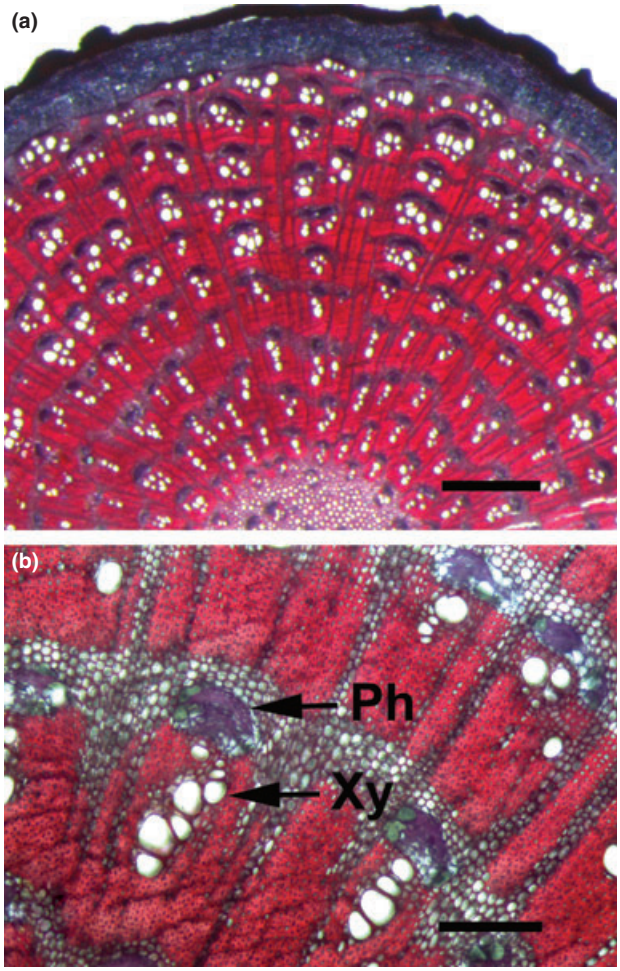

Fig. 5 Transverse sections from a *Bougainvillia* stem with successive cambia. The stem is characterized by multiple increments of secondary xylem and phloem within a background of conjunctive tissue formed by successive cambia (a). As shown in higher magnification (b), each increment contains secondary phloem (Ph) and secondary xylem (Xy) produced by an intervening vascular cambium. Bars: (1), 2 mm; (b), 12 mm. Images are courtesy of Ciera Martinez, University of California, Davis, CA, USA.

parenchyma that can function in both carbohydrate and water storage. A familiar example is the storage root of *Beta vulgaris* (sugar beet), which contains large parenchyma cells in the regions between successive cambia (Rapoport & Loomis, 1986) and an intricate conducting network of phloem (Zamski & Azenkot, 1981a,b). Lianas include a disproportionate number of species with successive cambia, suggesting potential mechanical advantages such as flexibility and compartmentalization of vessels sheathed by fibers (Carlquist, 2007). Lianas with abundant parenchyma have been shown to resist loss of xylem conductivity under severe twisting far more effectively than similar-sized trees (Putz & Holbrook, 1991). Currently, the molecular mechanisms underlying the unique development of stems with successive cambia are unknown.

IV. Genes and mechanisms regulating secondary vascular growth and their evolutionary origins

The recent development and application of genomic and genetic tools in the model species of the genus *Populus* have begun to identify key genes and mechanisms underlying secondary vascular growth, and provide intriguing but still incomplete insights into the evolution of the development of cambia and secondary growth. As discussed below in Section 1, comprehensive gene expression profiling of cambium and secondary vascular tissues shows an overlap between the genetic regulation of primary meristems and that of vascular cambia. More detailed studies have characterized the function of key transcription factors and hormones in regulating specific aspects of secondary vascular growth, including the regulation of differentiation, patterning and polarity, as discussed in Section 3 below.

1. Gene expression studies reveal overlapping mechanisms regulating the shoot apical meristem and vascular cambium

Transcription is a primary level of regulation for secondary vascular growth, as revealed by comprehensive gene expression profiling using microarrays. Tangential sections of *Populus* spp. stems were taken from positions across the cambial region ranging from mature phloem through the cambial zone to the region of cell expansion in secondary xylem (Schrader *et al.*, 2004). Analysis of gene expression in these sections using cDNA microarrays revealed good correlation between developmental events (e.g. cell division, cell expansion and cell wall synthesis) and differential expression of genes from corresponding functional groups (e.g. cyclins, expansins and lignin-related genes, respectively). These results suggest not only that transcription is an important level of regulation of secondary vascular growth, but also that comparison of transcriptional profiles of secondary vascular tissues from species of interest will be an informative approach for future studies in other species.

Interestingly, known transcriptional regulators of key developmental processes associated with the shoot apical meristem (SAM) are also expressed in the cambial zone during secondary growth (Schrader *et al.*, 2004). Among SAM genes also expressed in the cambial zone are orthologs of the *A. thaliana* class I knotted-like homeobox (KNOX) gene *SHOOTMERISTEMLESS* (*STM*); class III homeodomain-leucine zipper (HD ZIP) genes *PHAVOLUTA/PHABULOSA* and *ATHB-15* (*CORONA*); *KANADI1*; *SHOOT-ROOT* (*SHR*); and potential orthologs of *AINTEGUMENTA* (*ANT*) and *PINHEAD* (*PNH*). These genes play fundamental roles in the SAM, including the control of cell proliferation (*ANT*), regulation of determinate vs indeterminate shoot growth (*PNH*), polarity and vascular

development (class III HD ZIPs and *KANADI1*), and specification of tissue identity (*SHR*).

The expression of major SAM regulatory genes in the cambial zone is consistent with the direct cooption of these genes and mechanisms from the SAM during the evolution of cambia and secondary vascular growth (Groover, 2005). This hypothesis not only addresses the acquisition of major regulatory elements associated with the innovation of secondary vascular growth during land plant evolution, but also speaks to the previously mentioned rapid gain of secondary growth from herbaceous ancestors in secondarily woody species. If genes necessary for secondary vascular growth are also required for SAM function, there is strong pressure for them to be maintained in herbaceous plants. Expression of these genes could thus be readily recruited to function during secondary growth by relatively simple steps, for example by change of expression of key transcription factors. The overlapping expression of key regulatory genes in both primary and secondary growth may also underlie the gradual developmental transition between primary and secondary growth in many species.

