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Chapter 2

Controlling Maturation and Flowering for Forest Tree Domestication

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Abstract

Maturation refers to programmed age-related changes in developmental processes that occur in all organisms. In trees, where maturation occurs over years to decades, numerous morphological and physiological changes associated with this process have been described. However, there has been little progress in elucidating the mechanisms that control maturation, and only limited capability to alter maturation state for horticultural plants and forest trees. The ability to prevent the acquisition of competence to flower during maturation could enable the broad use of genetically engineered trees in plantations with acceptable ecological and social consequence. Conversely, the ability to speed the onset of flowering could allow the use of breeding methods now considered untenable in trees. In addition, the modification of cambial maturation state could allow directed improvements in wood quality,

could allow facile clonal propagation of elite, proven genotypes. New genomic information and methods are providing many fresh avenues for probing mechanisms and identifying control points. More so than any other forest tree, *Populus* possesses the genomics infrastructure, and facile transformation and clonal propagation, that could allow rapid progress in elucidating the regulatory networks that control maturation.

Introduction

All higher plants exhibit maturation or developmental phase change. The most obvious example of phase change is the transition to reproductive development, though phases can also be defined quantitatively rather than qualitatively. Maturation in vegetative characteristics varies among species, and these changes are most obvious and numerous in long-lived, woody plants. Vegetative changes that commonly occur in trees include a decline in the ability to form adventitious roots, and changes in wood, branching and leaf characteristics (Figure 1). A defining feature of phase change is that maturity in a trait is relatively stable—it cannot be easily reversed under normal growth conditions. However, juvenility is ultimately restored in the next generation. These characteristics suggest a role for epigenetic mechanisms (i.e., gene-regulating activities that do not involve changes in DNA sequence, but can be mitotically and/or meiotically inherited) in the regulation of maturation states (e.g., Poethig, 1990; Greenwood and Hutchinson, 1993; Hackett and Murray, 1993).

Rejuvenation in at least some species and traits is possible using various treatments (e.g., *in vitro* culture and serial grafting). For trees, the stability criterion distinguishes vegetative phase change from developmental changes that recur seasonally. For example, in addition to age-related changes, cottonwoods and other trees produce morphologically distinct early- and late-flush leaves each season (e.g., Eckenwalder, 1996). Wood characteristics change during a single growing season, resulting in earlywood and latewood in many species, in addition to the well-known transition from juvenile to mature wood as trees age.

Several reviews of phase change in woody plants have made a distinction between maturation and aging (e.g., Greenwood and Hutchinson, 1993). We follow this convention, as have studies of metazoan systems (reviewed in Kirkwood and Austad, 2000). Aging is considered to be a stochastic process that occurs after maturation. It may result in part from the accumulation of somatic damage and is manifest by a slow progressive decline in vigor or productivity. Some of this decline is due to increased size and complexity, particularly in trees, but separating age responses from size and complexity

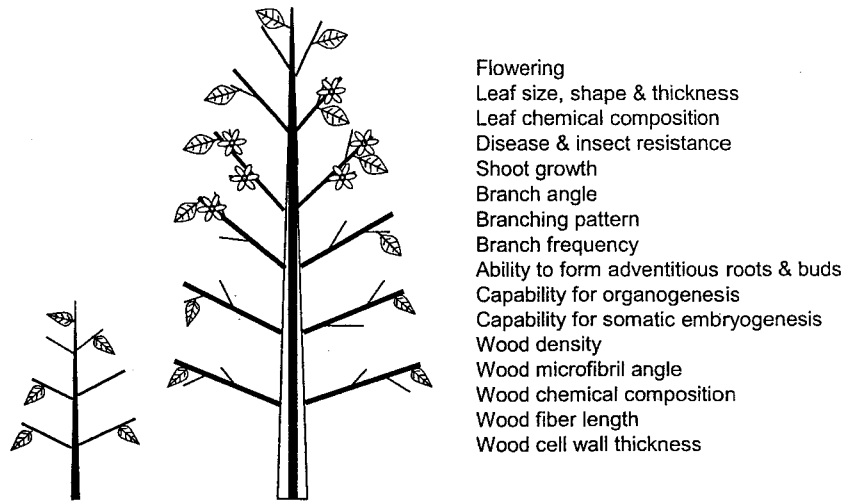


Figure 1. Phenotypic changes associated with maturation in trees. Stylized juvenile (left) and mature angiosperm trees display some maturation characteristics, and additional changes are listed on the right (reviewed in Hackett and Murray 1993, Greenwood and Hutchison 1993). Mature trees show within-tree maturation gradients for some traits. Juvenile (or core) wood is depicted in black, mature wood is white.

is difficult. Whatever the underlying causes, characteristic changes in physiology and morphology occur after many years of reproductive and vegetative maturity, up to and through the 'old-growth' stage of trees (Bond, 2000). Nonetheless, though they are distinct processes, maturation and aging can alter the same traits. For example, declines in height and diameter growth with age can be the result of maturation, aging, or both (Greenwood and Hutchison, 1993).

Studies in the model annual plant *Arabidopsis* have defined at least three post-embryonic phases—a juvenile vegetative phase, an adult vegetative phase, and a reproductive phase (reviewed in Simpson *et al.*, 1999). The vegetative phases are distinguished by changes in leaf traits; most notably the distribution of trichomes, and only the adult vegetative meristem is usually competent to respond to floral induction. While *Arabidopsis* and other plants progress in a coordinated manner through vegetative maturation to the reproductive phase, the relationships between vegetative phase change and reproduction are variable among species and are particularly complex in trees. The timing of the onset of flowering in trees typically varies from a few years to many decades. While we may know when a tree first initiates

flowers, in the vast majority of cases, we do not know precisely when the tree becomes competent to respond to floral inductive signals. Moreover, maturation of a particular vegetative trait may occur years before the onset of reproduction, or even after flowering occurs. For these reasons, we define juvenile vs. adult or mature trees by their reproductive status, and do not generally relate maturation in vegetative traits to specific phases (e.g., adult vegetative) of the whole organism.

We will discuss the relationships between vegetative phase change and the floral transition in the context of the whole tree, and how this may influence transgenic manipulation of maturation traits. Rather than a comprehensive review of maturation in various trees, we focus on maturation traits that are of central importance to the domestication of forest trees (see Bradshaw and Strauss, 2001). Our goal is to illustrate that the combination of forest tree genomics programs, tree-based systems for transgenic studies, and the wealth of information on regulatory genes and developmental processes in *Arabidopsis* and other annual plants, are providing powerful new tools to investigate and manipulate the maturation of trees.

Controlling Maturation: The Benefits

Breeding

Virtually all traits of economic and adaptive value change during the maturation and aging cycle in trees. Genetic improvement by breeding requires flowering for genetic recombination. The long delay before flowering and the very large size of trees when flowering occurs, preclude many options for breeding that might be considered were rapid turnover of generations feasible. This includes the introgression of a desired but rare gene into elite germplasm, especially when recessive, and the use of inbreeding/crossing to increase the efficiency of selection and better capitalize on both additive and non-additive genetic effects such as heterosis. In species threatened by exotic pests for which resistance alleles are rare, the capability for introgression could mean the difference between survival and extinction—at least from functional ecological and economic viewpoints. A means to reliably induce flowering in large numbers of very young trees, such as using an inducible transgenic system, might therefore revolutionize some kinds of tree breeding.

