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What genes make a tree a tree?

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Woody growth is evolutionarily ancient, yet has been gained and lost multiple times in plant evolution and is readily enhanced or minimized in eudicot speciation. New molecular genetic and genomic studies in *Populus* and *Arabidopsis* that are defining the genes responsible for cambium function and woody growth suggest that the genes regulating woody growth are not unique to woody plants. Surprisingly, key genetic mechanisms originally characterized as regulating the meristematic cells of the shoot apical meristem are also expressed in the vascular cambium during woody growth. This has important implications for the development of *Populus* as a model species and illustrates why forest trees constitute a contrived group of plants that have more in common with herbaceous relatives than we foresters like to admit.

A paradigm shift in the study of woody growth

Woody growth is fundamental to plant development and underlies important strategies in the evolution of vascular plants. The developmental process producing woody stems is known as secondary growth – the radial growth of stems that occurs after the elongation stage of growth subsides. At the heart of secondary growth is the vascular cambium [1], which consists of meristematic initials (stem cells) whose daughters are recruited to differentiate into secondary phloem (bark) and secondary xylem (wood). Forest trees exhibit extremes of secondary growth and have thus been the focus for most classical studies of cambium. However, trees are notoriously difficult to use in modern developmental genetic studies because of their large size and long generation times. In addition, secondary growth occurs late in development and thus complicates mutant screens in more tractable species because defects in early developmental events are expected to affect secondary growth indirectly. As a result, secondary growth is poorly defined at the molecular genetic level and the cambium remains the least understood plant meristem.

Two developments are facilitating a paradigm shift in the study of cambium and secondary growth. First, the realization by molecular geneticists that cambium and secondary growth are not unique to trees and can be studied in the model plant *Arabidopsis* [2,3]. Second, the development of genomic and molecular genetic tools for the model tree genus *Populus*, including the recent sequencing of the *Populus* genome (http://www.ornl.gov/sci/ipgc/). Early results from studies in these model eudicots are beginning to outline the classes of genes responsible for cambium function and secondary growth. Surprisingly, many of the genetic mechanisms well characterized for their role in regulating the shoot apical meristem are also at work during secondary growth, as discussed below. These results not only point to a framework for further exploring the developmental processes underlying secondary growth but also potentially explain features of the evolution of secondary growth, including how secondary growth can be readily recruited or modified during speciation.

Evolution of secondary growth and forest trees

A tree-like form has arisen multiple times during plant evolution and because preservation of woody tissues is favored there is a good fossil record describing key evolutionary events [4]. Plants exhibiting alternative forms of secondary growth include extinct arborescent lycopods and horsetails of the Carboniferous forests with unifacial cambium. However, only secondary growth as typified by extant tree species containing a continuous cylinder of cambium producing secondary xylem and secondary phloem will be considered in this Opinion article. This form of secondary growth is evolutionarily ancient and pre-dates the divergence of gymnosperms and angiosperms [4]. Thus, it is likely that some aspects of secondary growth in gymnosperms and angiosperms are homologous, (i.e. have a common evolutionary origin). Another striking feature of plant evolution is that secondary growth produced by a continuous cylinder of vascular cambium is largely absent in the monocots. Although they present fundamental questions for future studies, these larger order issues of woody plant evolution cannot be rigorously addressed with current molecular data. By contrast, studies in *Arabidopsis* and *Populus* can address the molecular genetics of secondary growth in eudicots using new genomic and molecular tools. However, meaningful molecular studies cannot be designed or interpreted without considering seed plant evolution and angiosperm speciation.

