Comparative and Evolutionary Genomics of Forest Trees

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Abstract Comparative and evolutionary genomic approaches can identify genes regulating biological processes, and describe how those genes have been modified through speciation to produce phenotypic variation. These approaches have the potential to address fundamental issues of forest biology, including the regulation of biological traits important to industry and conservation, but have not been widely applied because of technical limitations. Here, we argue that powerful "next generation" DNA sequencing technologies now make comparative and evolutionary genomic approaches not only tractable for basic biological research in trees, but also have the potential to be more informative and cost effective than traditional, one-species-at-a-time approaches. However, designing effective comparative studies for forest trees requires careful consideration of the evolutionary relationships of tree species and biological traits important to forest biology.

This chapter first provides an introduction to comparative and evolutionary genomics, followed by a brief review of some of the general features of the evolution and diversification of forest tree species. Next, two biological processes are discussed that are fundamental to forest trees: wood formation and perennial growth. We examine the varied evolutionary histories of these biological processes, and how these histories relate to the comparative genomic approaches used to research the genes and mechanisms underlying these processes. The chapter is concluded with discussion of practical issues that must be addressed to fully enable this new and powerful direction in forest genomics research, as well as how comparative genomics could support future research and applications for forest management.

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T. Fenning (ed.), *Challenges and Opportunities for the World's Forests in the 21st Century*, Forestry Sciences 81, DOI 10.1007/978-94-007-7076-8_26,

1 Introduction

Forest trees are defined by practical attributes, typically as woody perennial plants with a primary stem of some minimum height. The majority of forest trees share additional attributes. Forest trees typically go through a period of juvenility before undergoing phase change and becoming sexually mature. Forest trees tend to be highly out crossing, genetically heterozygous, and suffer from inbreeding depression. And forest trees are largely undomesticated.

Forest trees do not represent a monophyletic group of plants, however. Tree-like growth has been gained and lost in different seed plant lineages, and extant forest trees can be found among various gymnosperm and angiosperm taxa (Groover 2005; Spicer and Groover 2010). This situation raises important considerations for research aimed at understanding growth and development of the large number of taxonomically diverse tree species that are important to society and to ecosystems. As an example, the genus *Eucalyptus* is more closely related taxonomically to the herbaceous annual, *Arabidopsis*, than to the angiosperm trees ash, ebony, sycamore, and sweet gum (Angiosperm Phylogeny Group 2003). But even though *Eucalyptus* and *Arabidopsis* are more closely related, is *Arabidopsis* a suitable model for helping understand the traits associated with perennial, woody growth of *Eucalyptus*? Even more challenging, gymnosperms such as pines, yews, spruces, and firs are separated from angiosperm trees by more than 300 million years of evolution (Taylor et al. 2009). Does the woody, perennial growth of gymnosperms have a common (homologous) origin with that of angiosperms? If not, the mechanisms underlying growth must be identified independently in gymnosperms and angiosperms. On the other hand if they are homologous, comparative studies between gymnosperms and angiosperms could identify common, ancestral mechanisms regulating tree-like growth, and determine how those mechanisms have been modified during speciation to produce the variation we see today among extant tree species. Such a strategy could be more informative and cost effective than working within individual species, as has largely been the case to date, and would provide insights relevant to all tree species.

Genomic sciences are now providing the means to address previously intractable problems in forest biology. Two decades ago it was a significant feat to clone a single gene from a tree, but the sequencing of the entire genome of *Populus trichocarpa* (Tuskan et al. 2006) supported an explosion of new research and enabled the facile cloning and characterization of genes. Genome sequence also supported the development of powerful functional genomic tools for *Populus* such as microarrays capable of genome-wide assay of gene expression in tissue samples from individual trees (Tsai et al. 2010), and the identification of large numbers of genetic markers useful for association mapping studies that seek correlations between genotypes and phenotypes e.g. (Ingvarsson et al. 2008; Wegrzyn et al. 2010). Full genome sequence is now available for other woody perennial species, including *Eucalyptus* (http://www.phytozome.net/eucalyptus.php), grape (Velasco et al. 2007), and papaya (Ming et al. 2008). Excitingly, more genome sequencing is underway, including several conifers species that represent an important taxonomic group. The expectation is that genome sequence and extensive transcriptome (expressed gene sequence) information will be available in the near future for an increasing number of tree species.

