FLOWERING LOCUS T duplication coordinates reproductive and vegetative growth in perennial poplar

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Annual plants grow vegetatively at early developmental stages and then transition to the reproductive stage, followed by senescence in the same year. In contrast, after successive years of vegetative growth at early ages, woody perennial shoot meristems begin repeated transitions between vegetative and reproductive growth at sexual maturity. However, it is unknown how these repeated transitions occur without a developmental conflict between vegetative and reproductive growth. We report that functionally diverged paralogs *FLOWERING LOCUS T1* (*FT1*) and *FLOWERING LOCUS T2* (*FT2*), products of whole-genome duplication and homologs of *Arabidopsis thaliana* gene *FLOWERING LOCUS T* (*FT*), coordinate the repeated cycles of vegetative and reproductive growth in woody perennial poplar (*Populus* spp.). Our manipulative physiological and genetic experiments coupled with field studies, expression profiling, and network analysis reveal that reproductive onset is determined by *FT1* in response to winter temperatures, whereas vegetative growth and inhibition of bud set are promoted by *FT2* in response to warm temperatures and long days in the growing season. The basis for functional differentiation between *FT1* and *FT2* appears to be expression pattern shifts, changes in proteins, and divergence in gene regulatory networks. Thus, temporal separation of reproductive onset and vegetative growth into different seasons via *FT1* and *FT2* provides seasonality and demonstrates the evolution of a complex perennial adaptive trait after genome duplication.

perennialism | tree | dormancy | gene duplication | signaling

Life cycles of higher plants display a great diversity in morphological and seasonal adaptation. Annual plants grow, reproduce, and senesce within a growing season, whereas woody perennials display successive years of vegetative growth before reaching sexual maturity (1–3). After this time, shoot meristems begin cyclical transitions between vegetative and reproductive growth. Consequently, shoots may repeatedly form early vegetative buds (Vegetative Zone I), reproductive buds (Floral Zone), and late vegetative buds (Vegetative Zone II) in a sequential manner (3). However, our understanding of the mechanisms underlying such complex phenotypes, and thus variation in growth habits and adaptation, remain rudimentary. In the herbaceous perennial *Arabis alpina*, repeated transcriptional repression and activation of *PERPETUAL FLOWERING 1* (*PEP1*), an ortholog of the floral repressor *FLOWERING LOCUS C* (*FLC*) in annual *Arabidopsis thaliana* (4), controls recurring seasonal transitions between reproductive and vegetative phases (5). However, a true functional ortholog of *FLC* has not been reported in trees, nor does phylogenetic analysis point to a clear structural ortholog of *FLC* in poplar (*Populus* spp.) (6).

Previous results showed that *FLOWERING LOCUS T1* (*FT1*) (7) and *FLOWERING LOCUS T2* (*FT2*) (8) under the cauliflower mosaic virus 35S (CaMV 35S) constitutive overexpression promoter induce early flowering in poplar. Transcript abundance of both genes gradually increases in the growing season as poplar trees mature. These findings imply that *FT1* and *FT2* redundantly control the transition from juvenile to reproductive stage during the growing season. Moreover, short-day–induced growth cessation and bud set are attributed to the *FT1*/*CONSTANS 2* regulon in poplar (7). *FT1* and *FT2*, products of a whole-genome salicoid duplication event (9), are located on paralogous chromosomes VIII and X, respectively (Fig. S1*A*). *FT1* and *FT2* are homologs of paralogous *FLOWERING LOCUS T* (*FT*) and *TWIN SISTER OF FT* (*TSF*) (Fig. S1*B*). The onset of reproduction in *Arabidopsis* is induced redundantly by *FT* (10, 11) and *TSF* (12) under warm-temperature and long-day conditions. No other functions of *FT* or *TSF* have been reported. Through elucidating the detailed roles of *FT1* and *FT2* in reproductive and vegetative growth, we report a mechanism indicating that cycles of reproductive and vegetative growth in perennial poplar are coordinated by the transient expression of the functionally diverged paralogs *FT1* and *FT2* in contrasting seasons.

Results

***FT1* and *FT2* Diverged in Regulation.** To identify normal temporal and spatial expression of *FT1* and *FT2*, we first designed and tested gene-specific primers (Fig. S2 *A* and *B*). We then conducted year-round transcript analyses of *FT1* and *FT2* in the same tissues using normally growing mature *Populus deltoides*. In all five tissues analyzed, *FT1* transcripts were abundant only in winter (dormant season) when day length was the shortest (<12 h) and mean monthly low and high temperatures were <6 °C and <15 °C, respectively (Fig. 1 *A* and *B* and Fig. S2*C*). Conversely, *FT2* transcripts were abundant only in leaves and reproductive buds in the growing season when day length was >12 h and mean monthly low and high temperatures were >10 °C and >25 °C, respectively (Fig. 1 *A* and *C*). After abundant expression in spring, *FT2* continued

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to be expressed at lower levels in the same tissues until mid-fall, when day length became shorter (<12 h), and air temperature began dropping. These findings show that *FT1* transcripts were abundant in all tissues analyzed when the days were short and temperatures were cold, whereas *FT2* transcripts were abundant in leaves and developing reproductive buds when days were long and temperatures were warm. Similarly, in leaves of two other poplars (*Populus trichocarpa* and *Populus tremula* × *Populus tremuloides*), *FT1* transcripts were abundant in February, whereas *FT2* was abundant in May, suggesting similar regulation of *FT1* and *FT2* in different poplar taxa (Fig. S2*D*). These results suggest that transcription of *FT1* and *FT2* is temporally and spatially separated.

We then tested whether temperature, day length, and internal factors regulate *FT1* and *FT2* transcription in mature *P. deltoides*. Trees in the field were allowed to set terminal buds normally in late summer/early fall under short-day conditions. Then, in November, one group of dormant trees was moved to either warm (25 °C) or cold (4 °C) temperature under short-day conditions (8 h light) for 161 d. *FT1* transcription began to increase in preformed leaves enclosed in vegetative buds within 45 d at 4 °C but was undetectable at 25 °C throughout the experimental period (Fig. 2*A*). When some trees were transferred to 25 °C after 90 d at 4 °C, *FT1* transcription diminished rapidly, resembling the decline in normal *FT1* transcription from winter to spring (Fig. 1*B*). *FT2* transcripts were undetectable in the identical tissues in these experiments. The treatment of a second group of normally dormant trees in winter (November–March) showed that *FT1* transcripts were abundant in cold temperature under continuous darkness or ambient conditions (Fig. 2*B*). However, *FT1* transcription was significantly ($P \leq 0.0001$) less at 25 °C under short-day conditions. Day length did not affect *FT1* expression, because trees treated in short-day (8 h light) and long-day (16 h light) conditions in cold temperature showed no significant ($P = 0.45$) differences in transcript levels (Fig. 2*B*). Similarly, the presence or absence of light did not affect *FT1* transcription, because trees grown in dark and in light did not differ significantly ($P = 0.107$) in transcript abundance (Fig. 2*B*). *FT2* transcripts were not detected in the identical tissues in these experiments. A third group of actively growing trees was placed under long-day or short-day conditions at 25 °C for 42 d in spring, when *FT2* is normally induced. *FT2* transcripts were significantly ($P \leq 0.0001$) abundant in leaves in long-day conditions but were undetectable in short-day conditions (Fig. 2*C*). *FT1* transcripts were undetectable in the identical tissues. The fourth group of actively growing trees also was placed in long-day conditions at 25 °C or at 4 °C for 14 d in May. *FT2* transcripts in expanding leaves were abundant at 25 °C but were decreased significantly ($P \leq 0.001$) at 4 °C (Fig. 2*D*). *FT1* transcripts were slightly detectable in trees grown for 14 d at 4 °C. These results show that, although cold temperature activates and warm temperature suppresses *FT1* transcription, day length or presence or absence of light does not affect expression. Conversely, long-day conditions or warm temperatures promote *FT2* transcription, whereas short-day conditions or cold temperatures suppress expression. These findings are consistent with normal winter expression of *FT1* and growing-season expression of *FT2* (Fig. 1). Moreover, *FT1* expression does not show a rhythm in daily transcript abundance (Fig. S3*A*), whereas *FT2* expression shows a semidian rhythm with a periodicity of about 12 h (Fig. S3*B*). Taken together, these experiments reveal that *FT1* and *FT2* have diverged in regulation, implying changes in regulatory DNA regions of the paralogs after the duplication event.

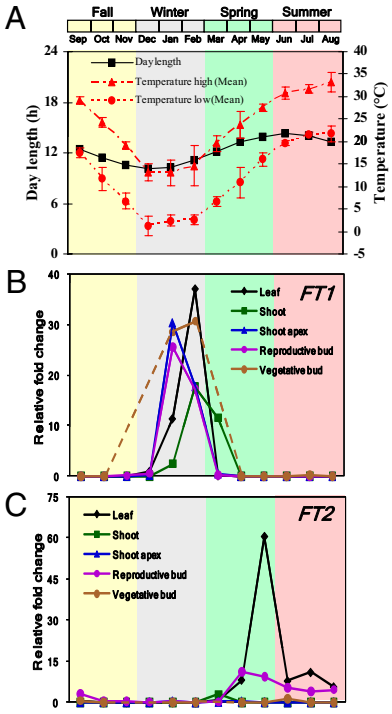

Fig. 1. Year-round normal expression of *FT1* and *FT2* in the same five above-ground tissues of mature *P. deltoides*. (*A*) Monthly high/low temperatures and day length in Mississippi, where experimental trees were grown. Error bars show SD about the mean. (*B* and *C*) Relative fold change in transcript levels of *FT1* (*B*) or *FT2* (*C*) relative to the lowest amount of expression within a tissue. (*B*) *FT1* transcripts are abundant in all the analyzed tissues in winter. Dashed lines indicate missing samples. (*C*) *FT2* transcripts are abundant in leaves and reproductive buds in spring and summer.