Visit your favorite plant nursery and you will find plants categorized by their appearance and function, including a group categorized as 'trees'. This categorization is intuitive and practical but contrived. When considering secondary growth in extant eudicot species, we find the plants termed trees scattered among diverse taxa and not within a monophyletic group (Figure 1). Similarly, species with various degrees of secondary growth ranging from trees to herbs are found within eudicot orders and families, suggesting that secondary growth is a measure of degree, rather than a trait that is present or absent or a trait that has arisen uniquely
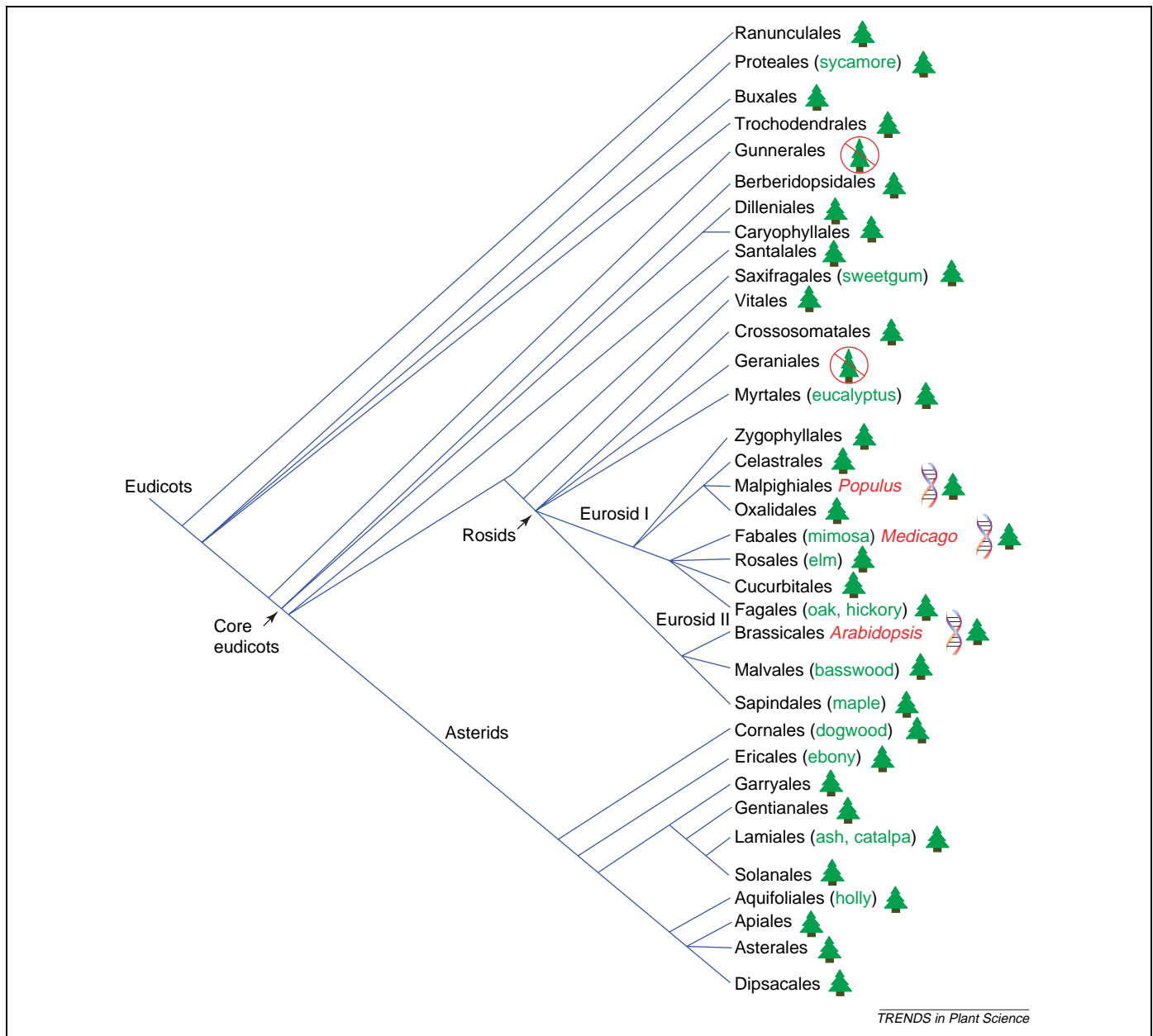
Figure 1. The distribution of tree species within eudicot orders illustrates that trees do not represent a monophyletic group. All orders containing one or more species annotated as having tree-like form by the Angiosperm Phylogeny Website database (Stevens, P.F. (2004) Angiosperm Phylogeny Website. Version 5, May 2004. http://www. mobot.org/MOBOT/research/APweb/) are indicated by a forest tree symbol. Only two orders do not contain tree-like species (Gunnerales and Geraniales). Species for which complete genome sequencing has been completed (*Arabidopsis* and *Populus*) or is underway (*Medicago*) are indicated by DNA helices. Some familiar forest tree species are indicated scattered among diverse taxa.

