New Phytologist

Research review

Revisiting tree maturation and floral initiation in the poplar functional genomics era

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Received: *27 April 2004*
Accepted: *21 June 2004*

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Key words: floral initiation, flowering time, genomics, juvenile phase, *Populus*, tree maturation.

Summary

The recent release of the *Populus trichocarpa* genome sequence will dramatically enhance the efficiency of functional and comparative genomics research in trees. This provides researchers studying various developmental processes related to the perennial and tree life strategies with a completely new set of tools. Intimately associated with the life strategy of trees are their abilities to maintain juvenile or nonflowering phases for years to decades, and once reproductively competent, to alternate between the production of vegetative and reproductive shoots. Most of what we know about the regulation of the floral transition comes from research on *Arabidopsis thaliana*, a small, herbaceous, rapid-cycling, annual plant. In this review, we discuss the similarities and differences between *Arabidopsis* and tree flowering, and how recent findings in *Arabidopsis*, coupled to comparative and functional genomics in poplars, will help answer the question of how tree maturation and floral initiation is regulated.

New Phytologist (2004) **164**: 43–51

© *New Phytologist* (2004) **doi**: 10.1111/j.1469-8137.2004.01165.x

Introduction

During their long life spans, as they attain great size and complexity, trees exhibit a complex array of developmental phases. The most obvious phase change is the transition to reproductive development, and trees that have reached the age of floral onset are typically referred to as mature or adult. Although the topic of this review is reproductive phase transition in trees, numerous morphological and physiological traits exhibit juvenile and mature phases that are maintained for years, including wood and leaf characteristics, crown architecture, pest resistance, and rooting ability (Greenwood & Hutchison, 1993; Hackett & Murray, 1993). In some cases the timing of a vegetative phase change appears to be correlated with reproductive maturation, but these changes also occur at very different times and the various phase changes are likely to be independently regulated to varying degrees (reviewed in Poethig, 2003). The genomics approaches we describe for studying reproductive phase change could also be applied to study the various vegetative phase changes in trees and the interrelationships among the different traits that undergo maturation.

Although the tradeoffs between reproduction, growth, and survival and the role of plant size in costs of reproduction are unclear (Obeso, 2002), the prolonged juvenile phase of trees is obviously a central component of their life strategy. The multiple traits that exhibit phase change during a tree's long

life span might be a strategy to enhance fitness as trees face different challenges to survival at different ages or sizes (Day *et al.*, 2002). From an applied perspective, regulation of forest tree flowering is of considerable interest for two contrasting reasons. First, the prolonged juvenility of trees has greatly limited tree domestication (Bradshaw & Strauss, 2001). The most advanced forest tree breeding programs are only in their fourth breeding cycle. Second, the prevention or delay of flowering is highly desirable in production plantations, especially if genetically modified trees are deployed, and most countries are likely to require some form of transgene confinement before many commercial applications of transgenic forest trees are approved (Strauss *et al.*, 1995). Tree pollen, and sometimes seed, can spread over very long distances and plantations are often near wild or feral relatives. Therefore, reproductive sterility appears to be the only feasible method for reliable mitigation of transgene spread in many cases.

Our ability to elucidate the genes and pathways regulating tree maturation and flowering have been severely limited for a number of obvious reasons, for example the long generation time of trees. The attributes of *Populus* (poplars, including aspens and cottonwoods), especially its facile transformation and propagation, combined with its genomic resources (reviewed in Bradshaw *et al.*, 2000; Brunner *et al.*, 2004) provides a way to circumvent many of these tree-inherent obstacles. In this review, we discuss our current knowledge of tree maturation and flowering and the studies enabled by poplar functional genomics in a comparative context to studies in *Arabidopsis*.

Functional conservation between *Arabidopsis* and trees?