***FT1* Signals Reproductive Onset.** To define *FT1* and *FT2* functions further, we genetically perturbed their expression in poplar. To avoid potential complications caused by constitutive overexpression using the CaMV 35S promoter, we used the heat-inducible promoter of *HEAT SHOCK PROTEIN* (*HSP*) gene to make Pro_{HSP}:*FT1* and Pro_{HSP}:*FT2* constructs for transformation. Unlike Pro_{HSP}:*FT2*, Pro_{HSP}:*FT1* induced flowers within 30 d of cyclical heat treatment at 37 °C (Fig. 3*A* and Dataset S1). Transcripts of both genes were significantly ($P \leq 0.0001$) abundant in transgenic trees. We note that, compared with extremely abundant overexpression of *FT1* and *FT2* under the CaMV 35S promoter (Pro_{35S}:*FT1* and Pro_{35S}:*FT2*, respectively), Pro_{HSP}:*FT1* and Pro_{HSP}:*FT2* constructs induced only a very moderate overexpression, much closer to normal peak expression of *FT1* and *FT2* (Fig. 3*A*). Pro_{HSP}:*FT1* trees continuously formed axillary inflorescences (catkins) and eventually formed a terminal inflorescence on the new shoot growth as long as *FT1* signaling was available (Fig. S4*A*). Axillary vegetative buds that had formed before heat treatment did not produce inflorescences or overcome dormancy. When the temperature was increased to 40 °C to test whether higher abundance of *FT2* transcripts triggers flowering, *FT2* transcript levels increased significantly ($P \leq 0.0001$), and trees showed a weak flowering phenotype, mainly forming incomplete inflorescences (Fig. 3*A*, Fig. S4*A*, and Dataset S1). Thus, in poplar relatively low *FT1* signaling induces reproductive onset in undifferentiated meristems, whereas abnormally abundant *FT2* transcripts are required for this process to occur. Our results suggest that a pulse of *FT1* expression in winter initiates the transition of vegetative meristems to the reproductive phase, resulting in a limited number of reproductive buds in the Floral Zone (Fig. S4*B*). Buds that are produced under warm temperatures before and after *FT1* expression are vegetative (Vegetative Zones I and II).

If *FT2* signal is required for reproductive onset in poplar, suppression of *FT2* transcription following *FT1* signaling should produce no reproductive buds. Because short-day conditions repress *FT2* transcription (Fig. 2*C*), we maintained branches of field-grown mature *P. deltoides* under short-day conditions in spring (March–May) when *FT2* expression normally is abundant (Fig. S5*A*). Control branches were kept under ambient long-day conditions (12–14 h). The short-day treatment was effective, because *FT2* transcription was significantly ($P \leq 0.005$) lower in short-day–treated shoots than in controls (Fig. S5*B*). The controls

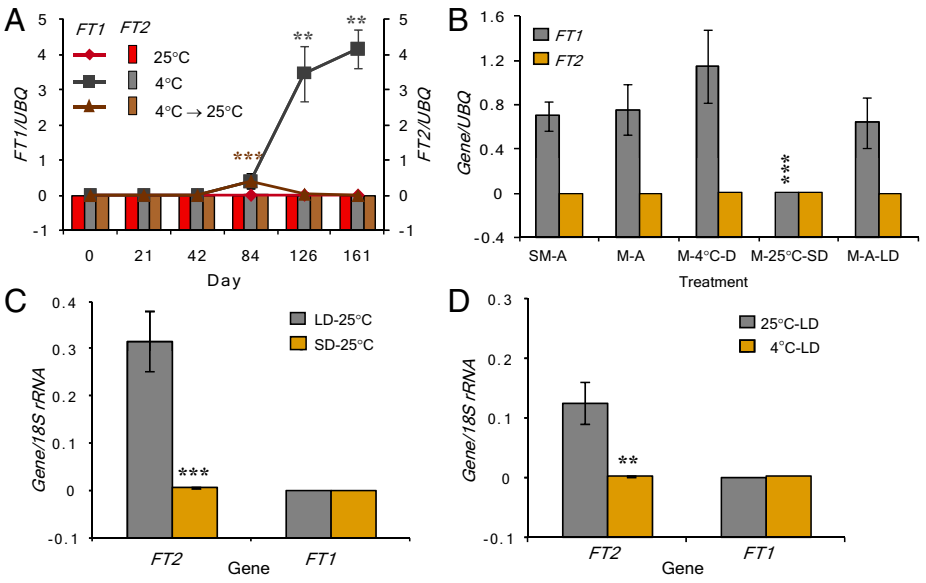

Fig. 2. Regulation of *FT1* and *FT2* in *P. deltoides*. (*A*) *FT1* transcript abundance increased in dormant trees ($n = 12$) at 4 °C under short-day conditions. When six trees were transferred to 25 °C after 90 d of 4 °C treatment, *FT1* was undetectable. *FT2* transcripts were undetectable in the identical tissues. (*B*) In winter, *FT1* transcripts were more abundant in mature dormant trees in the field at ambient conditions (SM-A; $n = 3$), in mature dormant trees in pots at ambient conditions (M-A; $n = 3$), in mature dormant trees in pots at 4 °C in continuous darkness (M-4 °C-D; $n = 3$), or in mature dormant trees in pots at ambient conditions in long-day conditions (M-A-LD; $n = 3$) than in mature dormant trees in pots at 25 °C under short-day conditions (M-25 °C-SD; $n = 3$). *FT2* transcripts were not detected in the identical tissues. (*C*) *FT2* transcripts were more abundant in long-day than in short-day conditions at 25 °C. *FT1* transcripts were undetectable in the identical tissues. (*D*) Treatment at 4 °C repressed *FT2* transcription ($n = 3$) in trees grown for 14 d at 4 °C and 25 °C. In contrast, *FT1* transcripts increased slightly in abundance at 4 °C. Error bars indicate SD. $^{**}P \leq 0.005$ and $^{***}P \leq 0.0005$ within a treatment.

ceased shoot growth within 56 d, but the short-day–treated shoots did so within 35 d and produced significantly ($P \leq 0.0001$) shorter shoots and fewer vegetative buds (Fig. S5 *C–E*). Reproduction was not eliminated; however, there were significantly ($P \leq 0.005$) fewer reproductive buds in the short-day treatment (Fig. S5 *C–E*). In the second experiment, Pro_{HSP}:*FT1* and *FT2*-RNAi constructs were coexpressed in the same trees to increase *FT1* and reduce *FT2* transcript abundance, respectively. *FT2* knockdown ranged from 15–45% compared with controls, and *FT1* transcripts were abundant during the heat treatment at 37 °C (Fig. S6 *A* and *B*). Unlike controls, 10 of 11 Pro_{HSP}:*FT1*/*FT2*-RNAi lines formed inflorescences (Fig. S6*C*), suggesting that *FT1* signaling is sufficient for reproductive onset for which *FT2* signaling is not necessary. In the third experiment, when Pro_{HSP}:*FT1* trees were heat-treated to 40 °C under short-day conditions in which *FT2* is not normally expressed (Fig. 2*C*), flowering still was induced (Fig. S6*D* and Dataset S1). Finally, poplar trees (*P. tremula* × *Populus alba*) with relatively less *FT2* overexpression (Pro_{35S}:*FT2*) produced inflorescences at the same age (5 y) as the controls in the field. We would have expected Pro_{35S}:*FT2* trees to transition to the sexually mature stage at an earlier age because of the greater *FT2* transcript output by both transgene and endogenous alleles. These results show that *FT2* signal is not essential for reproductive onset but may play a role in normal development of reproductive buds and/or flowers, because *FT2* transcripts are abundant in reproductive buds during the growing season (Fig. 1*C*).

***FT1* and *FT2* Molecular Networks Diverged.** To determine whether the molecular networks of *FT1* and *FT2* have diverged and reflect their function, we conducted microarray experiments to compare constitutive and inducible constructs with controls and subsequently to identify common genes downstream of Pro_{35S}:*FT1* and Pro_{HSP}:*FT1* or Pro_{35S}:*FT2* and Pro_{HSP}:*FT2* in poplar (Fig. S7*A* and Dataset S2). Leaf tissues from heat-treated (inducible constructs) plants were sampled on the day immediately following heat treatment (day 21). We then mapped year-round normal expression of such downstream genes in leaves of mature *P. deltoides* by conducting another set of microarray experiments, followed by cluster analysis and functional classification (Fig. 3*B*). Genes downstream of *FT1* mostly were down-regulated, whereas genes downstream of *FT2* and genes downstream of both *FT1* and *FT2* were mainly up-regulated. Unlike *FT2*, 18 genes downstream of *FT1* are related to reproduction (Fig. 3*B*), supporting *FT1*'s main function in reproductive onset. *FT1* up-regulated genes include *MADS49*, a homolog of *Arabidopsis SEPALLATA* involved in floral organ formation (Fig. S7*B*) (13). *MADS49* transcripts were abundant in reproductive buds throughout inflorescence development after the formation of floral meristems on flanks of inflorescence shoots (Fig. S7*C*) (3)]. In contrast, *MADS7*, similar to the *Arabidopsis* floral repressor *SHORT VEGETATIVE PHASE* (Fig. S7*B*) (14, 15), was down-regulated. *MADS7* was expressed mainly in juvenile trees (Fig. S7*D*) and showed an inverse relationship with *FT1* (Fig. S7*E*), suggesting that *MADS7* may be a negative regulator of reproductive onset. Moreover, 15 auxin-related genes involved in signaling and transport established a unique network with *FT1* and were down-regulated when *FT1* was up-regulated via Pro_{35S}:*FT1* or Pro_{HSP}:*FT1* (Fig. 3*B*). These genes were suppressed when *FT1* was normally activated in winter but were up-regulated in the following growing season (turquoise and red modules in Fig. 3*B*). Although the mechanism is not clear, auxin has been known since the 1940s to be a repressor of reproductive onset in leaves but a promoter of reproductive development (16–20). These auxin-related genes might act as negative regulators of poplar reproductive onset in winter, and thus need to be transiently repressed by *FT1*, but are subsequently needed during reproductive development in the growing season. Upon up-regulation of *FT1*, down-regulation of methyltransferase and histone genes (Dataset S2) indicates an epigenetic change in chromatin, probably enabling reproductive development. Of the 27% of the genes downstream of *FT1* that are involved in metabolism, 63% were down-regulated when *FT1* was activated, and 52% were up-regulated in the following growing season (turquoise and red modules in Fig. 3*B*), suggesting that *FT1* influences metabolic networks into the growing season that support rapidly developing reproductive buds. These results show that *FT1* and *FT2* molecular networks have diverged, are highly modulated, and show a dynamic year-round expression pattern.