within a single lineage. Even within species, the expression of secondary growth can depend on environmental conditions. For example, the normally herbaceous *Arabidopsis* can transition to secondary growth when vegetative growth is extended under short-day conditions [2,3]. Recent re-evaluation of angiosperm evolution suggests that the ancestral condition might have been characterized by woody growth [5], consistent with previous speculation that extant herbaceous angiosperms had woody progenitors [6]. But perhaps the most striking aspect of secondary growth in eudicots is that new species with woody habit can be derived from herbaceous species relatively quickly. For example, the introduction of new species to remote islands can present unique opportunities

to observe speciation events: woody members of the genus *Sonchus* [7] and *Echium* [8] native to the Macaronesian islands have evolved from herbaceous continental progenitors (Figure 2). Although it is possible that woody growth arises *de novo* in such cases by convergent evolution, this seems unlikely given the frequency and rapidity of such events. More likely, woody growth appears as a result of modifying the expression of genes already present in herbaceous progenitors.

The prediction from these observations is that genes regulating vascular cambia and woody growth in eudicots should be evolutionarily ancient and of a common origin, present in a broad range of taxa including herbaceous, non-tree species, and be readily modifiable to enhance or
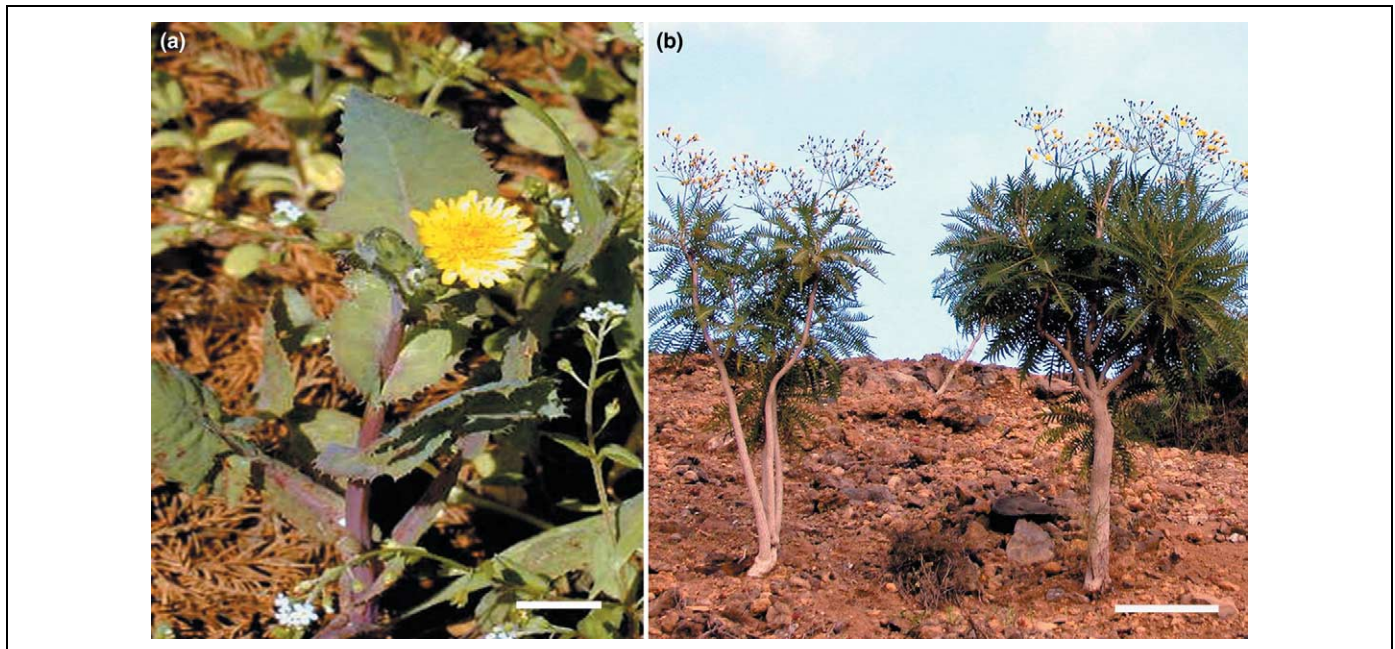
Figure 2. Woody species can be derived from herbaceous ancestors. **(a)** Continental *Sonchus asper* illustrates the herbaceous ancestral condition. **(b)** Derived woody species that evolved on Macaronesian islands include *Sonchus canariensis*. Photographs by S.C. Kim, Department of Botany and Plant Sciences, University of California Riverside. Scale bars: (a) =4 cm; (b) =0.5 m.

suppress secondary growth during speciation or in response to environmental inputs. Thus, the search for the genes that make a tree a tree should not be predicated on the notion that such genes will be present in trees and absent in herbaceous relatives. So, how do we find the genes underlying the tree-like growth habit?