Most of what we know about the molecular genetics of flowering time regulation comes from studies in the annual plant *Arabidopsis* (Mouradov *et al.*, 2002; Simpson & Dean, 2002). One approach to studying tree flowering is to take advantage of this wealth of information and to study the functional conservation of *Arabidopsis* genes regulating flowering in transgenic trees expressing these genes. The first example of such functional conservation was the finding that the *Arabidopsis* floral meristem identity gene *LEAFY* (*LFY*) could induce early flowering when expressed in transgenic hybrid aspen trees (Weigel & Nilsson, 1995), although it was later shown that this effect is highly variable between poplar clones (Rottmann *et al.*, 2000). *LFY* also induces early flowering in citrus trees (Pena *et al.*, 2001). This is not surprising given its central role in flower initiation and its high degree of conservation between plant species. However, these findings do not provide information about the natural regulation of flowering time in trees, because *LFY* is clearly downstream of this regulation. The same can be said for *APETALA1* (*AP1*) that stimulates flowering in both transgenic *Arabidopsis* and transgenic citrus trees (Mandel & Yanofsky, 1995; Pena *et al.*, 2001). However, *35S::AP1* is not effective in poplar (O. Nilsson *et al.*, unpubl. data) and this could be because citrus is more closely related to *Arabidopsis* than poplars, or that fruit trees may be more responsive than poplar trees because they have undergone selection for earlier and more intense reproduction. Moreover, the biological relevance of early flowering phenotypes induced by strong ectopic expression of MADS-box genes must be interpreted with some caution, because many MADS-box genes, when ectopically expressed, induce precocious flowering through nonspecific interactions with endogenous genes (Nilsson & Weigel, 1997).

Many of the *Arabidopsis* genes regulating flowering time have been shown to act in the photoperiod, autonomous, vernalization or gibberellin (GA) floral promotion pathways (reviewed in Mouradov *et al.*, 2002; Simpson & Dean, 2002). These include genes that are responsible for promotion of flowering by long days such as *CONSTANS* (*CO*), *FLOWERING LOCUS T* (*FT*) and *SUPPRESSOR OF OVEREXPRESSION OF CO1* (*SOC1*), and genes belonging to the autonomous pathway, acting under both long and short days, such as *FCA*, *FVE* and *FLOWERING LOCUS C* (*FLC*). In addition, *FT*, *SOC1* and *FLC* are integrators of two or more pathways. Crosstalk between pathways might explain how the multiple signals affecting flowering are coordinated, and differences in how pathways are integrated might underlie the diversity of plant flowering. Recent work has shown that photoperiod pathway genes, such as *CO* and *FT* are conserved among the divergent annual plants *Arabidopsis* and rice, but the activity of one component of this pathway is reversed (Kojima *et al.*, 2002; Hayama *et al.*, 2003). However, there is currently no direct evidence that these genes have similar functions in trees. Many of these genes induce early flowering when expressed in transgenic *Arabidopsis* (Kardailsky *et al.*, 1999; Lee *et al.*, 2000), but despite systematic efforts to induce early flowering by these genes in poplar trees, all attempts have thus far been unsuccessful (O. Nilsson *et al.* unpubl. data). It is possible that these genes could have a role in trees only after reproductive maturity has been reached, or that the function of the flowering time genes might be intimately associated with annual growth behavior. Despite the limited success gained to date, the complete genome sequence of poplar will make functional conservation studies between *Arabidopsis* and poplar much more straightforward, and is likely to give us important insights into the similarities and differences between these two species.

Candidate poplar orthologs of *Arabidopsis* flowering time genes

Populus and *Arabidopsis* are both rosids. Thus, comparison of whole genome sequences to identify putative poplar orthologs of *Arabidopsis* flowering time genes is relatively straightforward. Although the assembled and annotated genome sequence and expressed sequence tag (EST) unigene set were not available at the time of writing this review, searches of the EST database and poplar genome sequence reads give a preliminary indication of the conservation between poplar and *Arabidopsis* genes