Gene Flow

However, if transgenes are to be used widely in forestry, it may be necessary in some places and species to restrict the ability of these genes to enter wild

gene pools—either for social or ecological considerations. Thus, avoidance of flowering, which in most trees results in very wide dispersal of gametes via pollen and sometimes seed, may be required in operational plantations. This is likely to also have the desirable effect of increasing vegetative growth, especially later in life when flowering and fruit production are heavy. The simplest and most effective way to delay flowering would be to repress genes required for development of competence to flower or initiation of floral meristems, or to overexpress genes that antagonize development of these tissues. Examples of both of these kinds of genes are now known. Alternatively, strategies that do not affect reproductive maturation or meristem development could also be employed, such as via disruption of floral organs via tissue ablation or floral gene suppression (Strauss *et al.*, 1995).

Vegetative Propagation

Because of the extensive heterozygosity in tree populations and their intolerance to inbreeding (as a means of fixing desirable genotypes), large increases of genetic gain are often sought via clonal propagation of elite genotypes. However, because the genetic value of individual genotypes for yield traits cannot be reliably determined for a number of years, it is desirable to clone tissues from older trees. Alternatively, large numbers of genotypes can be maintained in a juvenile state via hedging or cryopreservation while field trials are performed. Cryopreservation is costly, can be technically challenging, and can only be applied to species for which there is a highly developed, cost-effective embryogenic propagation system in place. Hedging for long time periods, and propagation from older trees, is technically difficult. Most species show a loss of competence with age for the cellular redifferentiation required for propagation by rooted cuttings (discussed below). Epigenetic changes are also commonplace in trees, resulting in clonal propagules with undesirable mature characteristics that persist for years such as slow, branch-like growth form. If genes could be identified that enable regenerative competence to be maintained or restored, it thus would enable clonal production of juvenile-like “seedlings” from trees. Such knowledge and technology could radically increase the capability for cost-effective clonal deployment. Similarly, a system for induction of apomictic seeds in young trees would allow facile clonal propagation, and could take advantage of the already developed nursery infrastructure for seed and seedling culture. Genes related to apomixis are under intensive study in several herbaceous species (Grossniklaus *et al.*, 2001).

Wood Quality

Finally, wood quality, growth rate, pest resistance, and many other economic traits change markedly with tree age. Wood produced in young trees, and in the crown area of older trees, is referred to as juvenile wood, and differs in a large number of biochemical and structural ways from the “mature wood” produced toward the base of older trees. Nearly all of the characteristics of juvenile wood are inferior to those of mature wood for both pulp and solid wood products (Zobel and Sprague, 1998). Thus, means for accelerating the transition from juvenile to mature wood—if it could be done without compromising the rapid growth of juvenile trees—would appear highly desirable. Other targets for manipulation include crown structure; for example, more horizontal branches, common in older conifers, appear desirable for reducing knot size.

Poised To Make Major Progress

Candidate Regulatory Genes from Studies in Annual Plants

Since the sequencing of the *Arabidopsis* genome has completed, coordinated efforts are now focused on “understanding the function of all genes of a reference species within their cellular, organismal, and evolutionary context by the year 2010” (Chory *et al.*, 2000). Sequencing of the rice genome is approaching completion, and projects to construct saturation mutant lines for functional completion, and projects to construct saturation mutant lines for functional completion, are underway (reviewed in Yuan *et al.*, 2001; Hirochika, 2001). Thus, in the upcoming years, studies in *Arabidopsis* and other annual plants will provide an increasing number of candidate regulatory genes for the various traits that undergo maturation in trees (specific examples are discussed in later sections). From the standpoint of understanding tree maturation, one of the most intriguing functional genomic projects is focused on epigenetic regulation. The plant chromatin database (<http://chromdb.biosci.arizona.edu>) provides information on chromatin-level control of gene expression in plants, and a major project goal is to mutate and functionally analyze most of the maize and *Arabidopsis* genes that have a role in chromatin-level gene regulation.

Although studies in *Arabidopsis* and other annuals will continue to be a valuable guide, extrapolating from these studies to large, long-lived perennials has limitations. Sequencing of the *Arabidopsis* genome revealed that most genes are duplicated (*Arabidopsis* Genome Initiative, 2000). Moreover, study of the large MADS-box gene family in a variety of plant species has demonstrated that duplications specific to all taxonomic levels (e.g. orders, families, and genera) are common (e.g. Theissen *et al.*, 2000). Such duplications have resulted in genes with distinct functions, genes with highly

redundant functions, and genes with distinct but overlapping functions. Although computer programs to identify orthologous genes among the different plant databases (reviewed in Yuan *et al.*, 2001), and information from comparative mapping studies will help overcome this complication, identifying tree orthologs can still be problematic. This is especially true for the large conifer genomes, where amplification and dispersal of genes to form complex families appears to have been much more prominent than in angiosperms (Kinlaw and Neale, 1997).

Furthermore, vegetative phase change affects many traits in trees that may be poorly manifest in annual plants (e.g., wood characteristics). In addition, the degree to which the genes controlling phase transitions in *Arabidopsis* play a similar role in other annual species is largely unknown. Thus, a number of genes important in tree maturation may not be predicted based on studies in annual plants. An individual gene is part of a variety of complex genetic and developmental pathways, and a gene product may perform differently depending on its molecular, physiological, or developmental context (whole organism). All the functions of a gene over multiple years in a tree, may not be inferred based on studies of orthologs in annual plants. Clearly, tree genomics projects and the ability to functionally analyze genes in trees are needed.

Tree Genomics Projects and Approaches

A number of large genomics projects are underway in forest trees. The most advanced is a large EST (expressed sequence tag) sequencing-based project at Genesis (New Zealand). They have sequenced more than one hundred thousand ESTs from a diversity of tissues in *Pinus radiata* and *Eucalyptus grandis*, and have used transgenesis in herbaceous organisms extensively to analyze function (A. Shenk, pers. comm.). However, public access to these databases is highly restricted. Smaller EST projects for eucalypts are underway in Brazil, France, and Sweden.

A large EST sequencing project is underway at North Carolina State University (Whetten *et al.*, 2001). The sources of most of the RNA for the ESTs were xylogenic tissues of *Pinus taeda*, although some shoot tip RNA was included. The project has a goal of sequencing 70,000 ESTs and, as of August 2001, 55,000 have been completed (R. Sederoff, pers. comm.; <http://web.ahc.umn.edu/biodata/nsfpine> and <http://web.ahc.umn.edu/biodata/doepine>).

Populus is the other forest taxon for which there are significant genomics projects underway. Although of economic value for diverse forest, environmental, and agronomic products, it also is considered the model forest

tree for molecular genetic studies as a result of its small genome size (550 Mb), ease of clonal propagation, transformability, genome markers, and the existence of many interspecific pedigrees that facilitate QTL identification. It is therefore worthy of study in its own right as a model system for woody plants (Bradshaw, 1998). The main projects underway also focus on ESTs from xylem tissues (Mellerowicz *et al.*, 2001). The most advanced project is based in Sweden (<http://Poppel.fysbot.umu.se>), where a large number of scientists collaborate on diverse biological problems using the sequence and array hybridization methods developed there. Currently 49,000 EST sequences have been determined from 12 different libraries developed, mostly from aspen (*Populus tremula*), and another 7 libraries are under construction. A unigene array of 13,000 sequences has been constructed and is being used in hybridization experiments. Most of the ESTs that have been determined to date are derived from wood-forming tissues (>22,000) and leaf/apical meristems (>18,000). Other sequenced libraries derive from floral tissues (>7,000) and roots (>400). The target is 100,000 total ESTs, which they expect to produce within a year (B. Sundberg, pers. comm.).