Structure and function of the cambium
Consideration of cambium structure and function can guide the search for genes regulating secondary growth. Recasting the cambium in modern terms, it can be considered as a dynamic population of stem cells for which fundamental regulatory mechanisms can be anticipated. First, there must be a mechanism to identify which cells are to serve as cambial stem cells. Second, there must be mechanisms to maintain those cells in a stem cell fate. Third, there must be a means for radially patterning secondary vascular tissues, including identifying xylem fate from phloem. Fourth, there must be a balance between cells in a stem cell fate versus daughter cell differentiation.

Although these mechanisms have not been defined at the molecular level for the cambium, analogous mechanisms have been well characterized for the shoot apical meristem. Indeed, comparisons between these two meristems go beyond the anecdotal and indicate shared mechanisms or common origins. Anatomically, the pro-cambium, which gives rise to the vascular cambium, is derived from the shoot apical meristem [9]. Functionally, Marvin Bannan [10,11] inferred loss and replacement of cambium initials through elegant analysis of secondary xylem cell files, which implied that cambium stem cell specification occurs not through lineage but rather through cell-to-cell communication, as it does in the shoot apical meristem. Complex meristems, such as the vascular cambium, might be derived from simpler

meristems [12] and the evolutionarily ancient shoot apical meristem is a possible progenitor to the more recent vascular cambium.

Genes and mechanisms regulating secondary growth
New studies in *Arabidopsis* and poplar are defining the genes regulating the vascular cambium; they show that the conceptual analogies between cambium and shoot apical meristems reflect overlapping genetic regulation. Microarray analysis in *Arabidopsis* and poplar now provide a global view of gene expression during secondary growth, and developmental genetic studies in *Arabidopsis* are defining the function of individual regulatory genes. Strikingly, the most important gene families regulating basal shoot apical meristem functions are also expressed in the cambium region. A few examples are given here.

The best-characterized overlap between shoot apical meristem and cambium regulation is seen in the Class-III homeodomain-leucine zipper (HD-ZIP) and KANADI transcription factors. In *Arabidopsis*, KANADI and Class-III HD-ZIP genes establish the adaxial–abaxial polarity of lateral organs emerging from the shoot apical meristem [13]. These same genes also establish the radial patterning of primary vascular tissues [14]. Class-III HD-ZIP and KANADI genes are also expressed in the cambium region of poplar [15] and tobacco [16]. Intriguingly, Class-III HD-ZIP genes are regulated by MIR165/MIR166 microRNA gene families; together they define ancient genetic mechanisms that are at least 400 million years old [17]. Phenotypes resulting from disrupting microRNA regulation of the Class-III HD-ZIPs *REVOLUTA* [14] and *PHAVOLUTA* [16] implicate microRNA involvement in pro-cambium patterning.

Stem cell fate within the shoot apical meristem is dependent on homeobox genes. The *Arabidopsis SHOOT-MERISTEMLESS* (*STM*) gene encodes a Class-I KNOX

homeodomain protein required for shoot apical meristem stem cell maintenance [18]. The *Arabidopsis BREVIPEDICELLUS* (*BP*; also known as *KNAT1*) KNOX gene directly regulates genes involved in lignification and cell wall synthesis [19], and is partially redundant with *STM* [20]. The poplar orthologs of *STM* and *BP* are expressed in the stems of poplar [15] and *Arabidopsis* [21] during secondary growth, and promote stem cell fate in the cambium (A.T. Groover *et al.*, unpublished). The homeobox gene *WUSCHEL* (*WUS*) is also required for stem cell maintenance in the shoot apical meristem [22]. In contrast to *STM* and *BP*, *WUS* is not expressed during secondary growth in poplar [15] or *Arabidopsis* [21], although related family members are [15,21].

The spatial identification of which cells are to serve as cambium stem cells probably involves cell-to-cell signaling, as mentioned previously. In the shoot apical meristem, the analogous process involves receptor–ligand mediated cell-to-cell signaling requiring the *CLAVATA* (*CLV*) genes [23]. *CLV3* encodes a small, secreted peptide that serves as a ligand for a plasma membrane-localized receptor kinase complex containing the *CLV1* gene product. Loss-of-function *clv* mutants have an enlarged population of stem cells in the shoot apical meristem. *Arabidopsis CLV1* [21] and the putative *CLV1* poplar ortholog [15] are expressed during secondary growth. Although *CLV3* appears to be uniquely expressed in the shoot apical meristem, genes related to *CLV3* are expressed during secondary growth [15] and might provide the means for receptor–ligand signaling among secondary tissues.