The opportunities afforded by the ability to sequence the genomes and comprehensively genotype large numbers of individuals from various forest tree species should compel us to rethink our strategies for forest biology and forest genomics research. How can we optimize genomic research strategies to better understand the evolutionary histories and relationships among the thousands of forest tree species of interest? How can we use genomics to better predict future responses of forests to climate change, develop better forest-based biofuels feedstocks, or meet the demands for forest-based products? How do we best develop genomic research tools and applications to understand, monitor, and manage the growth, health, and conservation of forests? These and other fundamental questions are being pushed to the front of forest biology by competing pressures on forests by climate change, increasing needs for forest products and ecosystem services, and the desire to use forest trees as a source of bioenergy (FAO 2008).

We propose that comparative and evolutionary genomics will provide the next major technical and methodological advances that will produce fundamental breakthroughs in our understanding of the basic biology underlying traits important to forestry science, conservation, and management. We anticipate that the next chapter in forest biology will leverage approaches of comparative and evolutionary genomics, with experiments designed to explore the diversification of genes and the regulation of biological traits both within and among species. Such approaches are potentially more scientifically powerful than working within individual species. Such strategies can also address some very practical problems, including how a limited research community can make best use of increasingly large sequencing resources, and address complex biological problems in the large number of species of scientific, economic, and ecological interest. In the next section, we provide a brief introduction to the sequencing technologies that enable comparative and evolutionary genomics.

2 Comparative and Evolutionary Genomics in the Age of Next Generation Sequencing

“Next generation sequencing” (NGS) technologies are dramatically changing the strategies for forest genomics research. NGS technologies are developing with an enormous speed and increasingly produce longer and more numerous sequences, while at the same time the price per-base pair of sequencing is decreasing exponentially(Stein 2010). Others have reviewed technologies underlying NGS e.g. (Metzker 2010), which are quickly evolving. Regardless of the specifics of the underlying technologies, in general NGS provides increasingly cost effective methods for new genomics approaches and applications that rely on the ability to sequence the expressed genes or even entire genomes of multiple species and/or individuals within species (Mardis 2008; Schuster 2008). For example, “RNA sequencing” (RNA-seq) using NGS can comprehensively quantify the expression of all the genes in an individual organism for specific developmental time-points, among different tissues, or in response to different environmental conditions or treatments (Wang et al. 2009). NGS is also being used to produce large numbers of genetic markers within species that can be used for population genetic studies of genetic diversity, association mapping of genes regulating traits of interest, or any other marker-based approaches. For example, “genotype by sequencing” approaches using NGS can be used to simultaneously identify hundreds of thousands of genetic markers and genotype hundreds of individuals (Elshire et al. 2011). Such approaches enable research into previously intractable problems in forest biology including the dissection of the genetic regulation of complex biological traits, and understanding population-level genetic factors that underlie adaptation to specific environments (Neale and Kremer 2011).