Other genomics projects include ones in France (<http://mycor.nancy.inra.fr/poplardb/index.html>) and Canada. A French-based project also focuses on xylem tissues, and has a near-term goal of 20,000 ESTs and production of commercial *Populus* gene-chip that will be accessible by the scientific community. A project recently funded by Genome Canada is still being developed, but is likely to include a great deal of EST sequencing, and the construction of a detailed physical and genetic map based on BAC libraries and BAC-end-sequencing, as well as intensive QTL analysis. A parallel project is also underway there in spruce (C. Douglas, pers. comm.). Finally, it is expected the United States Department of Energy will determine the genomic sequence of *Populus* at a three to six-fold level of redundancy within the next two years (G. Tuskan and R. Dahlman, pers. comm.). This would obviously provide a quantum leap of capability for intensive genetic analysis in *Populus*.

Transgenic approaches are powerful means for functional genomic studies, and have been largely restricted to *Populus* among forest trees because of its ease of transformation and clonal propagation (e.g., Han *et al.*, 2000). Random insertional screens for genes have been carried out using *Agrobacterium*-mediated transformation and gene promoter traps, where the random insertion of a reporter gene such as GUS near to a regulatory element gives rise to cell or tissue specific expression patterns. In a preliminary study of several hundred lines, rates were observed to be similar to those of *Arabidopsis*, including a number of desired vascular and rooting-associated expression patterns (A. Groover and R. Meilan, pers. comm.). A pilot study

of activation tagging (Weigel *et al.*, 2000)—where a 4X cauliflower mosaic virus 35S enhancer element was “randomly” inserted into the poplar genome via *Agrobacterium*-mediated transformation—showed a rate of recovery of mutant phenotypes of about 1%, also similar to that observed in *Arabidopsis* (unpublished data). Large-scale mutagenesis programs using these methods are likely to be successful in identifying many genes that affect woody plant development that would be missed in screens of herbaceous plants.

It is also feasible to conduct large-scale, directed transgenic programs in *Populus* where specific candidate genes related to traits of interest are suppressed or overexpressed. Transgenes producing RNAs that form duplex structures (i.e., DNA inverted repeats) appear to be highly efficient in stimulating post-transcriptional gene suppression via double-stranded RNA interference (RNAi) (Smith *et al.*, 2000). With the wealth of functional genomics information coming from studies in *Arabidopsis*, rice, and other model plant species, transgenic manipulation of tree homologs would appear to be the most direct means for developing novel biotechnological applications from genomic information.

Although stably transformed conifers have been produced from a number of species (Huang *et al.*, 1991; Ellis *et al.*, 1993; Charest *et al.*, 1996; Tzfira *et al.*, 1996; Levee *et al.*, 1997; Walter *et al.*, 1998), the low frequency of transformant recovery and the time required to recover transformed plants limits the use of transformation for genomic research. These limitations appear to be more related to efficiency and speed of tissue culture regeneration system than to the introduction of DNA itself. In addition, some antibiotic selection systems that work well with angiosperms, are not very effective with gymnosperms (Merkle *et al.*, 2001). Thus, alternative selection systems could potentially improve efficiency and reduce the time until transformed plants are available. Most rapid progress will probably occur in those species for which reliable protocols exist for initiation of somatic embryogenic cultures and recovery of somatic embryos, such as *Picea abies* (Wenck *et al.*, 1999) and *Pinus radiata* (Walter *et al.*, 1999). However, transgenic approaches to functional genomics are likely to be concentrated in *Populus* for the foreseeable future.

Finally, new methods for studying genome-level methylation using arrays are under development (<http://www.epigenomics.com/>). Because of the expected importance of epigenetic regulation of all aspects of maturation in trees (discussed below), such methods could provide entirely new insights into the regulatory networks that govern maturation in trees.

Maturation, Meristems, Coordination, and Manipulation

The manifestation of maturation involves changes in the activity of meristems. Most of the trait transitions involve structures (e.g., leaves, branches, inflorescences) initiated by the shoot apical meristem (SAM). Because of the polar nature of shoot growth, some of these changes exhibit a spatial and temporal gradient along the axis of the shoot, such that basal regions of a mature tree may display juvenile characteristics (Figure 1). Although most adventitious root meristems are derived from vascular parenchyma cells in stems (Goldfarb *et al.*, 1998), the ability to form such roots generally declines along a gradient similar to other shoot characteristics. However, these various traits may mature at very different rates and not all show within-tree gradients.

As a product of a different meristem, the vascular cambium, wood maturation follows a different within-tree gradient; wood characteristics vary across the radius of the bole and along the height of the tree (Figure 1; reviewed in Zobel and Sprague, 1998). A young tree produces only juvenile wood, which is also called core wood, because it is formed within a given number of rings from the tree center. Wood characteristics (e.g., specific gravity, cell length) change gradually and at different rates in relation to the number of annual rings from the pith. The term “transition wood” is often used to describe intermediary phases between juvenile and mature zones. Though determinations of the mature wood zone vary depending on what particular wood trait is studied, all traits reach a relatively stable state so that the outer or mature wood is generally uniform. Because the type of wood produced is related to the age of the cambium at the point of wood formation, or distance from the pith, an older tree produces both juvenile (at the top of the bole) and mature wood (at the bottom of the bole); the overall proportion of juvenile wood decreases considerably with tree age.

Maturation in a trait involves two main regulatory steps—the initiation of phase change and the maintenance of the mature phase. Studies in a variety of plants have shown that the floral transition is regulated both by transmissible signals originating outside the SAM, and by competence of the SAM to respond to these factors (reviewed in Levy and Dean, 1998). Less is known about vegetative phase change, but studies in maize indicate that this transition is initiated by factors outside the SAM, though the adult phase may be maintained by intrinsic changes in the identity of the SAM (Irish and Karlen, 1998; Orkwiszewski and Poethig, 2000).

The long-standing hypothesis that epigenetic mechanisms (Poethig, 1990; Greenwood and Hutchinson, 1993; Hackett and Murray 1993), such as DNA methylation, have major roles in regulating phase change has not yet been proven, but accumulating evidence suggests that epigenetics is important in

maturation. Early studies attempting to correlate changes in total genomic methylation with tree maturation produced inconclusive results (reviewed in Haffner *et al.*, 1991). However, changes in specific genes would not be detected by such scans, and studies in *Arabidopsis* and other eukaryotes show a high degree of specificity in epigenetic regulation.

Studies in plants have shown the importance of DNA methylation in regulating gene expression, and have begun to link methylation to chromatin remodeling enzymes (reviewed in Habu *et al.*, 2001). This connection has already been clearly demonstrated in other eukaryotic systems (reviewed in Muller and Leutz, 2001), and it appears likely that reversible modifications of chromatin, such as DNA methylation and histone acetylation, act in a combinatorial fashion to provide multiple dimensions of transcriptional control in plants. For example, the *ddm1* mutation in *Arabidopsis* induces a severe reduction in genomic 5-methylcytosine level and various developmental abnormalities (Vongs *et al.*, 1993). *DDM1* does not encode a methyltransferase, but rather a protein homologous to SWI2/SNF2, a class of ATPases, which function in multiprotein chromatin remodeling complexes that regulate transcription (Jeddeloh *et al.*, 1999). Changes in chromatin structure and DNA methylation of a maize anthocyanin regulatory gene were shown to coincide with the shift from juvenile to adult growth (Hoekenga *et al.*, 2000), and reduction in the level of RPD3-type histone deacetylases in *Arabidopsis* delayed transition to the reproductive phase (Wu *et al.*, 2000; Tian and Chen, 2001).

The mechanistic relationships between phase change in various vegetative features and reproductive competence are unknown, but there are indications that changes, in at least a subset of vegetative traits and the floral transition, are often regulated independently (reviewed in Lawson and Poethig, 1995; Greenwood, 1995; Jones, 1999). In *Arabidopsis*, a loss-of-function mutation in a cyclophilin 40 gene resulted in accelerated transition to the adult vegetative phase, but did not change the timing of the floral transition (Berardini *et al.*, 2001). While mutations in some flowering-time genes alter both vegetative and reproductive phase changes, others appear specific to the floral transition (reviewed in Simpson *et al.*, 1999).