***FT2* Regulates Vegetative Growth.** What is the primary function of *FT2*? The abundance of *FT2* transcripts during rapid shoot growth in the growing season and the observation during aforementioned experiments that increased *FT2* transcription accelerated vegetative growth prompted us to conduct the following experiments to test whether *FT2* regulates vegetative growth. First, actively growing trees harboring Pro_{HSP}:*FT1* or Pro_{HSP}:*FT2* were transferred for 105 d into short-day conditions at 30 °C, which is compatible with growing-season temperatures (Fig. 1*A*) and is high enough to promote *FT1* and *FT2* transcription via Pro_{HSP} without inducing flowering. To repress endogenous expression of *FT1* and *FT2* and to ensure that the treatment effect is

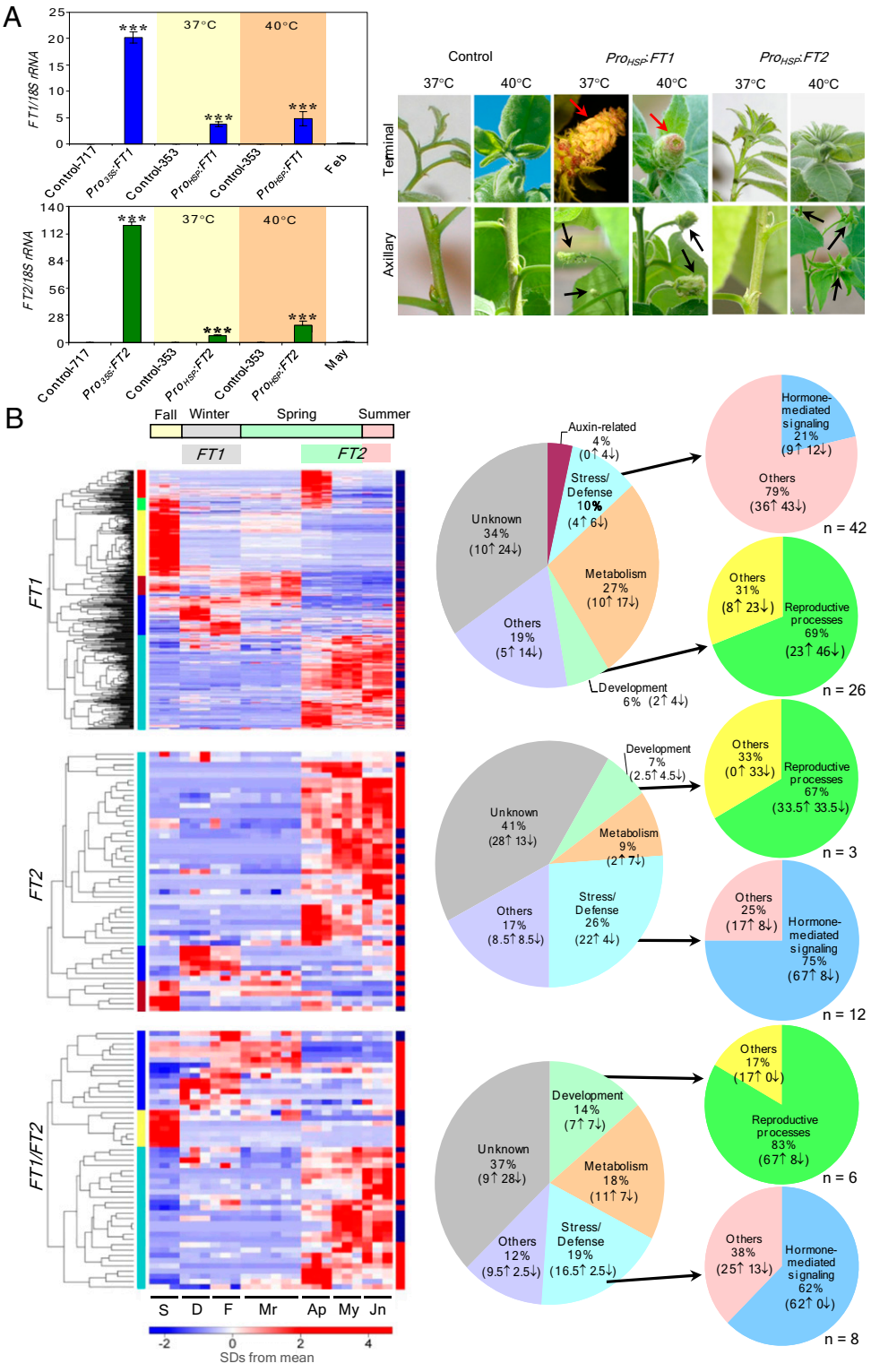

Fig. 3. Functional and network analyses of *FT1* and *FT2* in poplar. (*A*) Trees (*P. tremula* × *P. tremuloides* 353) harboring Pro_{HSP}:*FT1* and Pro_{HSP}:*FT2* (*n* = 30) were treated at 37 °C and 40 °C under long-day conditions to determine reproductive onset. (*Right*) (*Upper*) Red arrows show terminal inflorescences. (*Lower*) Black arrows show axillary inflorescences. (*Left*) *FT1* (*Upper*) and *FT2* (*Lower*) transcript abundance was determined in leaves of trees (*P. tremula* x *P. tremuloides* 353) harboring Pro_{HSP}:*FT1* and Pro_{HSP}:*FT2*, in leaves of trees (*P. tremula* × *P. alba* 717) harboring Pro_{35S}:*FT1* and Pro_{35S}:*FT2*, and in leaves of normally growing mature *P. deltoides* (controls) in February and May. ***$P \leq 0.0001$ within a treatment. (*B*) (*Left*) Heat maps showing year-round normal expression of genes downstream of *FT1* and *FT2* (Dataset S2) in mature *P. deltoides*. (*Left*) Clusters on the left represent modules. The column on the right shows up-regulated (red) and down-regulated (blue) genes downstream of *FT1*, downstream of *FT2*, or downstream of both *FT1* and *FT2* commonly expressed in Pro_{35S}:*FT1* and Pro_{HSP}:*FT1*, and Pro_{35S}:*FT2* and Pro_{HSP}:*FT2*. Months from September (S) to June (Jn) are identified below the heat maps. SDs are shown below the heat maps. (*Right*) Pie charts show functional categorization of similar Gene Ontology Biological Process terms. Numbers in parenthesis represent partitioning of overall percentages into up (↑) and down (↓) percentages. *n*, number of genes.

caused only by Pro_{HSP}, we used warm-temperature, short-day conditions, because *FT1* normally is not expressed in warm temperature (Fig. 2 *A* and *B*), nor is *FT2* normally expressed in short-day conditions (Fig. 2*C*). The treatment was effective, because *FT1* and *FT2* transcripts were significantly ($P \leq 0.001$) more abundant in transgenic trees than in controls (Fig. S8*A*). Control trees normally ceased shoot growth within 35 d because of short-day conditions. Pro_{HSP}:*FT2* trees grew continuously, whereas Pro_{HSP}:*FT1* trees ceased shoot growth by day 105. Consequently, Pro_{HSP}:*FT2* trees produced significantly ($P \leq 0.0001$) more shoot, internode, and stem diameter growth (Fig. S8*A*). When returned to 23 °C and short-day conditions, Pro_{HSP}:*FT2* trees ceased shoot growth within 35 d. Second, Pro_{35S}:*FT2* or $Pro_{35S2\times}$:*FT2*-C_{tag} trees with no early flowering did not cease shoot growth or form terminal buds in response to short photoperiods and cold temperatures in the field, resulting in no induction of winter dormancy (Fig. S8 *B* and *C*). Consequently, they grew year-round as long as air temperatures stayed above freezing. Winter frost killed

growing leaves and shoot tips on mature trees and often killed shoots and above-ground stems of juvenile trees. However, when the air temperature became warmer in the winter, undamaged axillary buds began to grow rapidly. Thus, constitutive expression of *FT2* is sufficient to prevent tree growth cessation induced by adverse environmental conditions (e.g., short days and cold temperature). In contrast, $Pro_{35S2\times}$:*FT1*-C_{tag} trees did not show year-round growth (Fig. S8*D*). Control trees normally induced dormancy in late summer or early fall and did not resume growth until the following spring. Third, Pro_{35S}:*FT2* trees showed strong apical dominance and produced significantly ($P \leq 0.0001$) shorter axillary shoots than controls (Fig. S9*A*). Finally, Pro_{HSP}:*FT1*/*FT2*-RNAi trees with fewer *FT2* transcripts (Fig. S6*A*) produced significantly ($P \leq 0.007$) less shoot growth than controls when grown at 30 °C and long-day conditions (Fig. S9*B*). A temperature of 30 ° C was used to drive *FT1* expression via Pro_{HSP}, and long-day conditions were used to enable normal expression of *FT2* so that the RNAi construct would reduce endogenous *FT2* expression. *FT2* knockdown resulted in less vegetative growth in trees. Considered together, these results reveal that vegetative growth, including growth cessation, bud set, and dormancy induction, is controlled by *FT2*, consistent with seasonal timing of its normal regulation in poplar (Fig. 1*C*).