Table 1 *Arabidopsis* flowering time genes and putative poplar orthologs

Arabidopsis gene	Locus	Encoded protein/putative molecular function	Poplar homolog[1]	Smallest sum probability[2]
PHYA	AT1G09570	Red/far-red light photoreceptor	AJ001318	0
PHYB	AT2G18790	Red light photoreceptor	AF309806	0
CRY2	AT1G04400	Blue light photoreceptor	BU813450	2.2e-48
ZTL	AT5G57360	F-Box/targeted protein degradation	BU829894	5.9e-108
LHY	AT1G01060	MYB related transcription factor	BU868664	1.9e-41
GI	AT1G22770	Nuclear protein	BU825475	3.1e-82
CO	AT5G15840	B-box Zn-finger transcription factor	AY515150	8.5e-82
FVE	AT2G19520	WD-40 repeat/metal ion binding, chromatin modification	CA927540	9.6e-103
FY	AT5G13480	WD-40 repeat/RNA-3′ end-processing	YFS369889.b1	1.6e-42
FLD	AT3G10390	Histone deacetylase complex subunit	XXI707719.g1	3.2e-116
FPA	AT2G43410	RRM motif/RNA binding	BU831648	4.5e-48
FLK	AT3G04610	KH domain/RNA binding	CA932357	1.9e-68
VRN1	AT3G18990	B3 transcription factor	BI073104	1.2e-42
GA1	AT4G02780	copalyl diphosphate synthase	ACSB133659.b1	3.4e-58
GA4	AT1G15550	GA3 beta-hydroxylase	AY433958	1.1e-118
GA5	AT4G25420	GA 20-oxidase	AJ001326.	3.9e-136
SPY	AT3G11540	Tetratricopeptide repeat/GA signal transduction	CF119204	6.4e-33
GAI	AT1G14920	GRAS transcription factor	CA933156	5.7e-87
FPF1	AT5G24860	unknown	CA826000	1.3e-32
FT	AT1G65480	Homology to RAF kinase inhibitor	AB109804	3.2e-75
SOC1	AT2G45660	MADS domain transcription factor	CA925124	1.2e-53
TFL1	AT5G03840	Homology to RAF kinase inhibitor	AY383600	1.8e-67
TFL2	AT5G17690	Heterochromatin Protein	BU831488	5.3e-32
EMF2	AT5G51230	Polycomb group transcriptional repressor	CK088668	1.6e-57
SVP	AT2G22540	MADS domain transcription factor	BU837680	5.2e-64
ESD4	AT4G15880	SUMO protease	CF232394	2.9e-65
SYD	AT2G28290	SWI/SNF ATPase	XXI955122.x2	2.1e-53
EBS	AT4G22140	PHD finger, BAH domain/chromatin remodeling	BU832465	4.5e-53
TOE1	AT2G28550	AP2 transcription factor	YFS457233.y2	9.7e-40

Genes are grouped by pathway or category, and alternate bold type delineates these groups. In order from top to bottom, the pathways or categories are: (1) photoperiod pathway (2) autonomous pathway (3) vernalization pathway (4) GA signaling pathway (5) integrators of multiple pathways, and (5) repressors not clearly associated with a particular pathway.

[1]GenBank accession number or *Populus trichocarpa* genome sequence identifier (http://genome.jgi-psf.org/poplar/). Poplar sequence databases were queried with *Arabidopsis* protein sequences using tBLASTX (Altschul *et al*., 1990), and then the best poplar sequence hit was used to query the AGI proteins database using the WU BLAST 2.0 BLASTX program (www.Arabidopsis.org/wublast/index2.jsp). Corresponding *Arabidopsis* genes or a very closely related paralog were the best hits to the listed poplar sequences.

[2]Probability score from WU BLAST 2.0 BLASTX query.

(Table 1). Phylogenetic analyses of entire gene families are especially useful for identifying lineage-specific gene gains or losses (Fig. 1). Although these preliminary comparisons indicate that most of the spectrum of *Arabidopsis* flowering-time genes are represented in poplar, some notable differences are also indicated. Many of the *Arabidopsis* MIKC-group of MADS-box transcription factors are key regulators of flowering (Parenicova *et al*., 2003), and poplar members of nearly all the subgroups of the *Arabidopsis* MIKC-type MADS-box gene family are readily identified (Fig. 1). A significant exception is the subclade that includes the central floral repressor *FLC*. Previous phylogenetic studies of plant MADS-box genes noted that the *FLC* subgroup appeared specific to the Brassicaceae lineage (Becker & Theissen, 2003). Given the number of genes in the autonomous and vernalization pathways (He *et al*., 2003; Ausin *et al*., 2004) that converge on *FLC*, and that many of these are conserved between poplar and *Arabidopsis* (Table 1), this raises a number of important questions regarding the function of these genes in poplar and how pathways regulating flowering have evolved.

Close homologs of most of the *Arabidopsis* photoperiod pathway genes are present in poplar. This is also the case for rice, where genetic studies have confirmed that the same genes function in the photoperiod pathway, but in contrast to *Arabidopsis*, the rice *CO* ortholog promotes flowering under short days (Kojima *et al*., 2002; Hayama *et al*., 2003). Although photoperiod pathway genes are present in poplar, whether or not flowering is under photoperiodic control in poplar is unclear (discussed below). It is possible that in poplar these genes are involved in regulating other processes, such as