Comparative genomics and the closely-related evolutionary genomics refer to a variety of approaches that ultimately seek to understand the diversification of genes, genomes, species, and biological traits within an evolutionary and taxonomic framework. There are increasing examples of comparative and evolutionary studies from both herbaceous model plants and forest trees, where analysis of DNA sequence and gene expression data have provided important insights into the evolution of genome properties as well as genes that ultimately underlie phenotypic differences among and within species. For example, gene discovery and annotation is a basic need for making any new forest tree genome sequence usable by the research community, and remains a challenging but increasingly automated task. Comparative genomic approaches played an important part in the annotation and interpretation of the first forest tree genome to be sequenced, *Populus trichocarpa* (Tuskan et al. 2006). Some 45,000 gene models in the *Populus* genome were discovered and annotated in part through comparison to other angiosperm genomes, including *Arabidopsis* (Tuskan et al. 2006). Sequence comparisons both among regions within the *Populus* genome and among other angiosperm genomes enabled the inference of genome duplication events within angiosperm and *Salicaceae* (order containing *Populus*) lineages (Sterck et al. 2005; Tuskan et al. 2006). Duplicated gene pairs (paralogs) are of interest because they provide the opportunity for divergence in expression or function of one or both paralogs, and thus provide fodder for evolutionary change. Use of microarray analysis of expression for duplicated gene paralogs in different tissues of *Populus* provided evidence for significant sub-functionalization of duplicated genes that have acquired distinct expression patterns (Segerman et al. 2007). Similar but more detailed studies of duplicated genes in *Arabidopsis* found that paralogous genes duplicated as part of large-scale events (i.e. duplication of entire or large segments of chromosomes) tend to have more similar expression pattern than genes duplicated on small-scale segments (Casneuf et al. 2006), and that most of the differences in expression between gene pairs occurs shortly after duplication (Ganko et al. 2007). Looking across species, comparison of expression for orthologous genes in *Arabidopsis* versus *Populus* organs found a range of congruity. The percentage of orthologous gene pairs expressed in both species was 60 % for genes expressed in roots, 58 % in young leaves, 69 % in mature leaves and stem nodes, and 76 % in stem nodes (Quesada et al. 2008). These same authors found that genes broadly expressed in multiple plant organs tend to be more conserved in their expression, in contrast to genes that are organ-specific (Quesada et al. 2008).

Changes in protein sequence can also play an important role in the evolution of gene function and phenotypic traits. For example, *Arabidopsis* gene families that contain at least one member that presents a loss-of-function morphological phenotype were used to examine the relative role of changes in expression pattern versus changes in protein sequence in driving morphological evolution (Hanada et al. 2009). Changes in protein sequence (59–67 % of changes), not changes in gene expression (33–41 % of changes), played the larger role in functional changes to duplicated genes that influenced morphological traits (Hanada et al. 2009). This stands in contrast to evolution of development in animals, where it has been argued that changes in gene expression play the prominent role in evolution of morphological traits (Carroll 2008). This could be a reflection of the history of prevalent genome duplication in plants versus animals. However, the relative number of examples in which the causative locus underlying morphological evolution in plants is low, and thus the relative contribution of changes in gene expression versus protein function in influencing evolution of traits remains uncertain.

While challenging, progress is being made in moving from comparative studies that primarily examine DNA sequence diversity, to studies that describe causative links between evolution of genes and phenotypic outcomes. For example, comparative approaches have been used to study the rapid diversification and speciation in *Aquilegia* (columbines) (Kramer 2009), including identification of loci involved in the diversification flower morphology (Voelckel et al. 2010) and adaptation to diverse habitats, and to identify miRNAs and their cognate target loci (Puzey and Kramer 2009). In *Mimulus* (monkey flowers), comparative genomic approaches were used to identify loci involved in parallel evolution of petal pigmentation in two species, *M. cupresus* and *M.luteus* (Cooley et al. 2011). A fascinating case in tomato illustrates how change in both gene expression and protein function for a single gene influenced morphological diversification (Kimura et al. 2008). In this case, changes in degree of complexity of compound leaves between wild tomato species of the Galapagos Islands has been ascribed to changes in the expression of a single transcription factor, PETROSELINIUM (PTS). PTS is a truncated KNOX transcription factor that lacks a homeodomain. PTS acts by competing with “normal” KNOX proteins for heterodimerization with a BEL-like Homeodomain protein. But in addition, in species with more complex compound leaves, *PTS* expression is upregulated by as a result of a single base pair change in the gene’s promoter, thus promoting the antagonistic effects of the truncated PTS protein.

NGS sequencing technologies are greatly expanding the previously limited scope of experimental approaches for comparative and evolutionary genomics. But how do we apply NGS-based comparative methods to the study of forest trees? To do so, it is vital to first consider the relationships among tree species. In the next section, we discuss a foundation topic for comparative genomics in forest trees – the evolutionary history and diversification of forest trees.