Evolution of secondary growth re-examined

The overlapping genetic regulation of the cambium and shoot apical meristem suggests possible evolutionary steps leading to secondary growth. For genes such as *STM* that are expressed in the cambium and the shoot apical meristem, direct co-option [24] of shoot apical meristem functions during the evolution of the cambium seems likely because stem cell functions of the shoot apical meristem presumably pre-date the cambium. Similarly, in the case of the Class-III HD-ZIP and KANADI genes, the ancestral function was likely patterning of tissues originating from the apical meristem [17], followed by co-option for radial patterning of secondary vascular tissues. *WUS* and *CLV3* are uniquely expressed in the shoot apical meristem but have paralogs that are expressed in the cambium. Such instances would be consistent with co-option of a duplicated element [24] in which a duplicated gene acquired new functions in the cambium. It is not unusual to find such recycling of genes and mechanisms; indeed this has proven to be the rule rather than the exception in the evolution of development in plants and animals.

Given the recent molecular insights into cambium and secondary growth, some of the peculiar aspects of secondary growth can be re-examined. Many of the key genes and mechanisms regulating secondary growth are required for primary growth and shoot apical meristem functions, which might explain why secondary growth has been gained and lost so readily during eudicot speciation.

Even in plants with little or no secondary growth, the genes required for secondary growth would be retained because of selective pressure for their role in primary growth. For plants such as *Arabidopsis* that transition to secondary growth only under certain environmental conditions, the required genes must be present – secondary growth is merely a reflection of gene expression.

Prospects

The arguments presented here suggest that secondary growth is homologous among diverse eudicot species, and perhaps even between eudicots and gymnosperms, and that within eudicots, genes required for secondary growth are not unique to forest trees and are usually present in herbaceous species, including *Arabidopsis*. Several practical implications flow from these suggestions. Obviously, in the search for which genes make a tree versus a herbaceous plant, it would be folly to look for genes present in poplar and absent in *Arabidopsis*. More likely, tree forms reflect differences in expression of a similar suite of genes to those found in herbaceous relatives. Although diverse tree species have great ecological and economic value, it is not possible to develop advanced genetic and genomic tools for all tree species of interest. However, if the basal mechanisms regulating the cambium and secondary growth in diverse species are similar, initial gene discovery and characterization can occur in model species and then be extended to less tractable species. Even though the basal regulatory mechanisms might be shared, significant variation in wood anatomy occurs among tree species and the details of secondary growth will have to be worked out on a species-by-species basis.

Our understanding of woody plant development should advance rapidly. Genomic tools are being developed that make effective use of the *Populus* genome sequence for discovering candidate genes regulating secondary growth, including insertional mutagenesis [25], gene trapping [26] and microarray resources [15]. The overlap of genetic mechanisms regulating shoot apical and cambium meristems should be further explored. Detailed knowledge of shoot apical meristem regulation can be paired with cambium gene expression patterns to identify key candidate genes regulating cambium functions. However, overlapping regulation of multiple developmental processes (e.g. the cambium and shoot apical meristem) presents a vexing problem for determining gene function. If a gene regulating the cambium is also required for a crucial process occurring earlier in development, it becomes difficult to study the role of the gene in the later process. A striking example is *STM*, which plays a role in regulating the cambium, but loss-of-function alleles condition a seedling lethal phenotype.

New approaches should be developed for studying the function of genes specifically during secondary growth. The ability to evaluate the effect of changing gene expression or function specifically during secondary growth in otherwise wild-type plants is crucial. Strategies that can alter gene expression specifically in secondary tissues are being developed for *Populus*, including chemical induction of gene expression (S. Strauss, personal

communication) and genetic mosaic strategies (A.T. Groover *et al.*, unpublished). Ultimately, hypotheses developed using model species must be tested in an evolutionary context. Once candidate genes regulating processes underlying secondary growth are in hand, a survey of a broad range of taxa could address broader issues, including the absence of secondary growth in monocots, and the relationship of secondary growth in eudicots and gymnosperms.

Acknowledgements

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