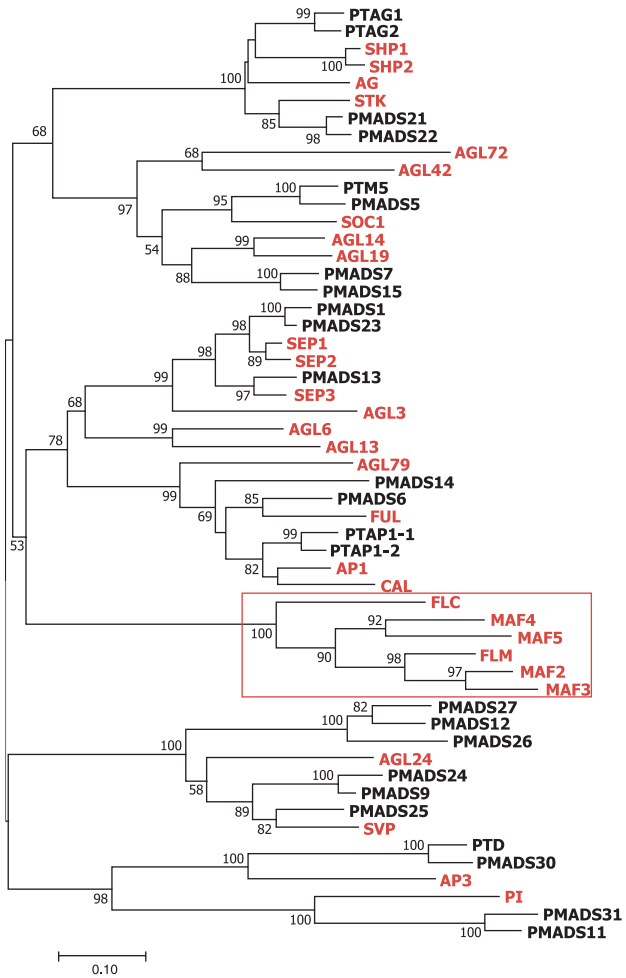

Fig. 1 Phylogenetic relationships of *Arabidopsis* and poplar MIKC-type MADS domain proteins. Included were *Arabidopsis* MIKC group proteins belonging to subclades that contain known floral regulators (Parenicova *et al*., 2003) and their poplar homologs identified via tBLASTn searches of GenBank and the poplar genome sequence (http://genome.jgi-psf.org/poplar/). Accession numbers for full-length poplar sequences are AF052570 (*PTAG1*), AF052571 (*PTAG2*), AF057708 (*PTD*), AF377868 (*PTM5*) AY615964 (*PTAP1-1*) and AY615966 (*PTAP1-2*). *Poplar MADS* (*PMADS*) genes were derived from contigs of ESTs and genomic sequences. An alignment of the MIK domains was used to construct a neighbor-joining tree, and the predicted MIK sequences for all poplar genes are provided in supplementary information. Poplar proteins are shown in black type. Poplar members of all major subclades except one (boxed) were identified. Bootstrap values (percentages based on 1000 replicates) of 50% or higher are shown at nodes.

the short day-induced growth arrest and bud set occurring in autumn, but not floral initiation. Thus, sequence-based comparative genomics is a valuable starting point. However, to reveal the genes and regulatory pathways that control flowering in poplar and how these compare with floral regulation in annual plants, this approach needs to be combined with physiological studies, microarray expression analyses and studies of gene function via transgenics.

The long juvenile phase of trees

The prolonged maintenance of both the juvenile and mature phases in trees, and the fact that maturity is not easily reversed under normal growth conditions yet juvenility is restored in the next generation, led to the hypothesis that epigenetic mechanisms play a central role in the regulation of tree phases (Greenwood & Hutchison, 1993; Hackett & Murray, 1993). Many recent studies have shown that factors involved in chromatin-mediated control of gene expression are key regulators of the floral transition in *Arabidopsis* (reviewed in Sung *et al*., 2003). For example, Polycomb group proteins (PcG) maintain stable states of gene repression via multiprotein complexes. The plant PcG genes *EMBRYONIC FLOWER2* (*EMF2*) and *FERTILIZATION-INDEPENDENT ENDOSPERM* (*FIE*) repress floral homoeotic gene expression during embryo and vegetative development in *Arabidopsis*, suggesting that the transition to flowering involves resetting chromatin structure by disruption of PcG complexes at target loci. Another example is the PHD finger protein EARLY BOLTING IN SHORT DAYS (EBS) that represses *FT*, a key integrator of floral promotion pathways (Pineiro *et al*., 2003). Similarly, *TERMINAL FLOWER2* (*TFL2*) encodes a homolog of HETEROCHROMATIN PROTEIN1 and appears to counteract the promoting effects of *CO* on *FT* expression in leaves to ensure the photoperiodic regulation of flowering in *Arabidopsis* (Takada & Goto, 2003). Although similar genes and processes might maintain the juvenile phase in trees, the differences that result in a juvenile phase of multiple years in trees are not easily explained. It is possible that the roles of repressors such as *EBS* and *TFL2* are much more pronounced in trees than in *Arabidopsis*. One hypothesis is that each annual cycle of growth and dormancy in trees leads to a gradual release of the chromatin-based repression. But what is the mechanism that requires many years for derepression instead of only weeks or one winter as is the case for annuals and biennials? How chromatin-based repression is overcome by floral promoters in *Arabidopsis* is still largely unknown, and the answer is potentially key to understanding the extreme differences in juvenile phase length between *Arabidopsis* and poplar.