Independence of vegetative and reproductive phase change increases developmental plasticity, and thus, the potential for heterochronic evolution (discussed in Lawson and Poethig, 1995; Diggle, 1999). Recent studies in *Eucalyptus globulus* have shown that the transition from juvenile to adult leaves and the onset of flowering are under independent quantitative genetic control, and quantitative genetic analysis also suggests that the timing of this vegetative phase change is an adaptive trait (Jordan *et al.*, 1999; 2000). There are a number of alternative models that may explain the complex and prolonged maturation in trees (see Greenwood, 1995; Hackett and Murray,

1997). Nonetheless, the proposal (e.g., Poethig, 1990) that phase changes in different traits are controlled by independent pathways that share a few common regulatory elements appears to be generally consistent with our current knowledge. This model allows for coordination in the maturation of two or more traits, but maturation in one trait is not a prerequisite for maturation in a second trait. It is important to note that if there is a universally applicable model, there are still likely to be taxon-specific deviations. For example, in some species, the floral transition may be dependent on maturation in one vegetative trait, but be independent of other vegetative phase changes.

Short-distance communication via the plasmodesmata, and long-distance signaling via the phloem play a role in specific phase changes (e.g., Bernier *et al.*, 1993), and could potentially provide a mechanism for coordinating the maturation of various traits. Long-distance movement of signaling molecules such as phytohormones and sucrose have long been recognized. Sucrose signaling has an important role in the floral transition (reviewed in Bernier *et al.*, 1993), and also in wood development (Uggla *et al.*, 2001). Auxin has a major role in wood development (Sundberg *et al.*, 2000), branching (e.g., Chatfield *et al.*, 2000), and adventitious root formation (Hackett, 1988), and integrates developmental processes throughout the plant (Berleth and Sachs, 2001). An important aspect of distance signaling is that while a molecule may be widely transported via phloem, there are mechanisms for locally modulating the activity of the molecule. Plasmodesmata alter their transport capacity both temporally and spatially in different regions of the plant (reviewed in Zambryski and Crawford, 2000). In addition, the activity of transported phytohormones may be modulated locally via mechanisms such as degradation and the formation and hydrolysis of inactive conjugates. For example, the *Arabidopsis* gene *SUPERSHOOT*, which is strongly expressed in leaf axils, appears to suppress axillary meristem initiation by locally attenuating cytokinin levels (Tantikanjana *et al.*, 2001).

Recent studies have shown that mRNAs are also transported via the phloem and that a translocated mRNA can be functional (Ruiz-Medrano *et al.*, 1999; Kim *et al.*, 2001). Moreover, the delivery and exit of phloem transcripts appears to be selective. Another potential mechanism for coordinating maturation processes via long-distance signaling is RNA-mediated gene silencing (reviewed in Matzke *et al.*, 2001). Epigenetic states of genes can be transferred from one part of a plant to another via a mobile silencing signal; however, this mechanism has not been shown to play a role in normal plant development.

The mechanisms underlying maturation in various traits, how they are coordinated, and whether or not phase change in different traits is independently regulated influence our ability to manipulate maturation traits

for tree improvement. Independent regulation of individual traits or suites of traits is desirable, because it allows greater flexibility. For example, this may allow genetic engineering of trees to delay reproductive phase change, and at the same time accelerate wood maturation. Conversely, it may be desirable to maintain juvenility in two or more traits, such as adventitious rooting ability, reproductive capability, and shoot growth. In such cases, targeting one common regulatory gene for transgenic manipulation rather than several different trait-specific genes may be the preferred approach. As discussed above, maturation in traits may be genetically separable, and at the same time, coordinated to various degrees at the whole plant level. Thus, genetic manipulation of one maturation trait could have indirect effects on other maturation traits and plant processes. As a whole, these possibilities highlight the need for research including transgenic manipulation in trees (rather than annual plant models) and testing of transgenic trees over multiple years. Also of importance is the development of facile conditional gene expression/suppression systems. For some traits, it may be expedient for a particular phase to be maintained, but the alternate phase to be inducible at particular times. The prevention of the floral transition is desirable when trees are grown in production plantations, but accelerated flowering is needed to advance tree breeding.

The Switch from Vegetative to Reproductive Development

Physiological and genetic studies in a variety of plants indicate that the transition to flowering is under multifactorial control, involving perception of environmental cues and internal signals related to the developmental state of the plant, changes in gene expression, and mobile signals that must be transported to the SAM (Bernier *et al.*, 1993). Different factor(s) of this regulatory network are predicted to become limiting factor(s) in different species or genotypes, or in a given genotype grown under different environmental conditions.

Most of the recent advances in unraveling the genetic networks that interact to control flowering have come from studies of the facultative long-day plant, *Arabidopsis* (reviewed in Simpson *et al.*, 1999; Araki 2001). Genetic analyses of mutants and natural variation in ecotypes have identified at least 80 loci that affect flowering time in *Arabidopsis* (Levy and Dean, 1998). Based on mutant responses to environmental cues and analyses of genetic epistasis, at least four pathways regulate flowering time in *Arabidopsis* (Figure 2; reviewed in Simpson *et al.*, 1999). Plants measure day length by integrating signals from photoreceptors and an endogenous circadian clock, and long days promote flowering via this photoperiod pathway. Extended

periods of cold temperature promote flowering in many ecotypes via the vernalization pathway. Genes in the autonomous pathway probably respond to an internal 'developmental clock'. Under short-day photoperiods, flowering depends on a gibberellic acid (GA) signal transduction pathway. Ultimately, the interplay among flowering pathways activates floral meristem identity genes and the competency of SAM to respond to floral induction signals.

Studies in *Arabidopsis* have also revealed that quantitative regulation of gene expression and redundancy are important features of the flowering pathway network. Additional characteristics of this regulatory network are that it includes both suppressors and promoters of the floral transition, that related genes may have opposite effects, and that regulation involves transcriptional, post-transcriptional, and epigenetic mechanisms. These pathways include genes, such as photoreceptors, that regulate a wide variety of plant responses as well as genes that appear specific to the floral transition. In addition, downstream genes that integrate multiple flowering pathways have been identified. Specific examples that illustrate these features are discussed below.

TERMINAL FLOWER 1 (TFL1) is a floral repressor that has a role in all growth phase transitions (Ratcliffe *et al.*, 1998), and also acts to maintain identity of the indeterminate inflorescence meristem (reviewed in Pidkowich *et al.*, 1999). While *tfl1* mutants progress more rapidly through all phases and apical meristems are converted to terminal floral meristems, all phases in *35S::TFL* plants are greatly extended, resulting in much larger plants with highly branched inflorescences. TFL1 belongs to the family of phosphatidylethanolamine-binding proteins (PEBP; Bradley *et al.*, 1997) that appear to act as regulators of kinase signaling pathways (Banfield and Brady, 2000). Six genes encode PEBP proteins in *Arabidopsis* and another of these, *FT*, promotes the floral transition (Kardailsky *et al.*, 1999; Kobayashi *et al.*, 1999).