At the same time, there are important anatomical distinctions between the radially organized vascular cambium and the conically organized apical meristems, many of the major regulatory genes predate the evolution of both the SAM and cambia and could have unique functions in the two meristems, and not all SAM regulatory genes are expressed in the cambial zone. *WUSCHEL* (*WUS*) and *CLAVATA3* (*CLV3*) encode a homeodomain transcription factor and secreted peptide ligand, respectively, which together with the CLV receptors form a feedback loop regulating the size of the stem cell population in the SAM (Sablowski, 2007). *WUS* is expressed in a small number of cells defining an 'organizing center' underlying the stem cells, which express *CLV3*. The CLV3 protein is detected by CLV1 and CLV2 receptors to limit the size of the organizing center by restricting *WUS* expression. While the putative *Populus CLV1* ortholog is expressed in the cambial zone, the presumed *Populus* orthologs of *CLV3* and *WUS* are not (Schrader *et al.*, 2004). Genes related to *WUS* (WUSCHEL related homeobox (*WOX*) genes) and other genes related to *CLV3* that encode CLAVATA3 (CLV3)/ENDOSPERM SURROUNDING REGION (ESR) (CLE) peptides are expressed in the cambial zone, however, consistent with cooption of duplicated genes related to *WUS* and *CLV3* during the evolution of secondary vascular growth. Recent studies have shown that downregulation of the *Populus WOX* gene *PttHB3* results in minimal secondary growth without affecting primary growth (O. Nilsson, pers. comm.), confirming that CLE-WOX-like signaling mechanisms are required for cambium initiation or maintenance. It is notable, however, that *PttHB3* has a relatively broad expression pattern that is not restricted to the cambial initials or even the cambial zone (Schrader *et al.*, 2004).

Indeed, it is not clear what cells in the cambial zone or secondary vasculature might correspond to cells analogous to an organizing center or stem cells in primary meristems, based solely on anatomical features or gene expression. One possibility is that the initials may represent the true stem cells, with xylem and/or phloem mother cells performing organizing center functions. Ongoing functional analysis of *WOX-CLE* gene expression and function may ultimately show this to be the case, but it is also possible that the radially organized cambial zone has fundamental differences from the three-dimensional organization of the shoot apical meristem. Notably, there has not been any report of a gene whose expression is limited to the initials. The class I KNOX gene *ARBORKNOX2* (*ARK2*), a *Populus* ortholog of *A. thaliana BREVIPEDICELLUS*, has an expression pattern that extends across the cambial zone and into developing xylem (Du *et al.*, 2009). It is interesting to note that class I KNOX expression in the SAM of simple-leaved dicots is repressed in developing organ primordia (Champagne & Sinha, 2004) but not in developing xylem during secondary growth, bringing into question direct analogies between organ primordia and developing secondary vascular tissues. Currently, there is little evidence for sharply defined compartments within the cambial zone or secondary vascular tissues, which may indicate that secondary vascular growth is defined by gradations of development rather than compartmentalization. Further understanding of the functional regions of the cambial zone and secondary vascular tissues will require additional, detailed characterization of genes and mechanisms regulating the cambium.

2. Class I KNOX genes are involved in regulating cell division and cell differentiation during secondary vascular growth

The balance of cell division vs cell differentiation is fundamental to the function of the cambium. This balance is dynamic and changes in response to environmental conditions and seasonal cues. For example, during vigorous growth in the spring, additional cell divisions occur in cambial daughter cells before differentiation, leading to a wider cambial zone. *Populus* orthologs of *A. thaliana* class I KNOX genes *SHOOTMERISTEMLESS* and *BREVIPEDICELLUS* (*ARBORKNOX1* (*ARK1*) and *ARBORKNOX2* (*ARK2*), respectively) play distinct roles in regulating the differentiation of cambial daughter cells.

ARK1 is expressed broadly in the cambial zone (Groover *et al.*, 2006). *Populus* mutants overexpressing *ARK1* have strong phenotypes that include greatly reduced leaf size, short internodes, and formation of ectopic meristems on the adaxial surface of leaves. In addition, *ARK1* overexpression plants have a severe inhibition of differentiation of lignified secondary xylem and lignified phloem fibers. These anatomical phenotypes are reflected in changes in the

expression of suites of genes involved in cell wall biosynthesis (Groover *et al.*, 2006). For example, several key genes in the lignin biosynthetic pathway are upregulated in *ARK1*-overexpressing *Populus*, with the exception of ferulate 5-hydroxylase (F5H) which is directly involved in the production of syringyl lignin monomers, which is downregulated. These changes in gene expression are reflected by slightly higher lignin content, but also a dramatic shift towards production of syringyl lignin (Groover *et al.*, 2006). Thus, changes in expression of a single transcription factor can result in complex changes in cambial daughter cell differentiation.

ARK2 also shows a broad expression pattern that includes not only the cambial zone but also developing secondary xylem and phloem fibers (Du *et al.*, 2009). Knockdown and overexpression mutants show that *ARK2* also plays a major role in regulating the differentiation of cambial daughter cells. Specifically, *ARK2* expression levels are positively correlated with the width of the cambial zone, and negatively correlated with the differentiation of lignified cell types in both secondary xylem and phloem fibers (Du *et al.*, 2009). Like *ARK1*, *ARK2* expression levels are correlated with expression of suites of genes involved in transcription, secondary cell wall synthesis, auxin-related processes, and cell division, although the individual genes misregulated in *ARK1* vs *ARK2* mutants are for the most part different. This observation is consistent with *ARK*s regulating co-evolved transcriptional modules that can alter complex secondary growth phenotypes, and with previous studies showing direct regulation of genes encoding cell wall-related proteins by *BREVIPEDICELLUS* (Mele *et al.*, 2003). Interestingly, while the cambial zone is wider in *ARK2* overexpression plants, the total number of cell layers in cambium and secondary xylem is actually reduced, and is correlated with downregulation of cell cycle-related genes (Du *et al.*, 2009). These observations could indicate a negative feedback mechanism, where delay in differentiation of cambial daughters results in inhibition of further cell division.