Although difficult to quantify, the ability to flower appears correlated with tree size, and another long-standing hypothesis is that shoot apices must attain a critical distance from the roots before maturation occurs (Fig. 2). Grafting to the upper crowns of mature trees has induced juvenile scions to produce flower buds in various tree species (Olivera & Browning, 1993). Trees often show within-tree maturation gradients such that basal branches of a mature tree have juvenile characteristics. In poplars, both reproductive and vegetative phase changes were correlated with a distance measure incorporating vertical position of a branch on the bole and position out on a branch (Kearsley & Whitham, 1997). Water stress and root chilling induced flowering in one poplar genotype and have also been effective in other tree species (Meilan *et al*., 2004). Reduction

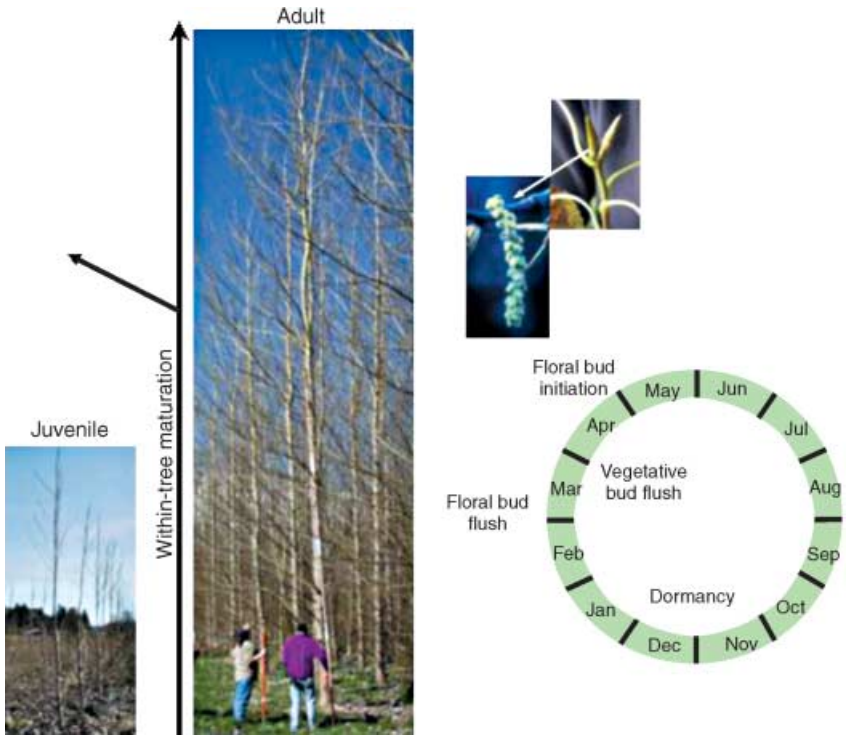

Fig. 2 The transition to flowering in *Populus* involves multiple, protracted temporal and spatial components. The juvenile, nonflowering phase of a poplar tree lasts several years. In addition, a gradient of competency to flower typically exists within an adult tree. The final components to the floral transition are the position of inflorescence meristems within a shoot and the seasonal time of floral initiation. Only lateral meristems in the axils of preformed leaves differentiate into inflorescence shoots (catkins), and the inflorescences initiate in spring, shortly after the preformed leaves have expanded. The seasonal development course depicted is typical of native *Populus trichocarpa* growing in the vicinity of Corvallis, OR, USA (Boes & Strauss, 1994).

in the transport of various molecules in the transpiration stream is one likely effect of both these treatments. Girdling, which blocks phloem transport, induces early flowering in some trees (Meilan, 1997). Whereas signaling from leaves to the shoot apex clearly has a role in floral induction, the role of signaling over longer distances in maturation is less certain. However, the long-hypothesized roles for various plant hormones are still plausible. Advances in our understanding of the complex signaling of plant hormones, which involves movement via phloem or xylem, cross-talk between different hormone signaling pathways and local modulation of hormone activity (Vogler & Kuhlemeier, 2003), seem to call out for a close re-examination of the possible roles of hormone signaling in tree maturation.