FT and the MADS-box gene, *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1 (SOC1)*, integrate flowering pathways (Samach *et al.*, 2000; Onouchi *et al.*, 2000; Lee *et al.*, 2000). Both genes are downstream targets of the long-day promotion pathway, and GAs also positively regulate *SOC1* expression (Borner *et al.*, 2000). *SOC1* and *FT* are also regulated by the autonomous and vernalization pathways via another MADS-box gene, the floral repressor *FLC* (Sheldon *et al.*, 1999; Michaels and Amasino, 1999). The level of *FLC* activity is proportional to flowering time and to the magnitude of the vernalization response. Moreover, the coding sequence of *FLC* alleles from early and late flowering ecotypes are identical, indicating that alleles differ in some aspect of their regulation (Sheldon *et al.*, 2000). Autonomous pathway genes that repress *FLC* expression, include two genes,

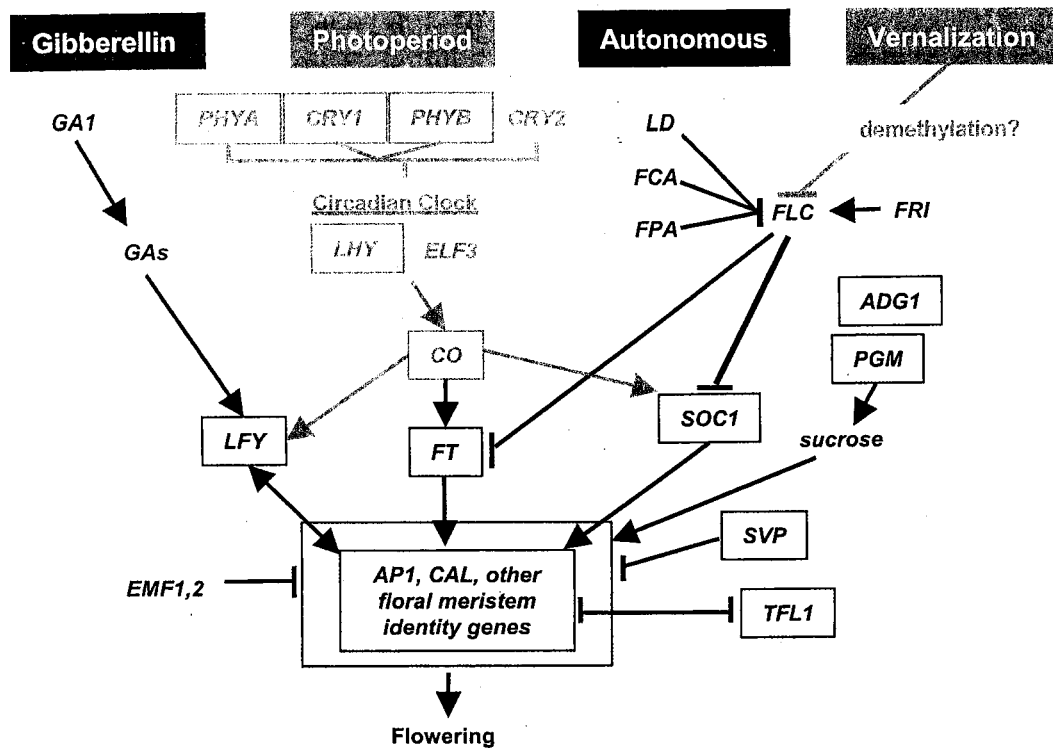


Figure 2. Model of pathways regulating the floral transition in *Arabidopsis*. The model is derived from Araki, 2001; Blazquez, 2001; and Simpson *et al.*, 1999, and does not include all interactions and genes. Arrows indicate positive regulation and short solid lines indicate repression. Some genes have not been placed in a particular pathway. Boxed genes indicate putative poplar orthologs that have been isolated via direct cloning (Brunner *et al.*, 2001, Rottmann *et al.*, 2000, Howe *et al.*, 1998) or searches of the poplar EST database (<http://Poppel.fysbot.umu.se/index.html>; Tandre *et al.*, 2001).

FCA and *FPA*, which encode proteins containing RNA recognition domains, and *LUMINIDEPENDENS (LD)*, which encodes a homeobox protein that may also bind RNA (MacKnight *et al.*, 1997; Auckerman *et al.*, 1999; Schomburg *et al.*, 2001). Thus, these genes may regulate *FLC* transcript levels post-transcriptionally.

A characteristic of the vernalized state is its mitotic, but not meiotic, stability, indicating epigenetic regulation. Similarly, the vernalization-induced reduction in *FLC* transcript levels occurs in all tissues, and is mitotically stable, but high levels of *FLC* transcript are restored in the next generation (Sheldon *et al.*, 2000; Michaels and Amasino, 1999). Evidence for epigenetic regulation via DNA methylation is indirect. Both chemical demethylation and antisense-*METHYLTRANSFERASE1*-induced demethylation accelerated flowering in vernalization-responsive plants, and *FLC* transcripts were also reduced (reviewed in Finnegan *et al.*, 2000). The late-flowering phenotype of dominant *fwa* mutants is the result of hypomethylation and the corresponding ectopic expression of *FWA* (Soppe *et al.*, 2000). In wild-type plants, *FWA* is expressed only in siliques and germinating seedlings, and its lack of expression in mature plants is associated with extensive methylation of two direct repeats in its 5' region, suggesting that *FWA* may promote establishment of the vegetative phase.

Still unknown is the extent to which genes that regulate the floral transition in *Arabidopsis* also regulate this transition in other annual plants. Only a few flowering-time genes have been cloned from other species, but it appears that the function of at least some genes is generally conserved among divergent species. For example, a major quantitative trait locus, *HDI*, controlling the photoperiodic response in rice, a short-day plant, was recently shown to be a homolog of the transcription factor *CONSTANS (CO)* (Yano *et al.*, 2000). *CO* acts downstream of the photoreceptors and clock-associated proteins in the long-day flowering promotion pathway (Putterill *et al.*, 1995). While GA signaling is an important flowering promotion pathway in *Arabidopsis*, it has a minimal effect in pea, another facultative long-day plant (Weller *et al.*, 1997). Moreover, GA inhibits flowering in the long-day plant, *Fuchsia hybrida* (King and Ben-Tal, 2001). Interestingly, GA appeared to inhibit the floral-promoting effect of sucrose by reducing sucrose content in the shoot apex, and also was associated with reduced import of assimilate from leaves. While GAs can induce precocious flowering in some conifers, they appear to have little effect in most angiosperm trees, and conversely, GA-inhibitors have been effective floral promoters in some angiosperm trees (reviewed in Meilan, 1997). Combined with the complex carbohydrate and sink-source relations in trees, the likely roles of sucrose and long-distance signaling in the floral transition and vegetative maturation (discussed in

previous section), these results are especially intriguing, and underline the great physiological and evolutionary diversity in control of flowering.

Another possible connection to changing sink-source relationships and transmission of signals via plasmodesmata and phloem comes from studies of a key regulator of the floral transition in maize, *INDETERMINATE1 (ID1)* (Colasanti and Sundaresan, 2000). *ID1* provides molecular genetic support for the florigen hypothesis—that a flowering signal is produced in the leaves and translocated to the SAM (Colasanti *et al.*, 1998). Day-neutral varieties of maize make the transition to flowering after initiating a particular number of leaves; *idl* mutants produce many more leaves, and eventually only produce aberrant inflorescences. *ID1* expression increases as plants approach the floral transition, and it is expressed in immature leaves, but not in the SAM. Within the leaves, *ID1* expression decreases as they emerge and become photosynthetically active (i.e., as the leaves transition from sink to source tissues) (Colasanti and Sundaresan, 2000). Moreover, plasmodesmata alter their within-leaf permeability along developmental gradients during the sink-to-source transition (reviewed in Pickard and Beachy, 1999).

While overexpression or suppression of a single gene can dramatically alter the time to flowering in annual plants, can such manipulations have a similar effect in trees without causing unwanted side-effects? The finding that overexpression of the *Arabidopsis* floral meristem identity gene *LEAFY (LFY)* induced the formation of flowers in transgenic poplar shortly after transformation generated much excitement, and indicated that flowering in trees might be usefully manipulated (Weigel and Nilsson, 1995). However, these flowers were not entirely normal, trees were dwarfed and highly branched, and additional studies showed that *LFY*'s ability to induce early flowering in poplar was highly dependent on genotype (Rottmann *et al.*, 2000). In contrast to poplar, overexpression of either *LFY* or *APETALA1* accelerated normal flowering and fruit production in a citrus cultivar (Pena *et al.*, 2001). Developmental differences between subtropical evergreen citrus, and temperate deciduous poplar were suggested as possible reasons for the different responses. Another possibility is that unlike forest trees, fruit trees are likely to have undergone selection for early and intense flowering, and thus, may be more amenable to induction by these genes.