What are the genetic mechanisms by which *FT2* controls vegetative growth? A majority (26%) of the known genes downstream of *FT2*, mainly expressed in the growing season (turquoise module in Fig. 3*B*), are related to stress defense (Fig. 3*B*). Growth cessation and bud set are induced when environmental factors are limiting (i.e., ecodormancy); thus, they may share regulatory elements (21). To determine whether genes downstream of *FT2* respond to stress that reduces or arrests shoot growth (22, 23), we conducted the following experiments in poplar. First, when day-length–treated tissues from mature trees grown in the field (Fig. S5) were reanalyzed, *FT2* and *JASMONATE-ZIM-DOMAIN PROTEIN 1* transcripts were significantly ($P \leq 0.05$) less abundant under short-day conditions that induced growth cessation (Fig. S9*C*). Second, poplar is a fast-growing pioneer species and normally is intolerant of shading by neighboring plants, but during the growing season, leaves in the interior tree crown often are shaded, or cloud covers shade trees. When the ambient light intensity was decreased from 1,700 to 500 μmol s^{-1} m^{-2} via shading of whole trees in the field, the transcript abundance of *FT2* and the antimicrobial extrusion efflux protein *ZF14* was reduced significantly ($P \leq 0.05$) (Fig. S9*D*). Shaded plants produced significantly ($P \leq 0.05$) shorter shoots. Third, trees often experience heat stress (temperatures >30 °C) coupled with water stress during summer days (Fig. 1*A*). *FT2* and *MAPK3* transcripts were significantly ($P \leq 0.05$) less at 38 °C (heat stress) than at 25 °C (Fig. S9*E*). Fourth, the abundance of *FT2* transcripts was significantly ($P \leq 0.05$) reduced, whereas that of *ETHYLENE RESPONSE FACTOR-APETALA2* was significantly ($P \leq 0.005$) increased under low, medium, and severe water stress that induced cessation of shoot growth (Fig. S9*F*). Finally, cold temperature significantly ($P \leq 0.001$) repressed *FT2* transcription (Fig. 2*D*). *FT1* transcripts were undetectable in these experiments (e.g., Fig. S9 *C–F*). These results demonstrate that *FT2* acts as a multistress sensor and selectively forms molecular networks with different genes in response to various stress factors to control vegetative growth during the growing season.

Discussion

Our results suggest that repeated cycles of reproductive and vegetative growth in sexually mature poplar are coordinated by the transient functioning of the duplication products *FT1* and *FT2*. Reproductive onset is determined by *FT1* signaling in response to winter temperature, resulting in the formation of a limited number of reproductive buds in the Floral Zone (Fig. 4). Cold-temperature signaling also is used by other trees for reproduction (24). The gradual onset of warm spring temperatures rapidly suppresses *FT1* transcription, ending reproductive onset and marking the beginning of reproductive bud development during the growing season when internal and external resources are abundant for rapid development. If *FT1* were expressed during the growing season, poplar could not form true vegetative shoots and buds, and all the buds would be reproductive, as our data show. In contrast to *FT1*, with the gradual onset of warm temperatures and long days in early spring, *FT2* signaling promotes rapid vegetative growth.

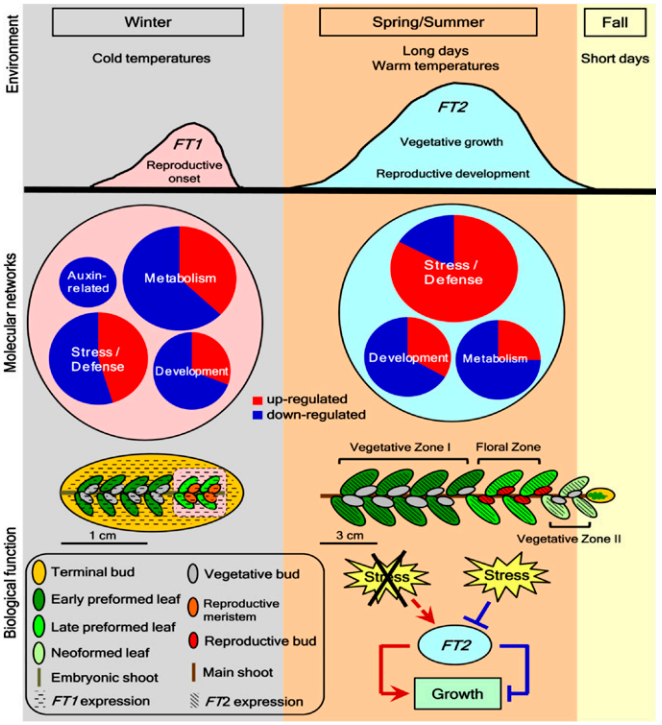

Fig. 4. A schematic integrated model showing that *FT1* and *FT2* regulate cycles of reproductive and vegetative growth. When *FT1* transcription is triggered by winter temperature, it induces reproductive onset through a network of downstream genes in a small number of axillary meristems in dormant buds, resulting in reproductive buds in the Floral Zone. Conversely, in response to warm temperatures, long days, and multiple stress factors in the following growing season, *FT2*, through its molecular networks, regulates vegetative growth.

However, *FT2* expression is either reduced or completely suppressed under stress, such as high temperature and drought that are prevalent in late spring and summer or the gradual shortening of days accompanied by cooling temperature that occurs in the fall, triggering growth cessation, bud set, and eventually dormancy induction (Fig. 4). The match between daily *FT2* rhythm and abiotic factors may allow poplar to detect and respond rapidly to such environmental changes. Consequently, *FT2* provides trees with adaptive properties important not only for growth under favorable conditions but also for survival under unfavorable conditions. Thus, temporal separation of reproductive onset and vegetative growth into different seasons via functionally diverged *FT1* and *FT2* appears to be one of the prominent features of poplar perennialism that enable formation of vegetative buds and shoots for future growth and allow trees to accommodate both vegetative and reproductive growth. These findings indicate a mechanism different from that previously reported for the herbaceous perennial *A. alpina*, in which repeated transcriptional repression and activation of *PEP1*, the *Arabidopsis FLC* ortholog, controls recurring seasonal transitions between reproductive and vegetative phases (5).

Unlike a previous report showing that *FT1* expression induces reproductive onset and controls growth cessation and bud set in the growing season (7), our findings clearly differentiate the regulation and function of the paralogs *FT1* and *FT2*. Specifically, we show that *FT1* expression in winter initiates the transition of vegetative meristems to the reproductive phase, whereas *FT2* controls vegetative growth, including growth cessation, bud set,

and dormancy induction, in the growing season. Our data indicate the following four reasons for this discrepancy: First, the *FT1* primer pair used for expression analysis by Böhlenius et al. (7) cross-reacts with *FT2* transcripts in PCR reactions (Fig. S2*B*). Thus, their *FT1* gene expression data during the growing season [e.g., figures 2 I and J, 3 C and F, S6A, and S7 in Böhlenius et al. (7)] probably reflect *FT2* expression. Second, Böhlenius et al. (7) did not conduct an extensive year-round transcript analysis, as we did, to determine the spatial and temporal expression of both *FT1* and *FT2* in normally growing trees (Fig. 1). Thus, their expression analysis missed a piece of information that *FT1* normally is expressed only in winter or in response to cold temperatures. Third, in interpreting their results, Böhlenius et al. (7) relied primarily on Pro_{35S}:*FT1* trees. As our current results show, the CaMV 35S constitutive promoter causes abnormal gene expression, resulting in additional phenotypes (e.g., vegetative growth) not necessarily associated with the primary function of the gene under normal conditions. Furthermore, their RNAi construct was not *FT1* specific and thus would be expected to knockdown both *FT1* and *FT2*. Finally, Böhlenius et al. (7) did not conduct extensive, long-term field tests on their genetically manipulated trees. Moreover, previous findings by Hsu et al. (8) showed that *FT2* induced reproductive onset when both poplar and *Arabidopsis* were transformed with the Pro_{35S}:*FT2* construct. Our current results suggest that induction of reproductive onset is not *FT2*'s primary function. However, we do not dismiss the possibility that *FT2* might be involved in reproductive development, because *FT2* normally is expressed in reproductive buds during the growing season (Fig. 1*C*). As we did in the current study, Hsu et al. (8) should also have used weaker and/or inducible promoters in their constructs along with suppressing the expression of *FT2*. Thus, we suggest that experimental designs concerning the duplicated genes in duplicated genomes should carefully consider all these aspects as appropriate.

Our results imply that changes in both gene expression and protein sequence have contributed to diverged functions of *FT1* and *FT2*. Transcription of *FT1* and *FT2* is temporally and spatially separated and is under the regulation of contrasting environmental and internal factors. Similarly, under the same inducible promoter, different phenotypes resulting from heat treatment of trees harboring constructs overexpressing *FT1* or *FT2* indicate diverged protein functions, which can be attributed to 16 amino acid changes between the two paralogs (Fig. S1*C*). One of the changes (alanine to proline in *FT2*) is located in a C-terminal external loop (residues 128–145) that contributes to antagonistic activity of *FT* and *TERMINAL FLOWER 1* on flowering time in *Arabidopsis* (25). This change makes the *FT2* external loop more hydrophilic based on hyropathy index, potentially affecting protein–protein interactions. A recent report shows that in biennial sugar beet (*Beta* spp.), the *FT* duplication products *BvFT1* and *BvFT2* have diverged in function (26). *BvFT1* and *BvFT2* are expressed mainly in leaves but differ in temporal expression: *BvFT1* is expressed at the juvenile stage, and *BvFT2* is expressed at the reproductive stage. *BvFT1* expression represses reproductive onset and bolting (vernalization response); similar to *Arabidopsis FT*, *BvFT2* function is needed during the growing season for flowering. The functional difference between BvFT1 and BvFT2 proteins results in part from three amino acid changes in the external loop area of BvFT1 (Fig. S1*C*), making this region more hydrophilic. In contrast to these two examples, a single amino acid change (asparagine to glutamine) in TSF does not appear to affect the external loop hydropathicity, thus showing a structure similar to that of *FT* in annual *Arabidopsis*. In addition, *FT* (10, 11) and *TSF* (12) not only show similar temporal and spatial expression patterns and redundantly control reproductive onset under warm-temperature and long-day conditions but also appear to have similar biochemical functions by interacting with the same transcription factors (27). These advances provide a framework for understanding how changes in *FT* genes have contributed to the evolution of plant life forms and adaptation.

In conclusion, our findings in perennial poplar suggest that *FT* duplication and subsequent changes in gene expression patterns, proteins, and molecular networks leading to adaptive functional differentiation between the paralogs appear to have increased phenotypic flexibility for responding to seasonal and yearly environmental variation. Given that divergence in the expression patterns of many other duplicated gene pairs on paralogous chromosomes VIII and X, as well as in the whole genome, is widespread in poplar (Fig. S10), gene duplication followed by expression pattern shifts, adaptive changes to proteins, and divergence in gene regulatory networks appears to be one of the important elements for the evolution of complex perennial life-history traits.

Materials and Methods

Details of year-round transcript analysis, transcriptional regulation, functional studies, molecular network analysis, and growth and stress experiments are described in *SI Materials and Methods*.