3. Plant hormones as well as genetic mechanisms regulate patterning and polarity during secondary vascular growth

The regulation of patterning and polarity during secondary vascular growth is still poorly understood, but recent studies point to possible mechanisms. Auxin is the best studied hormone regulating secondary vascular growth, and a gradient of auxin exists across the cambial region and developing xylem (Uggla *et al.*, 1996, 1998; Tuominen *et al.*, 1997). While once proposed to represent a morphogen gradient, a recent study suggests that this attractive hypothesis may be an oversimplification or incorrect (Nilsson *et al.*, 2008). Auxin-responsive genes were identified in *Populus* wood-forming tissues using microarrays and shown to respond to changes in cellular auxin concentrations, but the expression levels of auxin-responsive genes across the cambial zone and developing xylem were poorly correlated with the auxin gradient (Nilsson *et al.*, 2008). In addition, transgenic *Populus* expressing a dominant mutant form of *PttIAA3* have altered auxin responses, and have fewer cell divisions in the cambial zone and smaller lignified cell types in secondary xylem (Nilsson *et al.*, 2008). Nilsson *et al.* (2008) proposed that, rather than working as a simple morphogen, auxin may regulate the expression of a few downstream regulators to affect key aspects of wood formation, including cell division. Another role for auxin in secondary growth is indicated by changes in longitudinal auxin gradients associated with stem wounding, which are correlated with changes in the orientation of cambial initials and derived cells of secondary xylem (Kramer *et al.*, 2008). These changes are reflected in the grain pattern of the associated wood and indicate an important role for auxin in determining the orientation and relative rotation of cambial initials.

In addition to traditional hormones, well-conserved genetic mechanisms that have been identified as regulators of polarity and patterning in primary meristems are also expressed during secondary vascular growth. The class III HD ZIPs are a highly conserved gene family found in all land plants (Floyd *et al.*, 2006). Because these genes appear before the appearance of vasculature or polar lateral organs during land plant evolution, their ancestral function is believed to be one of regulating fundamental aspects of primary meristems. Interestingly, these genes are negatively regulated through degradation of transcripts by highly conserved miRNAs (Bowman, 2004). In *A. thaliana*, the class III HD ZIP family is comprised of five genes, *REVOLUTA* (*REV*), *PHAVOLUTA* (*PHV*), *PHABULOSA* (*PHB*), *ATHB8*, and *ATHB15/CORONA* (*CNA*). Roles for regulation of vascular development have been described for both *ATHB8* and *ATHB15*, while dominant mutants for *REV*, *PHB*, and *PHV* show striking phenotypes characterized by patterning and polarity defects (Emery *et al.*, 2003; Prigge *et al.*, 2005). For example, *REV* gain of function mutants have adaxialized vascular bundles, with xylem surrounding phloem (Emery *et al.*, 2003). *KANADI* genes work in opposition to class III HD ZIPs by promoting abaxial fates, and loss of *KANADI* gene function results in adaxialization of vascular bundles (Emery *et al.*, 2003).

In *Populus*, class III HD ZIPs orthologous to *A. thaliana PHV/PHB*, *CNA*, and *ATHB8* are all expressed during secondary growth, with the highest expression levels found in adaxial xylem tissue (Schrader *et al.*, 2004). Similarly, the *Populus* ortholog of *REV* is also expressed during secondary growth, and *Populus* plants expressing a dominant, miRNA-resistant *Populus REV* transgene show patterning and polarity defects in secondary vascular tissues that include formation of ectopic cambia in the stem cortex, which produce secondary xylem to the outside rather than to the

inside of the stem (M. Robischon *et al.*, unpublished). These results suggest that class III HD ZIPs may be involved not only in regulating polarity in secondary vascular tissues, but also in cambium initiation from parenchyma. Similarly, putative *Populus* orthologs of the *A. thaliana KANADI* genes *KAN1* and *KAN2* are expressed during secondary growth, with the highest expression in the phloem (Schrader *et al.*, 2004). It thus seems likely that class III HD ZIP–KANADI systems are crucial for the patterning and polarity of secondary vascular tissues. These observations also lend credence to the notion that secondary xylem and phloem are functionally equivalent to adaxial and abaxial tissues, respectively.

V. Evolution of development approaches for the study of secondary vascular growth

Evolution of development studies are a logical next step in furthering our understanding of cambial function and evolution, through synthesis of anatomical, developmental genetic, and genomic approaches and data within a phylogenetic context (Cronk, 2001). Evolution of development approaches can address questions central to secondary vascular growth, such as: What are the evolutionary origins of secondary vascular growth in angiosperms and gymnosperms, and was there a single or multiple origins? What are the ancestral genes and mechanisms regulating secondary vascular growth? What is the genetic basis for observed phylogenetic variation in secondary vascular growth, including cambial variants? As discussed in Section three, a crucial missing component of current research is comparative studies of regulatory genes and mechanisms across taxa.

1. Evolution of development studies require synthesis of approaches and data types

Evolution of development studies of secondary vascular growth will require multiple steps. First, taxonomic relationships among plants must be determined with reasonable certainty and precision. Taxonomic relationships are increasingly well resolved at various taxonomic levels for large numbers of plants through the construction of DNA sequence-based phylogenies (Angiosperm Phylogeny Group, 2003; Palmer *et al.*, 2004). Second, developmental and anatomical variation in secondary vascular growth should be identified at different taxonomic levels, ranging from generalized traits at broad taxonomic levels to more unusual or subtle traits at lower taxonomic levels. Examples of such variation have already been discussed in section III, and there is an extensive wood anatomical literature and data that can be referenced (e.g. Carlquist, 2001; http://insidewood.Lib.Ncsu.Edu/search). Third, major regulatory genes and mechanisms must be identified using model plants for which extensive genomic tools and the ability to assess gene function (e.g. through transformation) are available. As discussed above, this work is incomplete but is accelerating with the availability of the *Populus* genome (Tuskan *et al.*, 2006) and functional genomic tools. Fourth, the development of new woody plant models at key taxonomic positions must be undertaken to enable comparative studies of gene function. Lastly, variation for both phenotypes and underlying genetic regulatory genes and mechanisms must be surveyed in species at appropriate taxonomic positions, which show variation for traits of interest (Soltis & Soltis, 2003).