The discovery of small RNAs, their roles in both transcriptional and post-transcriptional gene silencing, and that they can travel over long distances suggest an intriguing, possible link between long-distance signaling and epigenetic mechanisms (reviewed in Hunter & Poethig, 2003). Most plant microRNAs are predicted to target transcription factors, suggesting a major role for RNA-based gene regulation in plant development; however, long-distance movement of small RNAs has not been shown to play a role in plant development. *Arabidopsis* microRNAs have been implicated in the control of flowering via targeting of *AP2* family repressors (Aukerman & Sakai, 2003; Schmid *et al.*, 2003). Although studies in *Arabidopsis* provide many valuable inroads, the complexity of long-distance signaling, epigenetic regulation and their potential interactions, particularly at a whole-tree level, are definite challenges to our understanding of maturation in trees.

Vegetative and floral meristems in adult trees shoots

Tree branches that produce floral buds also continue to produce vegetative buds. In poplar, terminal apices always remain vegetative, and inflorescence meristems initiate during a particular time of the growing season in a subset of the leaf axils (Boes & Strauss, 1994; Yuceer *et al.*, 2003b) (Fig. 2). A winter vegetative bud contains several embryonic leaves and leaf primordia. The earliest of these leaves can initiate vegetative buds in their axils before dormancy, but floral buds initiate the following spring after bud flush. Additional vegetative buds can also initiate in preformed leaf axils during spring. Although mature poplars often have mostly short shoots with only preformed leaves, neoformed growth can occur after floral buds are initiated. However, only vegetative buds develop in the axils of neoformed leaves. How this alteration between production of lateral vegetative and reproductive buds is regulated is unknown. One possibility is that after release from dormancy, the shoot apical meristem is competent to respond to floral promoting signals, but this is temporary because epigenetic repression is reset later in the growing season.

Gibberellin signaling

In many annuals with a long-day regulation of flowering, including *Arabidopsis*, GA stimulates flowering, whereas it often inhibits flowering in diverse woody angiosperms (Metzger, 1995; Meilan, 1997). These include fruit trees such as cherry, peach, apricot, almond, and lemon, and GA

inhibitors have been used to promote early flowering and fruit set in these trees (Zeevaart, 1983). However, in conifers, GA applications are frequently used to stimulate flower formation for breeding purposes (Meilan, 1997). There are contrasting reports on the efficiency with which GA inhibitors can induce flowering on juvenile vs adult shoots. While the GA inhibitor paclobutrazol has been reported to induce flowering on both juvenile and mature citrus plants (Snowball *et al.*, 1994), it appears to be inefficient on juvenile *Eucalyptus nitens* and *Populus deltoides* plants (Williams *et al.*, 1999; Yuceer *et al.*, 2003a).

Although these studies have all been based on applications of GAs or GA inhibitors, it was recently confirmed that there is a genetic basis for the GA inhibition of flowering in grapevine. A precociously flowering grapevine dwarf mutant is defective in a grapevine homolog of the *Arabidopsis* gene *GA INSENSITIVE* (*GAI*), which is known to be involved in GA signal transduction (Boss & Thomas, 2002). However, all attempts to induce precocious flowering in transgenic poplars either through deactivation of active gibberellins (Busov *et al.*, 2003) or through inhibition of GA signal transduction by expression of the *Arabidopsis gai-1D* gene (O. Nilsson, unpubl. data), have thus far failed.

The effect of GA on shoot elongation appears very consistent across species, raising the question of where in signaling are the GA effects on elongation and flowering 'uncoupled'? In this context it is interesting to compare the promoters of the *Arabidopsis LFY* gene with that of its poplar ortholog *PTLF* (Rottmann *et al.*, 2000). In *Arabidopsis*, flowering under noninductive short-day conditions is dependent on GA (Wilson *et al.*, 1992). This is largely mediated through transcriptional regulation of *LFY* whose promoter has been shown to contain a GA response element with homology to a binding site for MYB transcription factors (Blázquez *et al.*, 1998). When this GA response element is mutated, *LFY* regulation and flowering is normal under inductive long-day conditions, but the plant fails to flower under short days. Interestingly, the *LFY* GA response element was originally identified through a sequence comparison with the *PTLF* promoter. There was little sequence similarity between the promoters, but an 8-bp motif was perfectly conserved and was shown to encode the GA response element (Blázquez *et al.*, 1998). This suggests the possibility that both *LFY* and *PTLF* are regulated by GA via interactions of a GA-MYB with this promoter response element, but that the effects differ between *Arabidopsis* and poplar. For example, this interaction might activate *LFY* transcription and flowering, but repress *PTLF* transcription. Further studies of *LFY/PTLF* regulation by GAs will help to show where the regulatory pathways controlling flowering and shoot elongation differs between *Arabidopsis* and poplars.