3 Evolutionary Origins and Diversification of Trees

A defining feature of all extant forest trees is the presence of a meristematic layer of cells inside the stem, the vascular cambium. The cambium is a thin layer of cells that divide over time to produce new cells that can develop into either wood or inner bark, processes collectively known as secondary vascular growth (Larson 1994). What are the advantages to secondary vascular growth and woody stems, and what factors drove the evolution of woody plant forms? Woody stems can confer great advantages in the competition for light, as woody stems allow for dramatic increase in height, and this has undoubtedly been a major factor in the diversification of woody plant forms. But the first appearance of woody growth is now attributed to diminutive plants of the early Devonian (ca 400 MYA), which are likely related to the extinct basal euphyllophyte genus, *Psilophyton* (Gerrienne et al. 2011). While these plants made small amounts of wood from a cambium, they only grew to modest height, supporting the hypothesis that wood may have evolved initially to provide increased water conduction needs rather than mechanical support (Gerrienne et al. 2011). The selective pressures for such innovation could have been driven in part by decreasing CO_2 levels during the early Devonian, which would have increased transpiration rates (McElwain and Chaloner 1995).

Through vascular plant evolution, there have been multiple, independent origins of arborescent plants that contained a vascular cambium. For example, extinct arborescent lycopsids (notably, *Lepidodendron*) were dominant species in swamps and wetlands of the Carboniferous (ca 300–360 MYA) that produced the massive coal deposits mined today (Taylor et al. 2009). In contrast to extant tree species, these plants had a unifacial vascular cambium that produced limited secondary xylem (wood) but no secondary phloem (Cichan 1985a; Eggert 1961). They did have extensive periderm, however, earning the term "bark stem." Similarly, extinct sphenopsid (group that includes extant horsetails) species produced arborescent forms (Cichan 1985b). These stems were tubes characterized by wide pith and a cambium that produced both secondary xylem and phloem (Eggert and Gaunt 1973; Taylor et al. 2009). However, these lineages were evolutionary dead ends, and are not ancestral to any living arborescent species.

The progymnosperms emerged in the Devonian as the first arborescent plants that had a bifacial cambium producing a woody stem similar in structure and anatomy to extant forest trees (Beck 1960). Although there are significant uncertainties about the relationships among progymnosperms, gymnosperms, and angiosperms, it is likely that modern gymnosperm and perhaps angiosperms have their evolutionary origins in progymnosperm lineages (Gifford and Foster 1989). If that is the case, it is possible that the vascular cambium and woody growth found in angiosperms and gymnosperms are homologous (have a shared evolutionary origin).

In extant species, forest trees can be found distributed among both angiosperm and gymnosperm lineages. While there is fossil evidence for extinct gymnosperms with herbaceous habit (Rothwell et al. 2000), all extant and most extinct gymnosperms are characterized by woody growth from a bifacial cambium. For angiosperms, there is a distinct possibility that the ancestral state was woody (but for a more nuanced view, see (Carlquist et al. 1996)). Basal angiosperm lineages include forest trees and woody plants. For example, *Amborella* is a genus of extant basal angiosperms that grow as shrubs or small trees with woody growth supported by a bifacial vascular cambium, and an *Amborella* genome is currently being sequenced (Soltis et al. 2008). Interestingly, the wood of *Amborella* lacks vessel elements (Feild et al. 2000), and given its basal angiosperm phylogenetic position *Amborella* provides insights into early angiosperm wood anatomy. Notably, while there are arborescent monocots (e.g. palms), their stem anatomy is distinctly different from forest trees: monocots lack a true vascular cambium and do not make wood.

Forest trees are found throughout most eudicot orders, and do not represent a monophyletic group (Groover 2005). Interestingly, eudicot tree species can be found that have close relatives with distinctly different growth habits, including herbaceous annuals. Examples of both gain and loss of woody habit can be found within eudicot orders. Gain of woody growth during speciation from herbaceous ancestors (so called secondarily woody species) are relatively common and have occurred independently in various eudicot taxa (Carlquist 2009), suggesting that woody growth form can re-evolve from herbaceous ancestors relatively rapidly.

4 Evolution and Diversification of Wood Formation

The woody growth of stems within angiosperm and gymnosperm taxa is characterized by a bifacial vascular cambium that produces both secondary phloem (inner bark) and secondary xylem (wood) (Esau 1977). Regardless of the evolutionary origins of woody growth in angiosperms and gymnosperms, there are some important differences in woody development and anatomy in these taxa (Esau 1977). Wood of familiar conifers (e.g. pines) is characterized as being comprised of long tracheary elements called tracheids that serve both water conducting and mechanical support functions. In most angiosperm woods, tracheary elements known as vessel elements are the primary water conducting cell type, and can have much wider lumens than tracheids. Mechanical support in angiosperm wood is provided by lignified fibers. Both angiosperm and conifer woods have rays, while only conifer woods contain well developed resin ducts. In response to gravity, angiosperm stems from tension wood to pull leaning stems upright, while conifers form compression wood to push leaning stems upright (Wilson and Archer 1977).