2. New woody model species should have desirable attributes and taxonomic positions

Currently there are significant genomic resources for a handful of woody species, most of which were selected because of their economic importance. For gymnosperms, there are large numbers of expressed sequence tags (ESTs) and genetic maps for several members of the Pinales, including species of *Pinus*, *Abies* and *Picea* (Dean, 2006). Ongoing development of existing Pinales models is accelerating, although transformation is limiting for many species. Similarly, there are a number of angiosperm species with significant EST sequences and other resources, although all are clustered within the Rosids. Notable are the forest trees of *Populus* (Tuskan *et al.*, 2006) and *Eucalyptus* (http://eucalyptusdb.bi.up.ac.za/), the secondarily woody perennial papaya (*Carica papaya*) (Ming *et al.*, 2008), and the woody vines of *Vitus* (The French–Italian Public Consortium For Grapevine Genome Characterization, 2007), for which full genome sequences are available, and members of the Fagaceae, for which extensive sequencing is underway (Fig. 3). However, some of these current models lack desirable attributes for model species (see below paragraph), and are not fully representative of taxonomic variation desired for robust comparative studies of gene function.

There are several desirable attributes of new woody plant models. Importantly, new models must be amenable to detailed analysis of gene function. In general for woody perennials, this requires the ability to transform with transgenes, which can be used to knock down or change the expression of genes of interest. Interestingly, novel approaches have been developed for transformation of cambial tissue with *Agrobacterium* after bark removal (Van Beveren *et al.*, 2006), which could be applicable even to currently recalcitrant species. In addition, it is highly desirable to be able to perform controlled crosses and produce pedigrees for genetic mapping, which supports efforts ranging from whole genome sequencing to analysis of quantitative trait loci. Important variables influencing construction of pedigrees for woody perennials include the time to sexual maturity and degree of inbreeding depression. It is encouraging that many other important

techniques, including sequence-based evaluation of gene expression and various 'omics' technologies, are relatively easy to apply to new organisms. There are, however, significant practical hurdles in establishing new models, including an often limited number of researchers working on the model who must establish and curate databases, annotations, bioinformatic tools, and germplasm while making research progress (Abzhanov *et al.*, 2008).

For addressing questions concerning the ancestral origins of vascular cambia in angiosperms and gymnosperms, *Ginkgo biloba* could be a valuable new model gymnosperm. Ginkgo is a basal gymnosperm that has changed surprisingly little over hundreds of millions of years, and culture methods have been reported that could support transformation (Dupre *et al.*, 2000). Ginkgo could be used as a highly complementary reference to models being developed for more derived Pinales.

There are a number of potential model woody species that could be developed to assess broader taxonomic variation within angiosperms (Fig. 3). These models would provide opportunities for comparative functional developmental and genomic studies that could address the degree of variation in core mechanisms regulating secondary growth, as well as evidence for homologous origins of such mechanisms. For example, the magnoliid *Liriodendron tulipifera* (tulip tree, order Magnoliales) is an attractive candidate, being a basal angiosperm and large forest tree for which a somatic embryogenesis-based transformation system is available (Dai *et al.*, 2004). There are also existing pedigrees from forest tree improvement programs, and *L. tulipifera* is interfertile with the Asian *Liriodendron chinense*. *Platanus* species (sycamores, order Proteales) are basal basal eudicots, can be transformed, and have existing pedigrees. *Liquidambar styraciflua* (sweet gum, order Saxifragales), within the core eudicots (but outside the rosids), also has the benefit of transformation systems (Dai *et al.*, 2004) and pedigrees. Within the core eudicots, existing rosid woody models (including fully sequenced *Populus* and *Eucalyptus*) would be complemented by comparisons with woody members of the asterids such as *Fraxinus* spp. or *Paulownia* spp. (see paragraph below) for which transformation (Giri *et al.*, 2004) and pedigrees are available. In addition, all of these species are of environmental and/or economic importance. Even more challenging will be comparative studies between angiosperms and gymnosperms. While initial comparisons have demonstrated the ability to recognize general homologies between genes from *A. thaliana* and pines (Kirst *et al.*, 2003), determining orthologous and functional relationships will be challenging.

Selection of new angiosperm woody models should also maximize the information gained from relationships to existing models, including comparisons with nonwoody species (Fig. 3). Selection of woody species within families with highly developed herbaceous annual models could allow for powerful comparative studies of herbaceous annuals and woody perennials. For example, the order Fabales includes both the sequenced *Medicago* (Cannon *et al.*, 2006) and several notable tree species of the *Acacia* family. Furthermore, selection of new models should include consideration of variation for secondary vascular growth in closely related plants, including cambial variants. For example, the order Lamiales occupies a key taxonomic position within the Asterids, and also contains important variation ranging from the forest trees of Oleaceae (e.g. *Fraxinus* spp. (ashes)) and Paulowniaceae (e.g. *Paulownia* spp.), to the previously mentioned lianas within the family Bignoniaceae with amazing diversity in secondary vascular growth (Pace *et al.*, 2009).

3. Genomic approaches can identify genes regulating development in model species, and survey phylogenetic variation

A comprehensive strategy for evolution of development studies of secondary growth will begin with detailed characterization of the regulation of secondary growth using functional genetic and genomic tools in taxonomically diverse model species. Currently, this strategy is best illustrated by *Populus*, where combinations of developmental genetic studies using transgenesis and genomics are revealing major regulatory genes and mechanisms. A desirable next step is to move from single-gene views of development resulting from transgenesis-based developmental studies and genomics studies that are largely descriptive, to modeling of biological networks (e.g. transcriptional networks) underlying key secondary growth traits (Du & Groover, 2010). Network-level models of secondary growth would provide new levels of resolution of regulatory mechanisms, provide predictive capabilities to inform new research, and directly support detailed comparative studies of gene expression and regulation. Specifically, network-level models of secondary growth regulation could identify both putative basal regulatory genes and regulatory modules that are shared among diverse taxa, as well as genes or modules whose expression or function may be variable and responsible for observed phenotypic variation.