Normally, *LFY* is activated by both long-day and GA floral promotion pathways (Blazquez and Weigel, 2000). Constitutive *LFY* expression accelerates flowering in *Arabidopsis*, but only after plants begin to produce adult vegetative phase leaves, indicating that the shoot first has to acquire competence to respond to *LFY* (Weigel and Nilsson, 1995). Functional comparisons between *LFY* and the *Populus trichocarpa* *LFY* homolog (*PTFL*) suggest that the regulation of floral genes in poplar is influenced by maturation (Rottmann *et al.*, 2000). Although *LFY* induced precocious flowering

Table 1. Examples of candidate reproductive and adventitious rooting maturation genes

| Gene | Source Organism | Encoded Protein | Function | Reference |
|------------------------------|--------------------|----------------------------------|--|--|
| <i>ID1</i> | Maize | Zinc-finger | Regulates a leaf-generated signal for the floral transition. | Colasanti <i>et al.</i> , 1998 |
| <i>FLC</i> | <i>Arabidopsis</i> | MADS domain transcription factor | Strong dosage-dependent repressor of flowering; downregulated by autonomous pathway genes and vernalization; possibly regulated via epigenetic mechanisms. | Sheldon <i>et al.</i> , 1999; Michaels & Amasino 1999 |
| <i>SVP</i> | <i>Arabidopsis</i> | MADS domain | Dosage-dependent repressor of flowering, largely independent of photoperiod and vernalization. | Hartmann <i>et al.</i> , 2000 |
| <i>SOC1/AGL20</i> | <i>Arabidopsis</i> | MADS domain | Promotes the floral transition, integrates signals from the autonomous, vernalization, photoperiod, and GA pathways. | Samach <i>et al.</i> , 2000; Lee <i>et al.</i> , 2000; Borner <i>et al.</i> , 2000 |
| <i>FT</i> | <i>Arabidopsis</i> | PEBP | Promotes the floral transition, integrates signals from the photoperiod and autonomous pathways. | Kardailsky <i>et al.</i> , 1999; Kobayashi <i>et al.</i> , 1999 |
| <i>TFL1</i> | <i>Arabidopsis</i> | PEBP | Delays the floral transition, appears to regulate the length of all phases. | Ratcliffe <i>et al.</i> , 1998 |
| <i>EMF1</i> | <i>Arabidopsis</i> | Novel | Mutants flower extremely early with essentially no vegetative phase. | Aubert <i>et al.</i> , 2001 |
| <i>FCA</i> | <i>Arabidopsis</i> | RNA-binding domains | Promotes flowering independent of photoperiod and vernalization. | Macknight <i>et al.</i> , 1997 |
| <i>FWA</i> | <i>Arabidopsis</i> | Homeodomain | Expression regulated by DNA methylation; possibly promotes establishment of vegetative phase. | Soppe <i>et al.</i> , 2000 |
| <i>PIN1/EIR1/AGR1</i> | <i>Arabidopsis</i> | Transmembrane protein | Important for polar auxin transport, which affects lateral root initiation. | Galweiler <i>et al.</i> , 1998; Luschig <i>et al.</i> , 1998; Chen <i>et al.</i> , 1998; Bennett <i>et al.</i> , 1998; Casimiro <i>et al.</i> , 2001 |
| <i>AtIAA3/AtIAA7/AtIAA17</i> | <i>Arabidopsis</i> | Transcription factors | Protein-protein or DNA protein binding; activators and/or repressors; can stimulate or inhibit lateral and adventitious root formation. | Nagpal <i>et al.</i> , 2000; Tian & Reed, 1999; Rouse <i>et al.</i> , 1998 |
| <i>AIR1</i> | <i>Arabidopsis</i> | Subtilisin Protease | Secreted in extracellular space; induced by auxin; expressed in lateral root primordia; up regulated by NAC1. | Neuteboom <i>et al.</i> , 1999 |
| <i>AIR3</i> | <i>Arabidopsis</i> | Proline/Glycine Rich Protein | Secreted in extracellular space; induced by auxin during lateral root formation. | Neuteboom <i>et al.</i> , 1999 |
| <i>NAC1</i> | <i>Arabidopsis</i> | Transcription Factor | Transcription factor; transduces the auxin signal for lateral root formation; regulates <i>AIR1</i> . | Xie <i>et al.</i> , 2000 |
| <i>CyclinB1;1</i> | <i>Arabidopsis</i> | Cell Cycle Regulation | Induced by auxin; expressed at the site of lateral root primordia formation. | Dubrovsky <i>et al.</i> , 2000 |
| <i>ACT7</i> | <i>Arabidopsis</i> | Actin Cytoskeleton | Induced by auxin; expressed at the site of lateral root emergence. | Kandasamy <i>et al.</i> , 2001 |
| <i>MtN21</i> | <i>Medicago</i> | Transmembrane protein | Expressed during nodule formation; pine homolog down regulated in mature shoots. | Gamas <i>et al.</i> , 1996; Busov <i>et al.</i> , 2001 |
| <i>SQN</i> | <i>Arabidopsis</i> | Cyclophilin40 | Promotes juvenile leaf morphology. | Berardini <i>et al.</i> , 2001 |

infrequently in most poplar genotypes, it induced vegetative alterations, such as increased branching, more often. On the other hand, *PTLF* induced early flowering in *Arabidopsis*, but only very rarely did so in poplar. However, some *35S::PTLF* transgenics began to show increased branching after a few years of growth. These differences between the *PTLF* and *LFY* transgenics suggest regulatory factors that constrain *PTLF* activity may be involved in maintenance of juvenility in poplar, and that heterologous *LFY* was less affected by these factors.

While modulating the expression level of *LFY* or its orthologs may not be a generally applicable way to manipulate flowering time in forest trees, there are an increasing number of candidate genes that may fulfill this goal (Table 1). Intuitively, genes that alter the competency of the SAM to respond to floral induction signals and/or genes whose functions are largely independent of environmental cues appear to be the strongest transgenic candidates. However, the alteration of gene regulation is an important evolutionary mechanism (Doebley and Lukens, 1998). Thus, a flowering-time gene that is regulated by photoperiod in a long-day plant, could exhibit constitutive or growth-regulated expression in a day-neutral plant. A fruitful approach to selecting candidate transgenes is to investigate tree genes homologous to the well-studied annual plant developmental genes.

However, informative expression studies of putative maturation genes in trees are not simple because of the long developmental time, and complex seasonal cycle of growth and differentiation. For example, some of the genes that regulate flowering time in annual plants may regulate the seasonal flowering time of mature trees or the within-crown distribution of flowers, but have little or no effect on the acquisition of competence to flower over years (i.e., the reproductive maturation process). To broach these constraints we have collected various tissues at different seasonal times from the upper crown of one female and one male *Populus trichocarpa* x *P. deltoides* genotype (Figure 3A). Ramets derived from juvenile trees of each clone were represented in a continuous age gradient of one to six years (i.e., they had been through one to six growing seasons when we began our collections). For both genotypes, inflorescences were first initiated at age four. We used RNAs extracted from these various tissues to determine whether changes in gene expression level could be correlated with reproductive maturation.