While conifer woods are relatively homogeneous in their anatomies, amazing variation can be found in angiosperm wood anatomy, including presence or absence of vessels, successive cambia, xylem furrowed by xylem, ring porous versus ring diffuse wood, storied versus unstoried cambia, and variation in the presence and structure of rays (Carlquist 2001). Many of these variations have arisen independently in multiple lineages, indicating that the developmental mechanisms regulating the cambium and wood formation in angiosperms are relatively plastic to evolutionary forces.

Important differences can be found between the biochemical makeup of angiosperm and gymnosperm woods that impact applications including pulp and paper production, biofuels, and mechanical properties important for use of wood in construction. For example, lignin is a biopolymer that imparts hydrophobicity and strength to cell walls (Boerjan et al. 2003), and has played a major role in land plant evolution and diversification (Peter and Neale 2004; Weng and Chapple 2010). Lignin is a major component of wood, and is the second most abundant biopolymer on earth. Angiosperm lignin typically consist primarily of guaiacyl and syringyl units, with traces of *p*-hydroxyphenyl units (Boudet et al. 1995). In contrast, gymnosperm lignin is composed primarily of guaiacyl units with significant *p-hydroxyphenyl* units, but lack syringyl units. These differences have major impacts on pulping efficiencies and end uses for softwoods (conifers) versus hardwoods (dicots).

So significant similarities and differences exist between conifer and angiosperm wood, and among angiosperm woods from different species. But how is that reflected by the genetic mechanisms that regulate wood formation among gymnosperm and angiosperm species?

5 Molecular and Genetic Regulation of Wood Formation

Genomic and molecular genetic tools have enabled a rapid increase in our understanding of the genes and mechanisms controlling the cambium and wood formation. Several reviews have summarized the current state of knowledge of mechanisms regulating the developmental aspects of the cambium and wood formation (Du and Groover 2010; Groover et al. 2010; Matte Risopatron et al. 2010), and the biosynthesis of secondary cell walls (Carpita 2011) and lignification (Boerjan et al. 2003; Zhong and Ye 2009). What follows is a brief synopsis of some of the major findings.

One interesting insight into the evolution of the vascular cambium is that some of the important regulatory genes that control the shoot apical meristem have been co-opted during the evolution of the cambium (Groover 2005). Extensive gene expression datasets from wood forming tissues of *Populus* showed that several important transcriptional regulators are expressed in both the shoot apical meristem and the cambium (Schrader et al. 2004). Since the shoot apical meristem predates the cambium in plant evolution (Gifford and Foster 1989), this indicates that these genes acquired a new expression pattern that extended into the meristematic cells of the cambium. Examples of these directly co-opted regulatory genes include the Class I KNOX transcription factors ARBORKNOX1 and 2, which regulate cell differentiation during wood formation (Du et al. 2009; Groover et al. 2006). Interestingly, this small family of transcription factors forms a separate clade in angiosperms and gymnosperm lineages (Guillet-Claude et al. 2004). In other examples, shoot apical meristem regulatory genes have undergone duplication, with a duplicate copy eventually acquiring unique expression in the cambial zone. For example, WUSCHEL (*WUS*) is well characterized for its role in maintaining the stem cells of the shoot apical meristem (Laux et al. 1996). *WUS* is not expressed in the cambial zone, but a related family member, WOX4, is and acts to regulate cambial divisions (Hirakawa et al. 2010). Undoubtedly there are also regulators that are unique to the cambium, but to date there is not a definitive view of the relative roles of co-option versus evolution of unique regulatory modules in the cambium and wood forming tissues.