As sufficient knowledge of regulatory genes and networks is developed in model species, surveys of additional species based on comparative gene expression during secondary growth can be used for comparative analysis outside of fully developed models. Genomic and sequencing technologies are increasingly extensible to new species, a feature that is highly supportive of comparative surveys that can include species that do not enjoy the full range of tools available for model species. For example, large-scale sequencing of ESTs can be accomplished now at reasonable cost using next-generation sequencing (Mardis, 2008; Schuster, 2008), and requires only the ability to isolate high-quality RNA from

appropriate tissues. Such sequencing efforts can provide a wealth of information, including evolutionary histories, such as gene duplication events that often underlie important new evolutionary novelties through acquisition of new protein function by a duplicated gene, subfunctionalization of the original functions between paralogs, or acquisition of new expression patterns by duplicated genes (Ganfornina & Sánchez, 1999). Importantly, high-throughput sequencing not only assays sequence variation, but can also provide information about gene expression levels through quantification of the frequency at which a given gene's transcript appears in a sequence run (Mardis, 2008). Changes in expression levels can be reflective of evolutionarily significant mutations in *cis* regulatory elements or activity of *trans*-acting factors (e.g. transcription factors). For sequencing-based surveys, a highly informative tissue for assay would be cambial and developing xylem tissue, which can typically be harvested in relatively large amounts from actively growing stems, and would allow simultaneous assay of both cambial meristem regulatory genes and genes involved in cell differentiation and wood formation.

4. Computational approaches and databases are central to evolution of development studies of secondary growth

Development of computational methods and bioinformatics tools, database creation and curation, gene and genome annotation, and curation of biological stocks will all present major challenges for establishing the field of evolution of development for secondary growth. Luckily, many of these needs are shared by other communities (Abzhanov *et al.*, 2008), and major efforts have been undertaken to address at least some of these needs by creation of generalized resources. For example, the Generalized Model Organism Database (GMOD) tools provide 'off the shelf' database and informatic tools which can be relatively easily extended to new species (http://gmod.org/wiki/Overview). Other efforts (e.g. TAVERNA; http://taverna.sourceforge.net/) are underway to allow users without extensive informatics resources to create data manipulation and analysis pipelines through selection, modification, and joining of modular scripts. Examples of sophisticated database and analysis tools for comparative genomics across taxa include PHYTOZOME (http://www.phytozome.net/). In short, while the challenge is significant, it seems likely that database and informatic tools are increasingly accessible and could be effectively leveraged to allow even a small community of researchers to undertake ambitious evolution of development studies for secondary growth. While beyond the scope of this review, projects using genomic approaches (e.g. association mapping; Neale & Ingvarsson, 2008) to understand genetic variation responsible for variation in wood traits are underway in several woody species including *Pinus* and *Populus* spp. These studies will be synergistic with the comparative studies described above.

VI. Conclusions

While significant advances have been made in our understanding of secondary vascular growth, comparative studies within the fields of evolution of development will ultimately provide insights into the core evolutionary histories and variation of secondary growth. This work will not only have important biological significance, but also be supportive of applied goals. For example, many woody species of potential use as bioenergy crops cannot be effectively developed as working model species, but knowledge from comparative genomic studies can translate knowledge of variation from related taxa to support breeding applications. The framework of models at key taxonomic positions paired with comparative, gene expression-based studies in additional species is the next generation of research for understanding secondary growth.

Acknowledgements

We thank Ciera Martinez for contributing the images of *Bougainvillea*. This work was supported in part by grant USDA NRI 2006-35304-17420 to A.G.

References

Abzhanov A, Extavour CG, Groover A, Hodges SA, Hoekstra HE, Kramer EM, Monteiro A. 2008. Are we there yet? Tracking the development of new model systems *Trends in Genetics* **24**: 353–360.

Angiosperm Phylogeny Group. 2003. An update of the angiosperm phylogeny group classification for the orders and families of flowering plants: APG II. *Botanical Journal of the Linnean Society* **141**: 399–436.

Bailey DC. 1980. Anomalous growth and vegetative anatomy of *Simmondsia chinensis*. *American Journal of Botany* **67**: 147–161.

Baldwin BG. 2007. Adaptive radiation of shrubby tarweeds (*Deinandra*) in the california islands parallels diversification of the Hawaiian silversword alliance (compositae-madinae). *American Journal of Botany* **94**: 237–248.

Ballard HE, Sytsma KJ. 2000. Evolution and biogeography of the woody Hawaiian violets (*Viola*, Violaceae): Arctic origins, herbaceous ancestry and bird dispersal. *Evolution* **54**: 1521–1532.

Bannan MW. 1951. The reduction of fusiform cambial cells in *Chamaecyparis* and *Thuja*. *Canadian Journal of Botany-Revue Canadienne De Botanique* **29**: 57–67.

Bannan MW. 1965. Ray contacts and rate of anticlinal division in fusiform cambial cells of some Pinaceae. *Canadian Journal of Botany* **43**: 487–507.

Bannan MW. 1968. Anticlinal divisions and organization of conifer cambium. *Botanical Gazette* **129**: 107–113.

Basson PW, Bierhorst DW. 1967. An analysis of differential lateral growth in the stem of *Bauhinia surinamensis*. *Bulletin of the Torrey Botanical Club* **94**: 404–411.

Beck CB. 2005. *Plant structure and development*. Cambridge, UK: Cambridge University Press.

Bohle U-R, Hilger HH, Martin WF. 1996. Island colonization and evolution of the insular woody habit in *Echium* l. (Boraginaceae). *Proceedings of the National Academy of Sciences, USA* **93**: 11740–11745.

Bowman JL. 2004. Class III HD-zip gene regulation, the golden fleece of argonaute activity? *Bioessays* **26**: 938–942.

Brugiere N, Jiao S, Hantke S, Zinselmeier C, Roessler JA, Niu X, Jones RJ, Habben JE. 2003. Cytokinin oxidase gene expression in maize is localized to the vasculature, and is induced by cytokinins, abscisic acid, and abiotic stress. *Plant Physiology* **132**: 1228–1240.