Environmental signaling

The role of environmental factors in the timing of tree flowering is unclear. It is obvious that a tree is unresponsive to flower-promoting environmental cues during its multiyear juvenile phase. However, environmental factors could have an important role in regulating flowering in a reproductively mature tree, for example, in regulating year to year variations in flowering or in regulating the specific seasonal time when flower buds are initiated. The role of these factors in floral initiation is difficult to study in a reproductively mature tree, because these generally need to be performed with large trees in the field. Many field studies have investigated the role of environmental conditions on tree flowering; however, these have studied floral bud flush or anthesis rather than floral initiation that would have occurred during the previous year. Therefore, there is no conclusive evidence linking environmental factors to floral initiation in poplars or the vast majority of trees. However, it can be speculated that photoperiod would be a suitable signal for regulating the particular time of the year when flower buds are initiated. In poplars, floral initiation is in spring, generally during rapid increase in day length.

Variations in temperature and light intensity during the suitable period for flower bud initiation could explain the large year to year variations in flowering (i.e. in some years mature trees do not flower) of many tree species such as aspen and Norway spruce (Owens, 1995). Not all poplar species exhibit year to year variations in flowering, but aspens are the *Populus* species that grow at the highest altitudes and the most northern latitudes. In many agricultural crops, the concept of thermal time or photothermal time is used to explain variations in flowering time (reviewed in Poethig, 2003). According to this concept, flowering time is determined by the accumulated amount of heat or heat plus light that the plant receives during a period preceding floral initiation. This concept might also apply to trees. Willow (*Salix*) and poplar are members of the Salicaceae, and a study of adult willow cuttings in controlled environments indicated that both photoperiod and temperature affect floral initiation (Junttila, 1980).

In many plants that survive the winter in a vegetative state, prolonged exposures to cold, also known as vernalization, makes the plant fully competent to respond to environmental factors inducing flowering during the next spring (Sung & Amasino, 2004). Whereas vernalization has not yet been shown to affect floral initiation in trees, chilling is required for the release of winter dormancy (i.e. bud break) and optimal shoot growth in temperate-zone trees (Rohde *et al.*, 2000). One possibility is that a tree gradually acquires competence to respond to environmental factors promoting flowering through the release of floral repression by multiple passes through dormancy and vernalization. In winter annual accessions of *Arabidopsis*, this repression is mediated through the floral repressor *FLC*. However, no clear homologs have yet been identified in *Populus*, though the complete genome sequence is now available (Fig. 1). It is possible that a different gene, perhaps a member of a different MADS-box subfamily, performs similar functions in other species. This appears to be the case in wheat, where vernalization downregulates a

floral repressor, *VRN2*, that is not a MADS-box gene, but a member of a different family of transcription factors (Yan *et al.*, 2004).

Comparing flowering in annuals with flowering in trees: the central questions

Comparative studies can answer to what extent diversity in floral transition is caused by alterations in gene function, gene regulatory networks or changes in gene number. As shown by recent studies comparing the floral transition in rice and *Arabidopsis* (Hayama *et al.*, 2003) complete genome sequences combined with genetic approaches are particularly powerful for addressing these questions. We have identified five different flowering characteristics of poplars and many other trees that are not easily explained based solely on studies of flowering in annual plants, and thus, also illustrate the value of model tree systems.

1 The prolonged juvenile period when a tree is reproductively incompetent. This could have similarities to the juvenile phase of annuals; however, tree juvenility persists for years of cycles through growth and dormancy.

2 In trees, flowering branches and nonflowering branches exist on the same tree. A reproductively mature tree will not initiate flowers on all branches, and the mechanism that controls this process is unknown. There is no counterpart in *Arabidopsis* where all branches are turned into inflorescence shoots after floral induction.

3 Individual tree shoots produce both vegetative and reproductive buds. In poplar, shoots alternate between the production of lateral vegetative and reproductive buds. There is no *Arabidopsis* equivalent. Once flowering is induced in *Arabidopsis*, the inflorescence shoot produces only flowers.