There is limited insight into the evolution of mechanisms regulating the cambium and wood formation in angiosperms versus gymnosperms. One study of gene expression in wood forming tissues of loblolly pine found evidence for a significant percentage of genes uniquely expressed in pine versus Arabidopsis or *Populus* (Kirst et al. 2003), but such studies are limited in their ability to detect orthologs that have significantly diverged.

6 Evolution and Developmental Regulation of Perennial Growth

The most extensive wood formation is found in perennial plants such as forest trees. The two traits are separable to some degree, however, as there are annual plants that can produce woody tissues from a vascular cambium (e.g. Arabidopsis (Chaffey et al. 2002)) as well as perennial plants that do not produce wood (e.g. red clover). As previously mentioned, most extinct and all extant gymnosperms undergo secondary growth, and are also perennial. With angiosperms, there is a more complex evolutionary history of woody, perennial growth. It has been proposed that early angiosperms were woody perennials, and that herbaceous annuals first arose as angiosperms were experiencing the challenges of harsh winters in higher latitudes during the Tertiary (Sinnott and Bailey 1915). Presumably this trend reflects that herbaceous annuals have the advantage of overwintering underground in the form of seed or roots, in contrast to woody perennials whose persistent above ground vegetation must bear the full brunt of winter weather. This trend is reflected in current plant distributions, where 85–90 % of angiosperm species in alpine regions are herbaceous, while in tropical regions only 25–40 % of angiosperm species are herbaceous (Sinnott and Bailey 1915). Additionally, shifts among growth habits and woodiness have been commonplace in the evolution of many angiosperm taxa (Carlquist 2009).

The growth habit of most forest trees in the temperate parts of the world is rather similar. As temperatures rise after the winter, trees will eventually start to grow laterally (i.e. wood formation will start) and apically (i.e. buds will flush), new leaves will develop and shoots will elongate (see e.g. Hänninen and Tanino 2011 for a review). A major challenge is to time growth initiation in the spring so that the growing season is maximized without risking premature growth and exposure to late winter/early spring cold spells. For example, in boreal forests there are periods when air temperatures during the day could be high while the ground is still frozen, and initiation of vegetative growth and transportation cannot be supported by the root system (Oquist and Huner 2003). To avoid desiccation, dormancy mechanisms must be in place to block growth until conditions are permissive.

The summer season is characterized by photosynthetic carbon fixation driving lateral growth of the stem and root growth. Some trees (e.g. pines and oaks) have a determinate growth in which vegetative buds set the previous season contain all the primordia of the new year's leaves, and are expanded to produce a flush of leaves and elongating stem tissue, before setting new terminal buds. Other species are characterized by an indeterminate apical growth habit in which new lateral buds and leaves continue being produced (Kozlowski and Pallardy 1979). In some species both determinate and indeterminate growth may be present on the same tree, and can often be very easily distinguished from each other. In aspen (*Populus tremula* and *P. tremuloides*), the characteristic rounded, serrated trembling leaves come from determinate growth of mature parts of tree, but if juvenile shoots emerge from the same trunk leaves are typically much larger and not rounded. A microRNA (miR156) seems to have a role in this phase change (Wang et al. 2011).

At the end of the growing season, forest trees react to environmental cues that start several processes; wood formation stops, terminal buds are formed, cold hardiness is acquired and – in deciduous species – leaves will senescence and abscise and dormancy is induced. The obvious cues to these developmental transitions are the shortening of the photoperiod and decreasing temperatures, and trees typically use both to correctly time these events (Garner and Allard 1923). Most trees flower in spring, some very early before vegetative budbreak (e.g. *Populus trichocarpa*), others later. The developmental decision to flower has therefore been taken the year before flowering and the buds that are formed in the autumn are either vegetative or reproductive (for an example in *Populus*, see (Yuceer et al. 2003)). Environmental conditions permissive for flowering may induce the formation of reproductive buds in parts of the tree that have reached maturity, but almost nothing is known about how this is regulated. The physiology of the tree is also a critical factor since many trees – especially in harsher climates – flower periodically, some years most trees in an area may flower, other years almost none. Needless to say, there exist a large amount of within-species variation in all these traits, and exploration of this natural variation has been one fruitful approach to dissect the processes behind different phenological traits e.g. (Frewen et al. 2000; Ma et al. 2010).