Cannon SB, Sterck L, Rombauts S, Sato S, Cheung F, Gouzy J, Wang X, Mudge J, Vasdewani J, Schiex T *et al.* 2006. Legume genome evolution viewed through the *Medicago truncatula* and *Lotus japonicus* genomes. *Proceedings of the National Academy of Sciences, USA* **103**: 14959–14964.

Carlquist S. 1969. Wood anatomy of Lobelioideae (Campanulaceae). *Biotropica* **1**: 47–72.

Carlquist S. 1975. *Ecological strategies of xylem evolution*. Berkeley, CA, USA: University of California Press.

Carlquist S. 1992. Wood anatomy and stem of *Chloranthus* – summary of wood anatomy of Chloranthaceae, with comments on relationships, vessellessness, and the origin of monocotyledons. *IAWA Bulletin* **13**: 3–16.

Carlquist S. 1995. Wood anatomy of Caryophyllaceae: ecological, habital, systematic, and phylogenetic implications. *Aliso* **14**: 1–17.

Carlquist S. 2001. *Comparative wood anatomy. Systematic, ecological, and evolutionary aspects of dicotyledon wood*. Berlin, Germany: Spinger-Verlag.

Carlquist S. 2007. Successive cambia revisited: ontogeny, histology, diversity, and functional significance. *Journal of the Torrey Botanical Society* **134**: 301–332.

Carlquist S. 2009. Xylem heterochrony: an unappreciated key to angiosperm origin and diversifications. *Botanical Journal of the Linnean Society* **161**: 26–65.

Champagne C, Sinha N. 2004. Compound leaves: equal to the sum of their parts? *Development* **131**: 4401–4412.

Cheadle VI. 1936. Secondary growth by means of a thickening ring in certain monocotyledons. *Botanical Gazette* **98**: 535–555.

Choat B, Pittermann J. 2009. New insights into bordered pit structure and cavitation resistance in angiosperms and conifers. *New Phytologist* **182**: 557–560.

Cichan MA. 1985. Vascular cambium and wood development in Carboniferous plants. I. Lepidodendrales. *American Journal of Botany* **72**: 1163–1176.

Cichan MA. 1986. Vascular cambium and wood development in Carboniferous plants. III. *Arthropitys* (Equisetales; Calamitaceae). *Canadian Journal of Botany* **64**: 688–695.

Cichan MA, Taylor TN. 1982. Vascular cambium development in *Sphenophyllum*: a carboniferous arthrophyte. *IAWA Bulletin* **3**: 155–160.

Cichan MA, Taylor TN. 1990. Evolution of cambium in geologic time – a reappraisal. In: Iqbal M, ed. *The vascular cambium*. New York City, NY, USA: John Wiley & Sons Inc, 213–228.

Coleman HD, Park JY, Nair R, Chapple C, Mansfield SD. 2008. RNAi-mediated suppression of p-coumaroyl-coa 3′-hydroxylase in hybrid poplar impacts lignin deposition and soluble secondary metabolism. *Proceedings of the National Academy of Sciences, USA* **105**: 4501–4506.

Cronk QCB. 2001. Plant evolution and development in a post-genomic context. *Nature Reviews. Genetics* **2**: 607–619.

Dai J, Vendrame W, Merkle S. 2004. Enhancing the productivity of hybrid yellow-poplar and hybrid sweetgum embryogenic cultures. *In Vitro Cellular & Developmental Biology – Plant* **40**: 376–383.

Dean JFD. 2006. Genomics resources for conifers. *Landscapes, Genomics and Transgenic Conifers*. Dordrecht, Netherlands: Springer, 55–74.

Dobbins D. 1981. Anomolous secondary growth in lianas of the Bignoniaceae is correlated with the vascular pattern. *American Journal of Botany* **68**: 142–144.

Domec J-C, Lachenbruch B, Meinzer FC, Woodruff DR, Warren JM, McCulloh KA. 2008. Maximum height in a conifer is associated with conflicting requirements for xylem design. *Proceedings of the National Academy of Sciences, USA* **105**: 12069–12074.

Du J, Groover A. 2010. Transcriptional regulation of secondary growth and wood formation. *Journal of Integrative Plant Biology* **52**: 17–27.

Du J, Mansfield SD, Groover AT. 2009. The *Populus* homeobox gene *ARBORKNOX2* regulates cell differentiation during secondary growth. *Plant Journal* **60**: 1000–1014.

Du S, Yamamoto F. 2007. An overview of the biology of reaction wood formation. *Journal of Integrative Plant Biology* **49**: 131–143.

Dupre P, Lacoux J, Neutelings G, Mattar-Laurain D, Fliniaux MA, David A, Jacquin-Dubreuil A. 2000. Genetic transformation of *Ginkgo biloba* by *Agrobacterium tumefaciens*. *Physiologia Plantarum* **108**: 413–419.

Eames AJ, MacDaniels LH. 1947. The origin and development of the secondary body and its relation to the primary body. In: Sinnott EW, ed. *An introduction to plant anatomy*. New York, NY, USA: McGraw-Hill Book Company, Inc, 175–203.

Eggert DA, Gaunt DD. 1973. Phloem of *Sphenophyllum*. *American Journal of Botany* **60**: 755–770.

Emery JF, Floyd SK, Alvarez J, Eshed Y, Hawker NP, Izhaki A, Baum SF, Bowman JL. 2003. Radial patterning of Arabidopsis shoots by Class III HD-zip and *KANADI* genes. *Current Biology* **13**: 1768–1774.

Esau K. 1943. Origin and development of primary vascular tissues in seed plants. *Botanical Review* **9**: 125–206.

Esau K. 1977. *Anatomy of seed plants, 2nd edn*. Hoboken, NJ, USA: Wiley.

Esau K, Cheadle VI. 1955. Significance of cell divisions in differentiating secondary phloem. *Acta Botanica Neerlandica* **4**: 348–357.

Esau K, Cheadle VI. 1969. Secondary growth in *Bougainvillea*. *Annals of Botany* **33**: 807–819.