A number of putative poplar orthologs of annual plant flowering time genes have been identified (Figure 2). As an example, preliminary quantitative expression studies of *P. trichocarpa ID1-LIKE5 (PTID1L5)* are shown in Figure 3 B. In contrast to maize, where leaves initiate, emerge and develop to maturity in one growing season, leaf development in poplar spans two growing seasons and is interrupted by dormancy. In addition, it is unknown when flowering signals occur, or when meristems in the leaf axils are

committed to forming an inflorescence rather than a vegetative meristem. Compared to juvenile ramets, *PTIDL5* was markedly upregulated in newly expanding spring shoots (SAM, leaves, internode) from mature ramets that would soon initiate inflorescences. Somewhat surprisingly, expression was highest in vegetative buds collected in the preceding fall from mature ramets, suggesting the possibility that signals for flowering may occur months before the initiation of flowers. Expanding this approach to study of a large number of genes via EST microarray hybridization might identify many additional genes, and networks of regulatory interactions, that take part in regulating vegetative to floral phase transition. The function of select genes or combinations of genes could then be studied via RNAi suppression or overexpression in transgenic trees.

An Example of Vegetative Phase Change: Competence for Adventitious Root Formation

The perennial vine *Hedera helix* (L) (English ivy) exhibits distinct juvenile (rooting) and mature (non-rooting) phases (Geneve *et al.*, 1988). Alternatively, for many other species, the decline in rooting ability occurs gradually, along with a progressive change in other traits (Wareing, 1959). This is the typical situation in many gymnosperms, such as loblolly pine (*Pinus taeda* L), hybrid larch (*Larix* spp.), radiata pine (*Pinus radiata* D. Don), and coast redwood (*Sequoia sempervirens* D. Don [Endl] (e.g., Peer and Greenwood, 2001). Many angiosperm tree species, such as *Quercus robur*, *Eucalyptus nitens*, *E. grandis*, *Fagus sylvatica*, and *Persea americana*, also exhibit a decline in rooting ability with increasing age (e.g., Maile and Nieuwenhuis, 1996). While the effects of maturation on rooting ability appear to be the same for gymnosperms and angiosperms, it is less clear whether the mechanisms of maturation are the same. There have been frequent reports of rejuvenation in angiosperms (e.g. Brand and Lineberger, 1992), but relatively few in gymnosperms (Monteuuis, 1987; Huang *et al.*, 1992). Thus, the changes in developmental competency associated with maturation appear to be more reversible in angiosperms than in gymnosperms.

Although many empirical studies have been conducted to define the effects of maturation in vegetative tissues (reviewed in Hackett and Murray, 1993), the underlying causes have proven more difficult to elucidate. Despite considerable recent progress in the study of adventitious rooting, many of the biochemical and genetic steps leading to the initiation and development of *de novo* root meristems are not yet known (Altman and Waisel, 1997). However, several groups have identified and studied the expression of tree

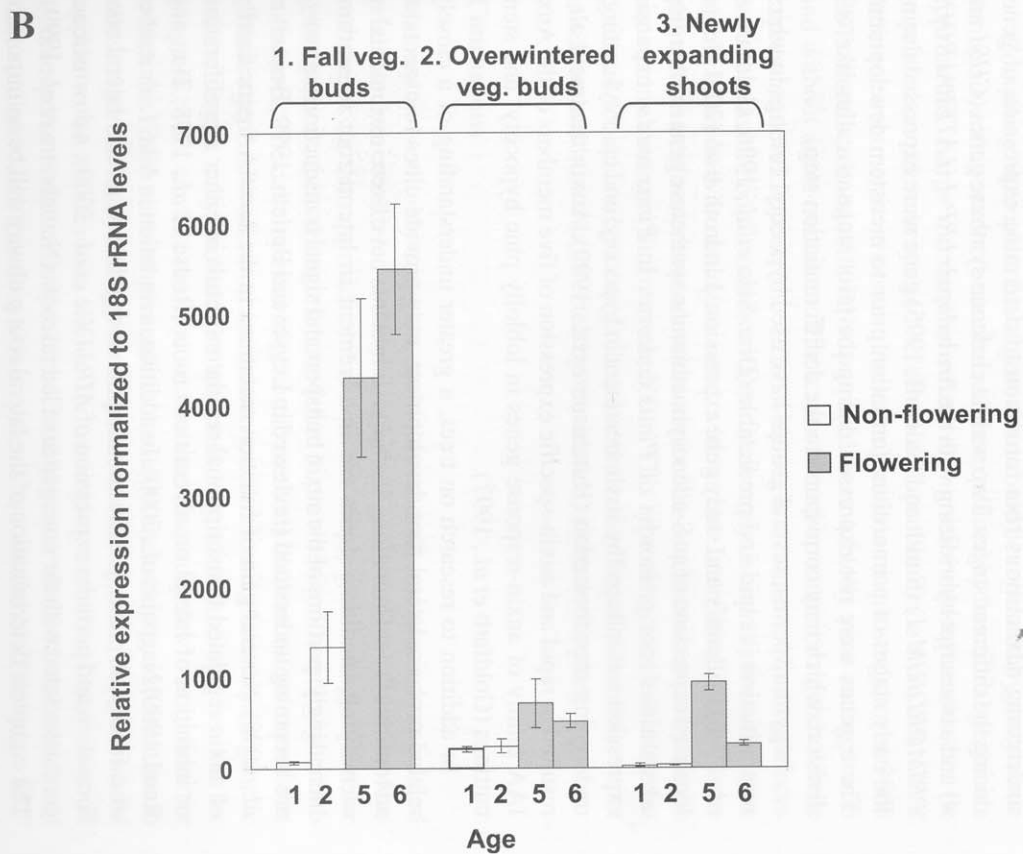
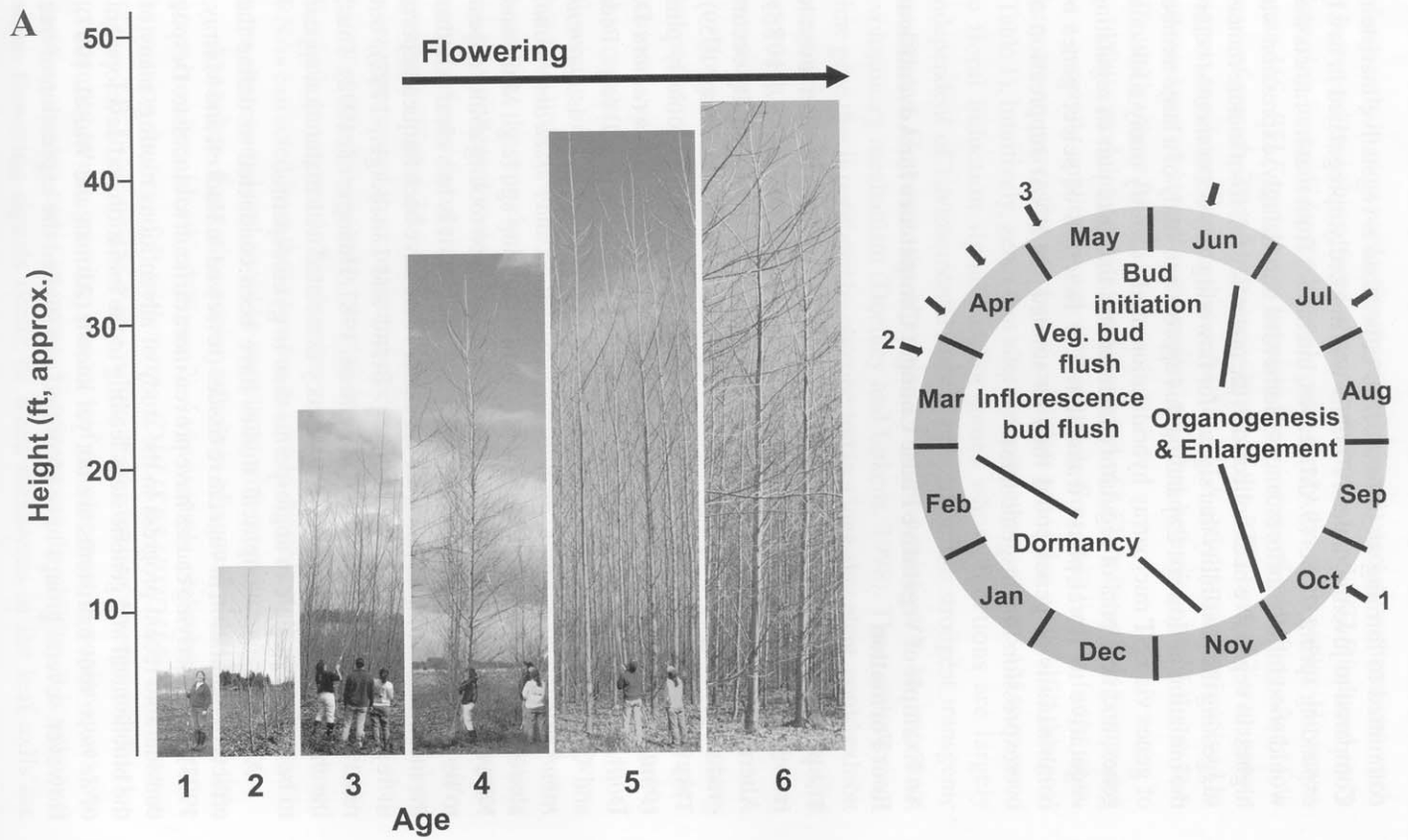