7 Regulation of Bud Set and Autumn Senescence

Several molecular details behind the regulation of bud set have been elucidated by studies in *Populus*. Photoreceptors phytochrome A and phytochrome B (Ingvarsson et al. 2008; Olsen et al. 1997) – and orthologs of the downstream components *CONSTANS*(*CO*) and *FLOWERING LOCUS T*(*FT*)(Böhlenius et al. 2006), have been shown to regulate bud set and dormancy in *Populus*. Also in *Populus*, orthologs of circadian clock components *LATE ELONGATED HYPOCOTYL1* and *2* and *TIMING OF CAB EXPRESSION1* have been implicated in cold hardiness and bud burst (Ibanez et al. 2010). In annuals, the main function of these components seems to be in the regulation of flowering time, which they also regulate in trees (Bohlenius et al. 2006). It is intriguing that the same system has been recruited for different purposes and in trees – at least in *Populus* – two different outputs are generated that regulates different key processes (see e.g. (Lagercrantz 2009)). Although photoperiodic control of bud set is very common among forest trees in temperate regions, some *Rosaceae* species like *Sorbusaucuparia* may instead rely on temperature cues (Heide and Prestrud 2005), and at extreme latitudes cases diurnal fluctuations in temperature may substitute for photoperiodic cues (Heide 1974). A *PopulusAINTEGUMENTA-LIKE 1* (AIL1) transcription factor appears to act downstream of the *CO*/*FT*regulon, and regulates the expression of key cell cycle genes, e.g. cyclins (Karlberg et al. 2011). Downregulation of the *PopulusAIL1* and/or other homologous genes seem to be a prerequisite for growth cessation. The Arabidopsis *Aintegumenta* has previously been shown to regulate cell cycle genes (Mizukami and Fischer 2000). The fact that in *Populus*, orthologs of *CO*/*FT* and *AIN1* together regulate growth cessation has been suggested to be an example of an "evolutionary mix and match" strategy (Karlberg et al. 2011). Hormones including GA and ABA e.g. (Eriksson and Moritz 2002; Ruttink et al. 2007) have crucial roles during growth arrest and bud set. More recently, changes of auxin response has been implicated in growth cessation, through stabilization of repressor auxin (AUX)/indole-3-acetic acid (IAA) proteins (Baba et al. 2011).

Less is known about the trigger(s) of autumnal senescence in deciduous trees. It is not obvious that photoperiod should trigger leaf senescence in milder climates, precocious senescence in a mild autumn would lead to significant losses in annual photosynthetic yield. Many trees do not shed their leaves until they do not contribute to net photosynthesis. However, there is a potential tradeoff between annual photosynthesis and nutrient status; if the senescence process has not reached completion, valuable nutrients – in particular nitrogen which often is the limiting factor from growth in many forests – are lost when leaves are shed (Keskitalo et al. 2005). Therefore, "safeguarding" by triggering autumnal senescence by photoperiod would be a useful adaptation to nutrient limitation at higher latitudes where photoperiodic cues are good predictors of freezing conditions. Studies in aspen in northern Europe indicates that, provided that the tree has reached a "competence to senescence" which seem to be related to completion of bud set and growth arrest, a second critical photoperiod may be sufficient to trigger onset of senescence (Fracheboud et al. 2009).

8 Dormancy

Meristem dormancy in buds and cambium is an essential trait for trees (see e.g. Cooke et al. 2012 for a review). Dormancy can be of different types, ecodormancy is provoked by certain environmental conditions and endodormancy occurs when the tissue itself has gone into a dormant state that cannot readily be activated by permissive growth conditions (Lang 1987). Dormancy is complex and it has recently been suggested that the terms endo – and ecodormancy need to be revised (Cooke et al. 2012). In any case, during the annual cycle of trees, ecodormancy is typically induced by a shortening of the photoperiod in the autumn, while the transition to endodormancy that occurs later in the season is less well understood. Induction of growth arrest and ecodormancy often happen in parallel suggesting that they are triggered by the same cues, although it is not easy to experimentally separate these processes. When ABA or ethylene signaling is impaired, it is however possible to induce dormancy independent of bud set (Rohde et al. 2002; Ruonala et al. 2006). Release from dormancy is, like vernalization, associated with periods of chilling temperatures. So far, no obvious molecular similarities between the two processes have been identified.