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Supporting Information

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SI Materials and Methods

Phylogenetic Analysis. Using the deduced amino acid sequences for *FLOWERING LOCUS T1* (*FT1*, POPTR_0008s07730.1) and *FLOWERING LOCUS T1* (*FT2,* POPTR_0010s18680.1), the related sequences were identified in the GenBank (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and poplar (http://genome.jgi-psf.org/Poptr1_1/Poptr1_1.info.html) databases via protein–protein BLAST with an E-value cutoff of $\leq 10^{-5}$. Selected genes and their alignment were extracted from the PlantTribes database (1) version 2.0, which includes 10 sequenced plant genomes [five eudicots: *Vitis vinifera*, *Populus trichocarpa*, *Medicago truncatula* (60% complete), *Carica papaya,* and *Arabidopsis thaliana*; two grasses: *Oryza sativa* and *Sorghum bicolor*; and two distantly related outgroups: *Selaginella mollendorffi* and *Physcomitrella patens*]. The alignment was examined manually and adjusted in MacClade 4.07 (2), and ambiguously aligned sites were excluded from the analysis. The relationship between these sequences was tested using maximum parsimony, maximum likelihood, and Bayesian inference and selecting the four *P. patens* sequences as the outgroup. A heuristic maximum parsimony search with 1,000 bootstrap replicates was performed with PAUP 4.10b (3) using 500 random-addition replicates and Tree-Bisection-Reconnection. Using GARLI version 0.951 (4), 500 maximum likelihood bootstrap replicates were performed, implementing the general-time-reversible model of sequence evolution + invariant sites + gamma distributed rate heterogeneity and the default settings for the genetic algorithm. Settings for the model of sequence evolution as implemented in MrBayes 3.1.2 (5) were determined using MrModeltest v2.2 (6). The Bayesian inference analysis was conducted for 5 million generations, with parameters and trees sampled every 1,000 generations; the first 100,000 generations were excluded as the burnin.

Development of Gene-Specific Primers for *FT1* and *FT2*. We aligned the coding regions of *FT1* and *FT2* from *Populus deltoides*, *P. trichocarpa*, and *P. tremula* × *P. tremuloides*. Two regions were identified with least similarity between *FT1* and *FT2* to develop forward and reverse primers. The forward primers for both genes were extended from exon 3 to exon 4; thus any genomic DNA with intron 3 cannot be detected. The reverse primers for both genes were located at the end of exon 4. We then tested the specificity of each primer pair via PCR using the recombinant plasmid DNA containing *FT1* or *FT2*. The amplicons from RT-PCR reactions were cloned into the pGEM-T easy vector (Promega), at least three individual colonies were sequenced, and the resulting sequences were compared with *FT1* and *FT2* sequences. The following PCR parameters were applied: 20 s at 94 °C, 20 s at 60 °C, and 20 s at 72 °C for 35 cycles using the Eppendorf Mastercycler ep gradient PCR system (Eppendorf). The following primers were determined to be gene specific and were used for conducting RT-PCR and quantitative PCR (qPCR) reactions for all poplar species in our experiments:

FT1 (POPTR_0008s07730.1):
Forward: 5′-CAACTGGGGCAAGCTTTGGCCATGAAAC-3′ [28 nt; melting temperature (T_m) 69 °C]
Reverse: 5′-TTATCGCCTCCTACCACCAGAGCCAC-3′ (26 nt; T_m 64 °C)

FT2 (POPTR_0010s18680.1):
Forward: 5′-CTACCGGGGCGAACTTTGGGCAAGAGGT-3′ (28 nt; T_m 70 °C)
Reverse: 5′-TCATGGTCTCCTTCCACCGGAGCCAC-3′ (26 nt; T_m 68 °C)

We also tested the specificity of the *FT1* primers (called "*PtFT1*") used in Böhlenius et al. (7) in the same manner as above, but we used a different PCR program, because the program mentioned above did not amplify either *FT1* or *FT2*. The PCR conditions were 20 s at 94 °C, 20 s at 57 °C, and 20 s at 72 °C for 40 cycles using the Eppendorf Mastercycler ep gradient PCR system. The primer sequences are:

PtFT1 (POPTR_0008s07730.1)
Forward: 5′-CAGAACTTCAACACCAGAGA-3′ (20 nt; T_m 44 °C)
Reverse: 5′-TCCTACCACCAGAGCCACT-3′ (19 nt; T_m 49 °C)

These primers are located in exon 4 and do not span an intron. They are short, with low T_m. Each *PtFT1* primer differs only by 1 nt in the *FT1* and *FT2* sequences in *P. tremula* × *P. tremuloides*, the species used by Böhlenius et al. (7). We also conducted year-round transcript analysis of *FT1* and *FT2* using leaf tissues from *P. deltoides*, *FT1*, *FT2*, and *PtFT1* primers, and qPCR as described below. T_m was calculated using Lasergene software (DNASTAR).

Year-Round Transcript Analysis of *FT1* and *FT2* Using a bucket truck with a hydraulic extending and elevating winch to reach the upper crown of ∼25-m-tall, 30-y-old, normally growing, sexually mature male *P. deltoides* trees located in Starkville, MS (33° 27′ 45″ N; 88° 49′ 12″ W), three independent replications of leaf, shoot, reproductive bud, shoot apex, and vegetative bud tissues were sampled for 12 mo, spanning all four seasons. Vegetative buds were not sampled in November, December, and March. We used one genotype to have a uniform data set, because gene expression often varies significantly among poplar genotypes. Shoot apex samples within a collection were pooled into one sample because of the minute amount of tissue. Sample collections were made 2 h after sunrise. A total of 147 samples was collected [(12 mo × three tissues of leaf, shoot, and reproductive bud × three replications) + (9 mo × one tissue of vegetative bud × three replications) + (12 mo × one tissue of shoot apex × one replication)]. Leaves were preformed and enclosed in terminal vegetative buds from September to March (8). Then they began unfolding from terminal buds, expanding in April and May, and were fully expanded in June, July, and August. Preformed leaves with embryonic shoots were sampled by removing bud scales. Expanding or fully expanded leaves were sampled at nodes 9, 10, and 11 (Floral Zone) from the base of a shoot and were pooled. Reproductive buds are axillary in *P. deltoides*, became visible for the first time in May, and continued to develop until anthesis (opening of reproductive buds) in March of the following growing season (8). The Floral Zone buds at nodes 9, 10, and 11 from the base of a shoot were sampled and pooled. Bud scales were removed (except in April, because of the very small size of buds). The axillary vegetative (shoot/leaf) buds in Vegetative Zone I (8) were sampled from April (early developmental stage) to February (late developmental stage) from shoots at nodes 4, 5, and 6 and were pooled. The newly extending shoots in April and May and fully extended shoots in June through March were sampled 2 cm below the shoot apex/terminal bud. The shoot apex was sampled by removing the bud scales and preformed/primordial leaves.

Total RNAs from 147 samples were isolated using the hot borate method (9) that was combined with the DNase I digestion and cleanup procedure using the RNeasy Mini Kit (Qiagen). Year-round transcript analysis via qPCR then was conducted for *FT1* and *FT2* by following a previously established protocol (10). *FT1* and *FT2* transcripts always were analyzed in the same tissues. The Power SYBR Green PCR Master Mix kit and the 7500 Fast Real-Time PCR system (Applied Biosystems) were used for qPCR reactions with three technical replications per RNA sample. Consequently, we conducted 1,323 qPCR reactions: 147 samples × three technical replications × three genes [*FT1*, *FT2*, and *UBIQUITIN* (*UBQ*)]; nontemplate controls (NTCs) were not included in counting. The *P. deltoides UBQ* transcript was used as an internal standard or reference gene. Each qPCR reaction mixture contained 0.5 μL of cDNA template, 5 μL of SYBR Green Mix, 0.25 μL of 10 μM forward primer, 0.25 μL of 10 μM reverse primer, and 4 μL of ddH_2O. The PCR was programmed to perform an initial incubation at 95 °C for 10 min, followed by 95 °C for 15 s, and 60 °C for 1 min, for a total of 40 cycles. A dissociation curve analysis was conducted after each run to verify the specificity of the amplicon and the formation of primer-dimers. A standard curve for each gene was generated by log [cDNA] (represented by the amount of total RNA used in the real-time reaction) versus the cycle threshold using a series of dilutions of the first-strand cDNA. The ratio between the expression levels of each transcript and *UBQ* (forward primer: 5′-CGATAATGTGAAGGCCAAAATTCAG-3′; reverse primer: 5′-GGTCAGGGGGTATTCCTTCCTTGTC-3′) for each sample was calculated using the relative quantitative analysis method based on a formula for the standard curve assay (11). Relative fold change was calculated by normalizing each expression data point for *FT1* or *FT2* with the lowest amount of expression data point. Daily high and low temperature data collected by a nearby weather station for 12 mo were obtained from 2004 to 2008 (http://ext.msstate.edu/anr/drec/weather.cgi), and the monthly average was calculated over a 5-y period. The data for day length were obtained from http://www.sunrisesunset.com for Starkville, MS.

Regulation of *FT1* and *FT2* Transcription. Approximately 140 dormant shoots, 80 cm long, with terminal vegetative buds, several axillary shoots, and multiple flower buds were cut from the upper crowns of normally growing, sexually mature female *P. deltoides* trees (clone ST-72) in late February. Thus, the cuttings were all clonally propagated from a single genotype. The cuttings were planted immediately in 20-L pots containing Pro-Mix HP (Premier Horticulture) and sand (3:1, vol/vol). Because the cuttings were mature and difficult to root, we dipped them in Hormex rooting powder (Brooker Chemical Corporation) to enhance rooting. The planted cuttings were maintained at 25 °C under drip irrigation and natural light. Once they were rooted and began growing shoots and leaves, the cuttings were moved outside into a shade house for acclimation. After 4 wk acclimation, they were moved into ambient conditions and were drip irrigated; 5 g of 13:13:13 (N:P:K) fertilizer was applied twice during the growing season. We used these trees in the following experiments.