Espinosa-Ruiz A, Saxena S, Schmidt J, Mellerowicz E, Miskolczi P, Bako L, Bhalerao RP. 2004. Differential stage-specific regulation of cyclin-dependent kinases during cambial dormancy in hybrid aspen. *Plant Journal* **38**: 603–615.

Fisher JB. 1973. Control of growth and development in the monocotyledons-new areas of experimental research. *The Quarterly Review of Biology* **48**: 291–298.

Fisher JB, Ewers FW. 1989. Wound-healing in stems of lianas after twisting and girdling injuries. *Botanical Gazette* **150**: 251–265.

Floyd SK, Zalewski CS, Bowman JL. 2006. Evolution of Class III homeodomain-leucine zipper genes in streptophytes. *Genetics* **173**: 373–388.

Friedman WE, Moore RC, Purugganan MD. 2004. The evolution of plant development. *American Journal of Botany* **91**: 1726–1741.

Ganfornina MD, Sánchez D. 1999. Generation of evolutionary novelty by functional shift. *Bioessays* **21**: 432–439.

Giri CC, Shyamkumar B, Anjaneyulu C. 2004. Progress in tissue culture, genetic transformation and applications of biotechnology to trees: an overview. *Trees* **18**: 115–135.

Groover AT. 2005. What genes make a tree a tree? *Trends in Plant Science* **10**: 210–214.

Groover A, Mansfield S, DiFazio S, Dupper G, Fontana J, Millar R, Wang Y. 2006. The *Populus* homeobox gene *ARBORKNOX1* reveals overlapping mechanisms regulating the shoot apical meristem and the vascular cambium. *Plant Molecular Biology* **61**: 917–932.

Groover A, Nieminen K, Helariutta Y, Mansfield SD. 2010. Wood formation in *Populus*. In: Jansson, Bhalerao, Groover, eds. *Genetics and genomics of Populus*. Dordrecht, Netherlands: Springer.

Judd WS, Campbell CS, Kellogg EA, Stevens PF, Donoghue MJ. 2008. Chapter 7. An overview of green plant phylogeny. In: Judd WS, ed. *Plant systematics: a phylogenetic approach*. Sunderland, UK: Sinauer Associates, Inc., 153–184.

Kidner C, Sundaresan V, Roberts K, Dolan L. 2000. Clonal analysis of the Arabidopsis root confirms that position, not lineage, determines cell fate. *Planta* **211**: 191–199.

Kim S, Soltis DE, Soltis PS, Zanis MJ, Suh Y. 2004. Phylogenetic relationships among early-diverging eudicots based on four genes: were

the eudicots ancestrally woody? *Molecular Phylogenetics and Evolution* **31**: 16–30.

Kirst M, Johnson AF, Baucom C, Ulrich E, Hubbard K, Staggs R, Paule C, Retzel E, Whetten R, Sederoff R. 2003. Apparent homology of expressed genes from wood-forming tissues of loblolly pine (*Pinus taeda* L.) with *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences, USA* **100**: 7383–7388.

de Kort I. 1993. Wood production and latewood percentage of Douglas-fir from different stands and vitality. *Canadian Journal of Forest Research* **23**: 1480–1486.

Kramer EM, Lewandowski M, Beri S, Bernard J, Borkowski M, Borkowski MH, Burchfield LA, Mathisen B, Normanly J. 2008. Auxin gradients are associated with polarity changes in trees. *Science* **320**: 1610.

Larson PR. 1975. Development and organization of primary vascular system in *Populus deltoides* according to phyllotaxy. *American Journal of Botany* **62**: 1084–1099.

Larson PR. 1976. Procambium vs cambium and protoxylem vs metaxylem in *Populus deltoides* seedlings. *American Journal of Botany* **63**: 1332–1348.

Larson PR. 1994. *The vascular cambium*. New York, USA: Springer-Verlag.

Larson PR, Isebrand JG. 1974. Anatomy of primary-secondary transition zone in stems of *Populus deltoides*. *Wood Science and Technology* **8**: 11–26.

Lee C, Kim S-C, Lundy K, Santos-Guerra A. 2005. Chloroplast DNA phylogeny of the woody *Sonchus* alliance (Asteraceae: Sonchinae) in the Macaronesian Islands. *American Journal of Botany* **92**: 2072–2085.

Lens F, Dressler S, Jansen S, van Evelghem L, Smets E. 2005. Relationships within *Balsaminoid ericales*: a wood anatomical approach. *American Journal of Botany* **92**: 941–953.

Lough TJ, Lucas WJ. 2006. Integrative plant biology: role of phloem long-distance macromolecular trafficking. *Annual Review of Plant Biology* **57**: 203–232.

Mardis ER. 2008. The impact of next-generation sequencing technology on genetics. *Trends in Genetics* **24**: 133–141.

Mele G, Ori N, Sato Y, Hake S. 2003. The knotted1-like homeobox gene *BREVIPEDICELLUS* regulates cell differentiation by modulating metabolic pathways. *Genes and Development* **17**: 2088–2093.

Melzer S, Lens F, Gennen J, Vanneste S, Rohde A, Beeckman T. 2008. Flowering-time genes modulate meristem determinacy and growth form in *Arabidopsis thaliana*. *Nature Genetics* **40**: 1489–1492.

Ming R, Hou S, Feng Y, Yu Q, Dionne-Laporte A, Saw JH, Senin P, Wang W, Ly BV, Lewis KLT *et al.* 2008. The draft genome of the transgenic tropical fruit tree papaya (*Carica papaya* Linnaeus). *Nature* **452**: 991–996.

Neale DB, Ingvarsson PK. 2008. Population, quantitative and comparative genomics of adaptation in forest trees. *Current Opinion in Plant Biology* **11**: 149–155.

Nilsson J, Karlberg A, Antti H, Lopez-Vernaza M, Mellerowicz E, Perrot-Rechenmann C, Sandberg G, Bhalerao RP. 2008. Dissecting the molecular basis of the regulation of wood formation by auxin in hybrid aspen. *Plant Cell* **20**: 843–855.

Olson ME. 2007. Wood ontogeny as a model for studying heterochrony, with an example of paedomorphosis in *Moringa* (Moringaceae). *Cambridge Journals Online* **5**: 145–158.