9 Evolutionary Insights from Molecular Mechanisms Regulating Perennial Growth

The intriguing observation that the same or similar molecular mechanisms that regulate flowing in both annuals and perennials, also regulate bud set in *Populus* illustrates well how comparative studies could give insights to the molecular evolution traits relating to the perennial lifestyle. The results of the studies of (Bohlenius et al. 2006) and (Hsu et al. 2011) show how complex the regulatory networks could be and how complicated it may to disentangle them (Ballerini and Kramer 2011). Output signals from the circadian clock can be used to regulate many – potentially all – traits under photoperiodic control, but it is still possible that there are other mechanisms, yet to be discovered, that help trees to accurately get information about the time of the year. Induction of dormancy coincide largely with bud set, it is likely that the initial photoperiodic trigger is the same despite the pathways diverge downstream of the clock. It should also be kept in mind that, while seed dormancy is considered by many plant biologists as a very old trait, it is more recent than the dormancy associated with perennial growth. For dormancy, knowledge transfer from herbs to trees has so far been less successful.

10 Towards the Future: Comparative and Evolutionary Genomic Studies in Trees

The future holds great promise for new insights into forest biology from evolutionary and comparative genomic studies. The advancement of our understanding of fundamental biological processes (e.g. wood formation and perennial growth as discussed here) in a limited number of model species will continue to provide a foundation for extending and testing models of regulatory pathways into other taxa. This work is already underway in *Populus*, eucalyptus, and a handful of conifer species. A promising approach in this regards is the modeling of the gene regulatory networks that control traits of interest. This is the level of complexity at which most traits of interest lie, and network models direct development and testing of hypothesis that can make research more targeted and effective. Once models of gene regulatory networks are established, a next step is to understand how these networks have been rewired and modified over evolutionary history to produce the diversity of form and function seen in extant species. Other approaches will start directly with comparative analyses. For example, comparison of gene expression in cambia across gymnosperm and angiosperm taxa could provide immediate insights into the evolutionary origins of cambia, identify the conserved or ancestral regulatory mechanisms, and characterize how regulatory mechanisms have been modified to produce phenotypic variation. Comparative methods can also be used to identify signatures of selection for genes within and across genomes, and provide insights into adaptive traits that are of fundamental importance to understanding how forest species may respond to climate change. Importantly, next generation sequencing technologies is highly supportive of comparative genomics, and is providing a new foundation of forest genomics-based research.

While genomics will provide new technical advances, comparative approaches also rely on knowledge of anatomy, morphology, physiology, taxonomy and other traditional disciplines. During the design of sequencing-based comparative genomic studies, it is imperative to consider the evolutionary origins, taxonomic distribution, and diversification of the trait under study. Knowledge of the physiological features, anatomical makeup, or developmental stages of a trait can be critical to experimental design. For example, comparative studies of fast-evolving mechanisms underlying disease resistance should take a different approach from studies addressing more evolutionarily conserved mechanisms, like wood formation. Clearly, collaboration among researchers is necessary to address the full range of technical issues surrounding comparative genomic studies. Another unappreciated aspect to comparative genomic studies that can require extensive collaboration is the practical issue of sourcing appropriate plant materials (Groover and Dosmann 2012).

Other practical issues include the fact that the relatively small research communities associated with forest biology must collaborate and communicate effectively. One example is a need to standardize the collection and processing of samples that are used to generate sequence data, and to provide the information about samples and sequencing libraries associated with high throughput sequencing datasets. Following such standards will help ensure that the data collected from different species or labs will be directly comparable, that the growth and other conditions associated with samples is well documented, and also ensure that experiments can be repeated and verified by other researchers. There is also an increasing need for empowering smaller labs with access to computer resources and informatics tools to make use of next generation sequence data, and to perform robust comparative analyses.

11 Conclusions

Sound forest management relies on scientific insights into the biological processes underlying tree growth and survival. An exciting new era of discovery is being ushered in by technological advances in genomics. But to make the most of these advances will require careful planning and coordination by the research community, funding agencies, and stakeholders who may ultimately benefit from the knowledge soon to come.

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