To determine whether *FT1* transcription is regulated by temperature, in mid-November we placed 24 dormant trees in warm (25 °C, $n = 12$ trees) or cold temperatures (4 °C, $n = 12$ trees) under short-day conditions (8 h light, ∼100 μmol s^{-1} m^{-2}) for 161 d. At day 90, six plants were transferred from 4 °C to 25 °C. Terminal buds were sampled in three replications (one replication per tree) per collection at days 0, 21, 42, 84, 125, and 161. A total of 42 samples was collected [(six collections × three replications at 25 °C) + (six collections × three replications at 4 °C) + (two collections × three replications at 4 °C→25 °C)]. Bud scales were removed, and total RNAs were extracted from the remaining preformed leaves and embryonic shoots as described in the previous section. A total of 378 qPCR reactions was performed as described previously [42 samples × three technical replications × three genes (*FT1, FT2,* and *UBQ*); NTCs were not included in counting]. The average ratio (*FT1*/*UBQ* or *FT2*/*UBQ*) for each biological replication was calculated and log2 transformed. A general linear model was used to analyze independently the effect of treatment at each time point on fold change using the the SAS software package V9 (SAS Institute). Means were separated by Fisher's protected least significant difference procedure in SAS.

To test whether *FT1* transcription is regulated by temperature, day length, and light, we kept six dormant trees under ambient conditions, six dormant trees at 4 °C in continuous darkness, six dormant trees at 25 °C under short-day conditions (8 h light, ∼100 μmol s^{-1} m^{-2}), and six dormant trees under ambient conditions in long-day conditions (16 h light) from mid-November to late February (winter). For the last treatment, the photoperiod was extended to 16 h with two 400-W high-pressure sodium lamps with an irradiance of 100 μmol s^{-1} m^{-2} at the canopy level. Terminal buds were collected in mid-February in three replications (one replication per tree). Fifteen samples were collected [five conditions (the four conditions above + one sample from a normally growing tree) × three replications]. After bud scales were removed, total RNAs were extracted from the remaining preformed leaves and embryonic shoots as described above. We included three naturally growing parent trees (clone ST-72) in the samples. A total of 135 qPCR reactions was performed as described above [15 samples × three technical replications × three genes (*FT1, FT2,* and *UBQ*); NTCs were not included in counting]. The average ratio (*FT1*/*UBQ* or *FT2*/*UBQ*) for each biological replication was calculated and log2 transformed. A general linear model was used to analyze the effects of temperature and day length on fold change. Means were separated as described above.

To identify whether *FT2* transcription is regulated by day length, 12 actively growing trees under long-day conditions (16 h light, ∼100 μmol s^{-1} m^{-2}) and 12 trees under short-day conditions (8 h light, ∼100 μmol s^{-1} m^2) were placed at 25 °C for 42 d in early April. Fully expanded leaves at nodes 9, 10, and 11 (Floral Zone) from the base of shoots were collected from three trees under each environmental regime at day 42 (mid-May). A total of six samples was collected (one sample per tree × three trees × two environmental regimes). Leaves were pooled within a sample, and total RNAs were extracted as described above. A total of 54 qPCR reactions was performed as described above [six samples × three technical replications × three genes (*FT1*, *FT2,* and *18S* rRNA); NTCs were not included in counting]. The average ratio (*FT2*/*18S* rRNA or *FT1*/*18S* rRNA) for each of the three samples per tree was calculated and log2 transformed. A general linear model was used to analyze the effect of day-length treatment on fold change across trees, and pair-wise differences were calculated between the treatments.

To test whether *FT2* transcription is regulated by temperature, six actively growing trees were grown at 4 °C, and six trees were grown for 14 d at 25 °C under long-day conditions (16 h light, ∼100 μmol s^{-1} m^{-2}) in early May. Fully expanded leaves at nodes 9, 10, and 11 (Floral Zone) from the base of shoots were collected from three trees under each environmental regime at day 14 (mid-May). A total of six samples was collected (one sample per tree × three trees × two environmental regimes). Leaves within a sample were pooled, and total RNAs were extracted as described above. A total of 54 qPCR reactions was performed as described above [six samples × three technical replications × three genes (*FT1*, *FT2,* and *18S* rRNA); NTCs were not included in counting]. The average ratio (*FT2*/*18S* rRNA or *FT1*/*18S* rRNA) for each of the samples per tree was calculated and log2 transformed. A general linear model was used to analyze the effect of temperature treatment on fold change across the six trees, and pair-wise differences between the treatments were calculated.

To determine whether the abundance of *FT1* and *FT2* transcripts fluctuates daily, we used the bucket truck to reach the upper crowns of three normally growing, sexually mature, ~25-m-tall male *P. deltoides* trees to sample three independent replications per tree at each collection. For *FT1*, terminal buds containing preformed leaves and embryonic shoots with bud scales removed were sampled in mid-February at six different time points within a 24-h period: 5:30 AM, 7:30 AM, 12:30 PM, 6:30 PM, 9:30 PM, and 12:30 AM. For *FT2*, fully expanded leaves in the Floral Zone were sampled in mid-May at 16 time points within a 48-h period: 6:30 PM, 9:30 PM, 12:30 AM, 3:30 AM, 5:30 AM, 7:30 AM, 9:30 AM, 12:30 PM, 3:30 PM, 6:30 PM, 9:30 PM, 12:30 AM, 3:30 AM, 5:30 AM, 7:30 AM, and 9:30 AM. We were careful not to introduce light during the night samplings. The sampling always was made from the same side of tree crowns (south) to be consistent with fluctuations in light intensity. Light fluence rate (μmol s^{-1} m^{-2}) was measured at each time point using a Li-Cor model LI-189 light meter. A total of 198 samples was collected [54 samples for *FT1* (six time points × three genotypes × three replications per genotype) and 144 samples for *FT2* (16 time points × three genotypes × three replications per genotype)]. Total RNAs were extracted as described above. A total of 1,782 qPCR reactions was performed as described above (198 samples × three technical replications × three genes [*FT1* or *FT2*, *LHY* (forward primer: 5′-CAGCTTCCGAATCTAGCTCTCG-CCAC-3′; reverse primer: 5′-GACCAGAGCAGCACTCCCAC-GTTTTAC-3′), and *18S* rRNA (forward primer: 5′-GGAATT-GACGGAAGGGCACCACCAGGC-3′; reverse primer: 5′-GG-ACATCTAAGGGCATCACAGACCTG-3′)]; NTCs were not included in counting]. *P. deltoides* 18S rRNA was used as an internal standard or reference gene. The average ratio (*FT1*/*FT2* or *LHY*/*18S* rRNA) for each of the three technical replications per sample per tree was calculated and log2 transformed. A general linear model was used to analyze the differences among time points for each genotype. Means were separated as described above.

Genetic and Physiological Experiments to Determine Functions of *FT1* and *FT2* For genetic alterations, we first made constitutively expressing constructs. The coding regions of *FT1* (forward primer: 5′-GTTCTAGAATGTCAAGGGACAGAGATCCTC-3′; reverse primer: 5′-TTGGATCCTTATCGCCTCCTACCACCAGAG-3′) and *FT2* (forward primer: 5′-CCGGATCCATGCCTAGGGA-TAGAGAACC-3′; reverse primer: 5′-TTGGTACCTCATGGT-CTCCTTCCACCGG-3′) cDNAs were amplified using *Pfu* DNA polymerase (Stratagene) and subsequently were cloned into the pBI121 binary vector (BD Biosciences) and the pYL436 vector (12) under control of two types of the cauliflower mosaic virus (CaMV) 35S promoter (Pro_{35S} or $Pro_{35S2\times}$). The 35S constitutive promoter in pBI121 (Pro_{35S}) is the original 835-bp promoter. However, a dual (2×) 35S promoter with a duplication of the enhancer region (−417 to −90) of the CaMV 35S promoter is present in the pYL436 vector ($Pro_{35S2\times}$). *FT1* and *FT2* in pYL436 were C terminally tagged with nine copies of myc repeat, six histidine residues, a human rhinovirus 3C protease cleavage site, and two copies of the Ig-binding domain of protein A from *Staphylococcus aureus*. The resulting constructs were named Pro_{35S}:*FT1*, Pro_{35S}:*FT2*, $Pro_{35S2\times}$:*FT1*-C_{tag}, and $Pro_{35S2\times}$:*FT2*-C_{tag}, respectively. Second, we made inducible constructs driven by a soybean heat-inducible promoter [*GmHsp17.6-L*; Severin and Schoffl ((13); Pro_{HSP}]. These inducible constructs were contained in the Gateway binary vector pK2GW7 (14). The resulting constructs were named "Pro_{HSP}:*FT1*" and "Pro_{HSP}:*FT2*," respectively. For the production of controls, we either made a vector-control construct (pBI101) with no promoter or produced wild-type plants at the same developmental stage as the genetically altered plants. All seven constructs were mobilized independently into the *Agrobacterium tumefaciens* strain C58 (constitutive and the vector-control constructs) and strain AGL1 (inducible constructs), which then were used for poplar transformation following the previous procedures (10, 15). Juvenile (30- to 60-d-old) hybrid poplars, *Populus tremula* × *Populus alba* [Institut National de la Recherche Agronomique (INRA) 717–1B4] and *P. tremula* × *Populus tremuloides* (INRA 353–53), were used for transformation because of their high transformation efficiency. Plant cultures were maintained in incubators at 25 °C with a 16-h photoperiod under cool-white fluorescent lamps. Regeneration of roots from preparation of leaf discs and preculturing took 210–231 d (7–8 mo) under our experimental conditions. Following the in vitro production of shoots and roots, 2-mo-old plants positive for transgene insertion (PCR confirmation) were planted in ~0.25-L pots and grown in a growth room or a greenhouse until they either were used in experiments or were planted in the field. Some plants were transplanted into 4-L pots. If the trees with the Pro_{35S}:*FT1*, Pro_{35S}:*FT2*, $Pro_{35S2\times}$:*FT1*-C_{tag}, or $Pro_{35S2\times}$:*FT2*-C_{tag} construct flowered early, they were planted in 4-L pots along with controls. The nonflowering trees and controls were planted in the field and grown under drip irrigation.