Olson ME, Rosell JA, Geber M. 2009. Using heterochrony to detect modularity in the evolution of stem diversity in the plant family Moringaceae. *Evolution* **60**: 724–734.

Pace MR, Lohmann LG, Angyalossy V. 2009. The rise and evolution of the cambial variant in Bignonieae (Bignoniaceae). *Evolution & Development* **11**: 465–479.

Palmer JD, Soltis DE, Chase MW. 2004. The plant tree of life: an overview and some points of view. *American Journal of Botany* **91**: 1437–1445.

Poorter L, McDonald I, Alarcón A, Fichtler E, Licona J-C, Peña-Claros M, Sterck F, Villegas Z, Sass-Klaassen U. 2009. The importance of wood traits and hydraulic conductance for the performance and life history strategies of 42 rainforest tree species. *New Phytologist* **185**: 481–492.

Prigge MJ, Otsuga D, Alonso JM, Ecker JR, Drews GN, Clark SE. 2005. Class III homeodomain-leucine zipper gene family members have overlapping, antagonistic, and distinct roles in arabidopsis development. *Plant Cell* **17**: 61–76.

Putz FE, Holbrook NM. 1991. Biomechanical studies of vines. In: Putz FE, Mooney HA, eds. *The Biology of Vines*. Cambridge, UK: Cambridge University Press, 73–97.

Rapoport HF, Loomis RS. 1986. Structural aspects of root thickening in *Beta vulgaris* L.: comparative thickening in sugarbeet and chard. *Botanical Gazette* **147**: 270–277.

Rothwell GW, Karrfalt EE. 2008. Growth, development, and systematics of ferns: does *Botrychium* sl (Ophioglossales) really produce secondary xylem? *American Journal of Botany* **95**: 414–423.

Rudall P. 1991. Lateral meristems and stem thickening growth in monocotyledons. *Botanical Review* **57**: 150–163.

Ryberg PE, Taylor EL, Taylor TN. 2007. Secondary phloem anatomy of *Cycadeoidea* (Bennettitales). *American Journal of Botany* **94**: 791–798.

Sablowski R. 2007. The dynamic plant stem cell niches. *Current Opinion in Plant Biology* **10**: 639–644.

Schrader J, Nilsson J, Mellerowicz E, Berglund A, Nilsson P, Hertzberg M, Sandberg G. 2004. A high-resolution transcript profile across the wood-forming meristem of poplar identifies potential regulators of cambial stem cell identity. *Plant Cell* **16**: 2278–2292.

Schuster SC. 2008. Next-generation sequencing transforms today's biology. *Nature Methods* **5**: 16–18.

Sharma VK, Carles C, Fletcher JC. 2003. Maintenance of stem cell populations in plants. *Proceedings of the National Academy of Sciences, USA* **100**: 11823–11829.

Soltis DE, Soltis PS. 2003. The role of phylogenetics in comparative genetics. *Plant Physiology* **132**: 1790–1800.

Stewart WN, Rothwell GW. 1993. *Paleobotany and the evolution of plants*. Cambridge, UK: Cambridge University Press.

Sundberg B, Uggla C. 1998. Origin and dynamics of indoleacetic acid under polar transport in *Pinus sylvestris*. *Physiologia Plantarum* **104**: 22–29.

Taylor TN, Taylor EL, Krings M. 2009. *Paleobotany. The biology and evolution of fossil plants*. London, UK: Academic Press/Elsevier.

The French–Italian Public Consortium For Grapevine Genome Characterization. 2007. The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. *Nature* **449**: 463–467.

Tomlinson PB, Zimmermann MH. 1967. The "wood" of monocotyledons. *IAWA Bulletin* **2**: 4–24.

Tuominen H, Puech L, Fink S, Sundberg B. 1997. A radial concentration gradient of indole-3-acetic acid is related to secondary xylem development in hybrid aspen. *Plant Physiology* **115**: 577–585.

Tuskan GA, DiFazio S, Jansson S, Bohlmann J, Grigoriev I, Hellsten U, Putnam N, Ralph S, Rombauts S, Salamov A *et al.* 2006. The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray). *Science* **313**: 1596–1604.

Uggla C, Mellerowicz EJ, Sundberg B. 1998. Indole-3-acetic acid controls cambial growth in scots pine by positional signaling. *Plant Physiology* **117**: 113–121.

Uggla C, Moritz T, Sandberg G, Sundberg B. 1996. Auxin as a positional signal in pattern formation in plants. *Proceedings of the National Academy of Sciences, USA* **93**: 9282–9286.

Van Beveren KS, Spokevicius AV, Tibbits J, Wang Q, Bossinger G. 2006. Transformation of cambial tissue *in vivo* provides an efficient means for induced somatic sector analysis and gene testing in stems of woody plant species. *Functional Plant Biology* **33**: 629–638.

Wang H, Moore MJ, Soltis PS, Bell CD, Brockington SF, Alexandre R, Davis CC, Latvis M, Manchester SR, Soltis DE. 2009. Rosid radiation

and the rapid rise of angiosperm-dominated forests. *Proceedings of the National Academy of Sciences, USA* **106**: 3853–3858.

Willis KJ, McElwain JC. 2002. *The evolution of plants*. New York, NY, USA: Oxford.

Wilson JW. 1978. The position of regenerating cambia: auxin/sucrose ratio and the gradient induction hypothesis. *Proceedings of the Royal Society of London. Series B, Biological Sciences* **203**: 153–176.

Zamski E, Azenkot A. 1981a. Sugarbeet vasculature. I. Cambial development and the three-dimensional structure of the vascular system. *Botanical Gazette* **142**: 334–343.

Zamski E, Azenkot A. 1981b. Sugarbeet vasculature. II. Translocation of assimilates in the supernumerary phloem. *Botanical Gazette* **142**: 344–346.

Zanis MJ, Soltis DE, Soltis PS, Mathews S, Donoghue MJ. 2002. The root of the angiosperms revisited. *Proceedings of the National Academy of Sciences, USA* **99**: 6848–6853.

Zobel BJ, Van Buijtenen JP. 1989. *Wood variation: its causes and control*. New York, NY, USA: Springer-Verlag.