4 Some reproductively competent trees do not flower in certain years. Annual plants only have one growing season to flower; therefore, they do not display such variation in flowering.

5 In trees, the acquisition of reproductive competency and seasonal initiation of flowering occur over much larger temporal and spatial scales than in annual plants. It remains to be determined whether the signaling pathways regulating these different components of the floral transition are less integrated in trees compared with annuals. It is not yet known if, in trees, the juvenile phase, the maintenance of nonflowering branches within an adult tree, and the maintenance of vegetative meristems within flowering branches are regulated by the same or different pathways.

Searching for answers: exploiting the poplar genomics toolkit

With the release of the *Populus trichocarpa* genome sequence, poplar functional and comparative genomics is poised to make a gigantic leap forward. First, the sequences of all genes belonging to a particular gene family will be available, and owing to their phylogenetic proximity, it is relatively easy to identify putative poplar orthologs of *Arabidopsis* genes or cases where there is no poplar counterpart (Fig. 1). Genome-wide expression analysis using microarrays will also facilitate the identification of candidate poplar flowering genes. This approach has already proven to be very powerful for elucidating the transcriptomes regulating wood formation (Hertzberg *et al.*, 2001; Israelsson *et al.*, 2003) and autumn senescence (Andersson *et al.*, 2004), and is likely to provide important insights into the specific transcriptional changes occurring during the juvenile to mature transition in poplars. Transcriptome profiling will help elucidate the regulatory networks controlling flowering and these can then be compared with similar studies in other plants. The conservation of regulatory pathways can be tested in transgenic poplars by expressing key regulatory genes under constitutive or inducible promoters combined with global expression studies to identify downstream targets. Additional tests of functional conservation can be performed by studying poplar genes in *Arabidopsis* and vice versa. One major weaknesses of the poplar system is that forward genetic screens (i.e. screening for loss-of-function mutants) are virtually impossible given the outcrossing behavior of poplars coupled with their long generation times. However, a complete genome sequence can provide an alternative by allowing the efficient generation of large-scale RNAi mutant screens (Waterhouse & Helliwell, 2003). Analysis of RNAi-induced loss-of-function transgenics coupled with microarray expression analysis will provide important information about the role of these genes in tree flowering.

The results from transgenic experiments can be compared with results obtained using poplar clones of various ages growing in plantations (i.e. a continuous age gradient of a single genotype) and early-flowering genotypes grown in the glasshouse. This will be especially important for the characterization of floral repressors and genes influencing the formation of floral organs. Grafting can be another important tool in elucidating the nature of the flower-inducing substance ('florigen') that moves from the leaf to the apex before floral initiation and the possible root–shoot signals that are involved in tree maturation. Isolation of large amounts of phloem and xylem sap is relatively easy in poplars compared with *Arabidopsis*, and could, in combination with metabolic profiling and proteomics techniques, aid in the characterization of these processes.

Finally, the positioning of the poplar genetic map on the completed genome sequence will greatly aid quantitative trait locus and association mapping studies, providing a link between key flowering regulators identified in the lab and natural variations in flowering time and sex determination. Because of the expected low level of linkage disequilibrium in poplars, use of candidate genes to narrow the search for adaptive polymorphisms might be the most fruitful approach. Although floral initiation has not yet been examined, there is abundant genetic variation in other aspects of phenology that for at least some traits has been associated with latitude or

other indices of geographic origin (Pellis *et al.*, 2004). A common garden study of variation in juvenile phase length and seasonal time of floral initiation is obviously a time- and space-consuming project. However, poplars grown on optimal plantation sites often initiate flowering in their third to fifth growing season. Thus, if ecotypes are carefully selected, this is not an unrealistic experiment, especially given that valuable information on vegetative trait variation could also be obtained. Moreover, progeny tests of a cross between an early flowering female poplar genotype and a male clone from the same half-sib family are in progress that might identify polymorphisms associated with juvenile phase length (Meilan *et al.*, 2004). With the very powerful suite of tools at hand for poplar, it seems likely that within the next few years we will have found some of the first answers to the long-standing questions of how maturation and flowering are controlled in trees.

Supplementary material

The following material is available as Supplementary material at http://www.blackwellpublishing.com/products/journals/suppmat/NPH/NPH1165/NPH1165sm.htm

Appendix S1 Poplar MADS domain protein sequences used to construct the phylogenetic tree shown in Fig. 1 are provided in FASTA format.

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