The trees with the Pro_{HSP}:*FT1* or Pro_{HSP}:*FT2* construct and controls were subject to heat treatment in a 196 × 195 × 90 cm growth chamber (Model AR75L; Percival Scientific) at 37 °C. Heat was applied daily for 60–90 min at the same time each day for 10–28 d, and plants were moved back to the greenhouse immediately after each daily treatment. Lighting within the growth chamber was provided by a combination of fluorescent (TL70 E32T8/TL735; Philips) and 60-W soft-white incandescent bulbs with a light intensity of 110 μmol s^{-1} m^{-2}. Then we selected the best poplar lines (based on flowering response and/or transgene expression) for downstream experiments. Line 17 of the poplar 353 clone carrying Pro_{HSP}:*FT1* and line 60 of the poplar 353 clone carrying Pro_{HSP}:*FT2* were clonally propagated along with wild-type 353 to produce 10 plants (ramets) of each that were grown under long-day conditions (16 h; light intensity, 100 μmol s^{-1} m^{-2}) in a growth room at 25 °C until they attained a size of 20 cm. Then 30 plants were moved into a chamber (Model CMP3246; Conviron) for heat treatment at the following settings: heat treatment at 37 °C for 1.5 h/d from 8:00 AM to 9:30 AM for 21 d during the light period (85 μmol s^{-1} m^{-2}) and 22.5 h of normal temperature (25 °C). The chamber was kept under long-day conditions (16 h light). At the end of the treatment period, plants were moved back to the growth room for phenotypic observations for 90 d. With the same experimental design, a separate set of plants was subjected to heat treatment at 40 °C. We observed or measured the following response variables on each tree: number of days to inflorescence formation, number of reproductive and vegetative buds, locations of inflorescences and vegetative buds, morphology of inflorescences/flowers, shoot length, and height. Leaves (at nodes 5, 6, and 7 from the shoot tip) from three controls and three trees harboring Pro_{HSP}:*FT1* or Pro_{HSP}:*FT2* were sampled at day 21 immediately after the last heat treatment for the analysis of *FT1* and *FT2* transcript abundance using qPCR as described above. We included leaves from three trees carrying the Pro_{35S}:*FT1*, Pro_{35S}:*FT2*, $Pro_{35S2\times}$:*FT1*-C_{tag}, or $Pro_{35S2\times}$:*FT2*-C_{tag} construct along with controls. We used the poplar *18S* rRNA as an internal standard. A total of 51 samples was collected:

For *FT1*: three samples were collected from controls (717) corresponding to Pro_{35S}:*FT1*; three samples were collected from Pro_{35S}:*FT1* (717) with a flowering phenotype; three samples were collected from controls (353) at 37 °C; three samples were collected from Pro_{HSP}:*FT1* at 37 °C; three samples were collected from controls (353) at 40 °C; three samples were collected from Pro_{HSP}:*FT1* at 40 °C; three samples were collected from a normally growing sexually mature *P. deltoides* tree in mid-February when *FT1* normally expresses; three samples were collected from controls (717) corresponding to Pro_{35S2X}:*FT1*-C_{tag} (717); and samples were collected from three independent lines of Pro_{35S2X}:*FT1*-C_{tag} (717).

For *FT2*: three samples were collected from controls (717) corresponding to Pro_{35S}:*FT2*; three samples were collected from Pro_{35S}:*FT2* (717) with a flowering phenotype; samples were collected from controls (353) at 37 °C as in *FT1*; three samples were collected from Pro_{HSP}:*FT2* at 37 °C; samples were collected from controls (353) at 40 °C as in *FT1*; three samples were collected from Pro_{HSP}:*FT2* at 40 °C; three samples were collected from a normally growing sexually mature *P. deltoides* tree in mid-May when *FT2* normally expresses; three samples were collected from Pro_{35S}:*FT2* (717) with a nonflowering phenotype; three samples were collected from controls (717) corresponding to Pro_{35S2X}:*FT2*-C_{tag} (717); and samples were collected from three independent lines of Pro_{35S2X}:*FT2*-C_{tag} (717).

A total of 324 qPCR reactions with three technical replications of each sample was performed as described above [(27 samples × three technical replications × two genes, *FT1* and *18S* rRNA or *UBQ*) + (30 samples × three technical replications × two genes, *FT2* and *18S* rRNA or *UBQ*) − (six samples × three technical replications × one gene, *18S* rRNA or *UBQ*); NTCs were not included in counting]. The average ratio [*FT1*/*18S* rRNA (or *UBQ*) or *FT2*/*18S* rRNA (or *UBQ*)] for three technical replications per biological replication was calculated and log2 transformed. A general linear model was used to analyze the effect of treatment on the expression of *FT1* and *FT2*. Means were separated as described above.

To determine whether *FT2* signal is needed for the normal onset of reproduction in *P. deltoides*, individual shoots and large branch units were maintained under short-day conditions from March 25 to May 31 in 2009. March 25 was marked as the beginning of terminal bud break. We selected two normally growing, sexually mature *P. deltoides* trees (~25 m tall) and built a wooden tower with a platform on top beside each tree to access shoot and branches in the crown. We used a small (17 cm × 16 cm × 12 cm × 39 cm) double-layer pollination bag (Seedburo Equipment Co.) to cover the individual shoots and home-made large (1.0 m × 2.0 m) double-layer (black inside, white outside) bags made of polypropylene shade fabric with a single drawstring on one end to cover four large branch units. Twist ties were used to close the open ends of the small bags. We selected 42 individual shoots on one tree and 25 individual shoots on the other tree for small-bag treatment. Four large branch units with a total of 92 shoots were selected for large-bag treatment. A total of 234 control shoots was labeled and grown normally on both trees. The bags were placed manually over the individual shoots and branch units between 5:30 PM and 6:00 PM and were removed between 9:30 AM and 10:00 AM every day for 68 d. This treatment shortened the day length to ~8 h when day length normally was~12 h on March 25 and ~14 h on May 31 in Starkville, MS. The light fluence rate at midday under full sun was $<10\ \mu mol\ s^{-1}\ m^{-2}$ within a bag, whereas it was $>1{,}600\ \mu mol\ s^{-1}\ m^{-2}$ outside. To examine the effectiveness of our treatment, in mid-May we sampled recently expanded leaves (9, 10, or 11 nodes from the base of a shoot) from three treated and three control shoots with three replications within a shoot on each tree for analysis of *FT2* transcripts using qPCR as described above. The *P. deltoides* *UBQ* transcript was used as an internal standard. Twelve samples were collected (three shoots per tree × two treatments × two trees). A total of 72 qPCR reactions was performed [12 samples × three technical replications × two genes (*FT2* and *UBQ*); NTCs were not included in counting]. The average ratio (*FT2*/*UBQ*) for three technical replications per biological sample was calculated and log2 transformed. A general linear model was used to analyze the difference in *FT2* expression between short-day and long-day treatment effects for each shoot. Floral buds and vegetative buds were counted on shoots treated under short-day and long-day conditions from July to September. Shoot length and leaf length (lamina plus petiole) were measured. Timing of cessation of primary shoot growth was determined based on the formation of bud scales that enclose the terminal growing point to form a bud. A *t* test was used to test the differences between short-day and long-day effects on each of the four variables across biological replications.

To understand whether the *FT2* signal is needed for the onset of reproduction, transcript abundance of *FT1* was increased (Pro_{HSP}:*FT1*), and that of *FT2* was reduced via RNA interference (*FT2*-RNAi) in the same trees (Pro_{HSP}:*FT1*/*FT2*-RNAi). To make the RNAi constructs, we used an *Arabidopsis 7SL* RNA gene promoter-based expression vector (16). We synthesized an oligonucleotide set that targets a unique region of *FT2* transcripts in *P. tremula* × *P. tremuloides* INRA 353–53 (forward primer: 5′-CCTCTAGATACGATGGTGGAAAGACGGAGGTTGA-CCAGACCTCCGTCTTTCCACCATCTTTTTTT-3′; reverse primer: 5′-CGGGATCCAAAAAAAGATGGTGGAAAGAC-GGAGGTCTGGTCAACCTCCGTCTTTCCACCATCGTA-3′). Each set contained 19-nt sense and antisense target sequences separated by a 9-nt intron (or spacer). The Pro_{HSP}:*FT1* construct had a Kan^r selection marker (NPTII), and the *FT2*-RNAi construct had a Hyg^r selection marker (HPT). We transformed Pro_{HSP}:*FT1* and *FT2*-RNAi into the same juvenile 353 trees and selected 11 independent lines. Trees, including the wild-type controls, were grown to ~20 cm (~3 mo old). Eleven lines and 10 control trees then were subjected to heat treatment 1.5 h/d at 37 °C for 21 d. Before the heat treatment, one replication of leaves 4, 5, and 6 per line (all 11 lines) and of three controls from the shoot apex was sampled to measure *FT2* abundance using qPCR. The percentage of remaining *FT2* transcript abundance was calculated for each line in reference to wild-type control. Immediately after the heat treatment on day 21, one replication of leaves 4, 5, and 6 from the shoot apex of two lines and three controls was sampled to measure the abundance of *FT1* using qPCR. Number and types of inflorescences were counted after the treatment.

To examine further whether *FT2* signal is needed for the onset of reproduction, transcript expression of *FT1* was induced by heat treatment at 40 °C (Pro_{HSP}:*FT1*) under short-day conditions (8 h light; 85 $\mu mol\ s^{-1}\ m^{-2}$ light intensity) in the Model CMP3246 growth chamber (Conviron) as previously described. *FT2* is not normally expressed under short-day conditions. Line 17 (*P. tremula* × *P. tremuloides* 353) carrying Pro_{HSP}:*FT1* and wild type were propagated to produce nine trees of each group at the same developmental stage. Trees were grown as previously described to 15–20 cm under long-day conditions and were subject to a heat treatment with the following settings: 1.5 h/d heat treatment from 8:00 AM to 9:30 AM at 40 °C for 21 d during the light period and 22.5 h of normal temperature (25 °C). At the beginning and end of the treatment period, trees were measured for height growth, and leaves were counted. Trees then were moved to the growth room under long-day conditions (16 h light) to observe inflorescence formation, number of reproductive and vegetative buds, locations of inflorescences and vegetative buds, and morphology of inflorescences/flowers for 90 d. The difference in shoot growth and number of leaves was calculated from the beginning to the end of heat treatment.