Figure 3. Variation in gene expression with age and seasonal cycle in a poplar genotype for a poplar homolog of the maize flowering time gene *INDETERMINATE1*. (A), An age gradient of a female poplar clone in Spring 2001 (left), and a typical seasonal cycle of *Populus trichocarpa x deltoides* clones in NW Oregon showing times (arrows) and types of tissue collections. Time of collection of tissues used for experiments shown in (B) are indicated by numbers. (B), RNA expression levels of *PTIDIL5* measured by real-time reverse-transcriptase PCR and standardized based on ribosomal RNA expression levels for each tissue sample. Bars show one standard deviation based on three replicate measurements.

genes that could be involved. Using an *in vitro* apple (*Malus domestica*) stem disc system that previously has been useful for determining hormonal relations during root initiation (Van der Krieken *et al.*, 1992), Butler and Gallagher (2000) identified an *ARRO-1* gene that codes for a novel 2-oxoacid-dependent dioxygenase. This gene is up-regulated during root initiation and is induced by the root-stimulating auxins, IAA and IBA, but not by 2,4-D. While causing many other auxin effects, 2,4-D does not result in root initiation in this system. Further work with the apple disc system is underway to use microarrays to identify additional root formation-specific genes (Konings *et al.*, 2000).

Ermel *et al.* (2000) used cotyledon explants of walnut (*Juglans regia* L.) undergoing adventitious root formation to determine expression of genes during the different stages. Two walnut chalcone synthase genes (*CHS1* and *4*) and a transcript hybridizing with the *Arabidopsis* *LRP-1* (*LATERAL ROOT PRIMORDIUM-1*) (Smith and Federoff, 1995) gene were expressed during the early stages of primordium formation prior to meristem development. These genes were not expressed during the prior stage of callus-like cell division, which may correspond to the dedifferentiation step.

In gymnosperms, several groups have used hypocotyl cuttings in which root initiation is rapid and predictable (Diaz-Sala *et al.*, 1996; Goldfarb *et al.*, 1998) to identify and study gene expression. Lindroth *et al.* (2001) found localized expression of an S-adenosylmethionine synthetase gene in emerging adventitious root primordia of *Pinus contorta*. In *Pinus taeda*, expansin expression was induced by auxin treatment in hypocotyl and epicotyl cuttings undergoing root formation (Hutchison *et al.*, 1999). Auxin treatment also resulted in rapid and auxin-specific expression of five members of the Aux/IAA family of auxin-response genes in loblolly pine hypocotyl and stem cuttings (Goldfarb *et al.*, 1997).

In addition to research on trees, a greater understanding of a closely related process, lateral root development, may provide clues to aspects of adventitious root formation. *Arabidopsis* mutants have been instrumental in identifying candidate genes for involvement in lateral root formation. Increasingly, portions of the auxin transport and signal transduction pathways are becoming understood (reviewed in Leyser and Berleth, 1999; Bennett *et al.*, 1998). Several gain of function mutations in the Aux/IAA gene family of auxin regulated transcriptional regulators result in either a proliferation or inhibition of lateral or adventitious roots (Rouse *et al.*, 1998; Tian and Reed, 1999; Nagpal *et al.*, 2000). In addition, a mutation in *NAC1*, a member of a family of plant-specific transcriptional regulators, affects lateral root formation and perturbs expression of *AIR3* (Xie *et al.*, 2000), a downstream gene associated with the emergence of lateral roots (Neuteboom *et al.*, 1999). The complete determination of the lateral root pathway will be an important

step for scientists researching adventitious rooting and how it is influenced by maturation. However, because roots of mature trees continue to form lateral roots, but stems from mature trees lose the ability to form adventitious roots, maturation must be affecting steps in the adventitious pathway that are unique. Unlike lateral roots, which arise directly from cell divisions of root pericycle cells (Casimiro *et al.*, 2001), most adventitious root meristems organize only after preliminary cell divisions from vascular parenchyma cells in stems (Goldfarb *et al.*, 1998). Thus, it is possible that dedifferentiation occurs during these initial cell divisions. If so, it is tempting to speculate that maturation affects the adventitious root formation process by altering the ability of these cells to dedifferentiate.

Compiling a complete list of genes that could be regulated by maturation during adventitious root formation will be expedited by functional genomics approaches. Large numbers of relatively unselected sequences can be imprinted on microarrays and screened with probes made from mRNA of different treatments or tissues. For example, in a recent study, over 3000 ESTs were screened with RNA prepared from loblolly pine cuttings with and without auxin treatment. The screen turned up numerous genes whose expression level appeared to differ with auxin treatment. Nine sequences were selected for confirmation with northern analysis and all nine were more highly expressed in cuttings treated with auxin. Interestingly, there were also differences in expression level of some of the sequences between juvenile and mature cuttings (Busov *et al.*, 2001). This type of approach offers great potential for achieving a comprehensive understanding of adventitious root initiation and how it is affected by maturation of the donor plant.

Conclusions

Maturation is a highly complex, multidimensional, and precisely regulated process when considered at the phenotypic or genomic level. It is therefore not surprising that approaches that consider single genes, single traits, or gross genomic measurements, have failed to provide significant insights into its control. The development of genomic methods that can assess expression of entire gene networks over maturation gradients, and ultimately epigenetic state-changes, may provide the quantum leap of technology needed for progress. This work is best focused on one or a very few tree genera for which the entire suite of genomic technology, including transformation to allow rigorous analysis of gene function, can be applied. *Populus* would appear to be the obvious choice for an angiosperm forest tree. A good gymnosperm candidate is spruce, because genomic studies are planned, and tissue culture and transformation systems are available. The large EST

collections and arrays for other important tree species, especially pine and eucalypts, will also be a powerful resource for studying maturation if they are put in public domain. This would enable changes in networks of gene expression across maturation gradients to be studied in species where the ability to manipulate maturation might have major economic consequences. If support for genomic analysis of maturation in trees continues, the upcoming decade is expected to see some substantial progress in describing what maturation in trees is at a biological system level, as well as improved ability to manipulate it via transgenic and molecular breeding approaches.

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