Analysis of *FT1* and *FT2* Molecular Networks. To identify the genetic networks of *FT1* and *FT2*, we conducted microarray experiments. We used leaves because *FT1* and *FT2* are expressed abundantly in leaves (Fig. 1), and early signaling events for the onset of reproduction appear to begin in leaves (17–22). First, leaf samples were collected four times from one line harboring the constitutive construct Pro_{35S}:*FT1* and from four control trees harboring the empty vector (pBI101 with no promoter) in clone 717. Leaf samples also were collected from three trees propagated from one line harboring Pro_{35S}:*FT2* and three control trees (clone 717) harboring pBI101. All the leaves from these trees were sampled, because these trees were small and did not have

many leaves. Pooling of leaves within a tree was conducted before RNA extraction. Consequently, we had a total of 14 samples (eight for *FT1* and six for *FT2*) and used 14 microarrays. Second, a heat-inducible promoter was used to regulate each gene, because the constitutive overexpression (35S CaMV) may cause pleiotropic effects. The use of an inducible promoter would facilitate identification of the genes downstream of each gene. Leaf samples were collected from four trees of line 17 containing the inducible Pro_{HSP}:*FT1* and from four trees of line 60 containing the inducible Pro_{HSP}:*FT2* construct in clone 353 at day 21 immediately after the heat treatment (40 °C). Leaf samples from four trees of wild-type 353 were sampled under the same conditions and at the same developmental stage. Leaves 5, 6, and 7 from the shoot tip of genetically altered and control trees were sampled. Leaves within a tree were pooled before RNA extraction. Thus, we had a total of 12 samples (four for *FT1*, four for *FT2*, and four common controls) and used 12 microarrays. Finally, using the same bucket truck to reach the upper crown of a 25-m-tall, 30-y-old, normally growing, sexually mature male *P. deltoides* tree located in Starkville, MS, we collected three independent leaf samples on September 5, December 5, February 5, March 5, March 6, April 6, May 6, and June 6, spanning all four seasons. Thus, a total of 24 samples was collected (eight collections × three replications per tree). One genotype was used because of the significant variation among poplar genotypes in gene expression. Leaves were primordial during the first 4 mo and were expanding or fully expanded in the remaining months. Preformed leaves were separated from embryonic shoots and bud scales. Expanding and fully expanded leaves were sampled at nodes 9, 10, and 11 (Floral Zone) from the base of a shoot and pooled. Array experiments were conducted in two 12-chip sets. The first set included September 5, December 5, February 5, and March 5; the second set contained March 6, April 6, May 6, and June 6. This arrangement followed normal leaf development, because leaves were at their early developmental stage in September. The samples collected in March provided an overlap between the two sets.

Total RNAs were isolated as described above. A total of 3 μg of total RNA from each sample was used for the synthesis of double-strand cDNA using the One-cycle cDNA synthesis kit (Affymetrix) according to the manufacturer's protocol. After double-strand cDNA was cleaned up using the sample cleanup module (Affymetrix), biotin-labeled cRNA was synthesized by in vitro transcription from the double-strand cDNA using the Genechip IVT labeling kit (Affymetrix) according to the manufacturer's procedure. Then, 20 μg of the cRNA was fragmented at 94 °C for 35 min, 15 μg of which was hybridized with the GeneChip Poplar Genome Array (Affymetrix) in GeneChip Hybridization Oven 640 (Affymetrix) at 45 °C for 16 h. The arrays then were washed using GeneChip Fluidics Station 450 (Affymetrix) according to the manufacturer's procedure and were scanned using GeneChip Scanner 3000 (Affymetrix). One of the microarrays in the second experiment (Pro_{HSP}:*FT1*) was defective (because we did not have good focusing and imaging during scanning) and was removed.

For the first and second microarray experiments, all 25 microarrays [14 + (12−1)] were analyzed together using R (23) and Bioconductor (24) packages as indicated. After preprocessing the arrays with the GC robust multi-array average (GCRMA) algorithm (25), we evaluated the suitability of analyzing all arrays together and array quality by performing a principal component analysis (PCA)-based clustering from the affycoretools package (26). The experiments were separated into different clusters, and the replicates of each treatment group clustered together, indicating that the amount of variation was low and that no arrays were outliers. We were able to analyze all experiments together although there were large differences between experiments because direct comparisons were made only between treatment groups within an experiment, not between two treatment groups from two different experiments. Differential expression was assessed by fitting a cell-means model to the seven treatment groups using the limma package (27). Pairwise differences between overexpression of the *FT1* or *FT2* gene versus the appropriate control were pulled as contrasts from the model. After fitting the model, control probe sets and probe sets that were considered "not detectable" on any of the 25 arrays were filtered out. "Not detectable" was defined as either being called "absent" (Affymetrix's Call Detection Algorithm, *GeneChip Expression Analysis Data Analysis Fundamentals Manual*, Affymetrix; www.affymetrix.com) on all 25 arrays or not having at least one array with a GCRMA value above 3.5. Of the 61,413 probe sets on the poplar array, 35,150 survived the filtering and had their *P* values corrected for multiple hypothesis testing using the false discovery rate (FDR) method (28). For any pairwise comparison, probe sets were considered significantly different if they had FDR *P* values <0.05 and at least a twofold change, up or down.

For the third microarray experiment, quality control assessment, data processing, and statistical analysis of array data were conducted for all 24 microarrays in R using packages from the Bioconductor project. The raw probe-level data were transformed to one value per probe set ("Affymetrix ID"; "affyID") using the GCRMA algorithm (25), and a one-way ANOVA for time was performed using the limma package (27), which employs an empirical Bayes correction (29) that helps improve power by borrowing information across all 61,413 affyIDs. The ANOVA model also was adjusted for the average correlation (0.643) because of experimental block (27); PCA-based clustering from the affycoretools package (26) on GCRMA values after the block effect was removed showed no remaining block effect (all March samples cluster together) and no outlier arrays, indicating high-quality microarray data.

Annotation of the probe sets to Gene Ontology (GO) biological process terms was conducted using Bioconductor's GO.db package (version 2.3.5) via a multistep process. We first defined levels of GO terms using GO's hierarchical structure. Level 1 terms were defined as all the direct child terms of the root biological process term, GO:0008150. Level 2 terms were defined as all the direct child terms from any of the level 1 terms. We continued this process down to level 4. The complex, direct acyclic structure of GO terms is such that level 1 terms are not mutually exclusive from level 2 terms, and so on, but in general, the farther the level is from the root term, the more specific is the term. We then used a custom annotation source of the Affymetrix poplar (K.-H. Han and J.-H. Ko, Michigan State University, East Lansing, MI, January 9, 2009) that mapped the probe set sequences to the closest AGI from *Arabidopsis*. We used Bioconductor's At.tair.db package (version 2.3.5) to pull out the associated GO biological process terms for each Arabidopsis Genome Initiative's (AGI) gene ID. Once a gene has been associated with a specific GO term, by definition it also is associated with all the ancestor terms of the specific terms, even if they are not listed in the database. Therefore, we also pulled all the ancestor biological process terms for the listed biological process terms to annotate each probe set at our different GO "levels." The functional categorization of each probe set (pie charts) was conducted manually by clustering similar specific GO Biological Process terms. Microarray data were submitted to the National Center for Biotechnology Information Gene Expression Omnibus (GEO), ID nos.GSE24349 and GSE24609.

Growth and Stress Experiments to Understand *FT2* Function. To determine the effectiveness of *FT1* and *FT2* on shoot (vegetative) growth, we selected line 17 of Pro_{HSP}:*FT1* and line 60 of Pro_{HSP}:*FT2* in clone 353 with a flowering phenotype at 40 °C (see above). Each line, along with a wild-type control, was propagated to produce 20 plants; plants were transplanted into soil in a 0.25-

L pot, and grown under long-day conditions. When the shoots were about 7 cm in length, plants were moved into short-day conditions (8 h, light intensity 70 μmol s^{-1} m^{-2}) at 30 °C for 105 d. They were fertilized once at the beginning of the experiment and were watered daily. To examine the effectiveness of our treatment, we sampled recently expanded leaves at nodes 5, 6, and 7 from the shoot tip of three plants from each group at day 45 from the beginning of the experiment. We selected day 45 for sampling, because control trees had just ceased shoot growth. A total of nine samples was collected (three trees × three treatment groups). A total of 63 qPCR reactions [(nine samples × three technical replications × three genes, *FT1*, *FT2*, and *18S* rRNA) – (six samples × three technical replications × one gene, *FT1* or *FT2*); NTCs were not included in counting] was performed as described above to determine the abundance of *FT1* and *FT2* transcripts. The poplar *18S* rRNA was used as an internal standard. The average ratio (*FT1*/*18S* rRNA or *FT2*/*18S* rRNA) for each of the 12 samples was calculated and log2 transformed. A general linear model was used to analyze the differences in *FT1* and *FT2* transcript abundance among the three lines. Means were separated as described above. After shoot length and stem diameter were measured and the number of leaves on each shoot were counted at the end of the treatment period, trees were moved to short-day conditions at 23 °C for further observation. Internode length was calculated by dividing shoot length by number of leaves. The timing of cessation of primary shoot growth was determined based on the formation of bud scales that enclose the terminal growing point. A general linear model was used to test the differences among the three genotypes for each variable. Means were separated as described above.

To examine the effect of reduced *FT2* expression on shoot (vegetative) growth, we selected lines 1–4 (n = 4) and 2–2 (n = 5) of Pro_{HSP}:*FT1*/*FT2*-RNAi trees (*P. tremula* × *P. tremuloides* 353) (Fig. S6*A*). We included wild-type (n = 5) and Pro_{HSP}:*FT1* (*P. tremula* × *P. tremuloides* 353) (n = 13) trees as controls. Twenty-seven plants were grown to a height of 10–15 cm in 0.25-L pots at 23 °C under long-day conditions. Then trees were transferred to the growth chamber (Model CMP3246; Conviron) and treated at 30 °C under long-day conditions (16 h, light intensity 85 μmol s^{-1} m^{-2}) for 50 d. Shoot length and the number of leaves were recorded at the beginning and end of treatment. The increase in shoot growth and the number of leaves during the treatment was calculated. A general linear model was used to test the differences among the two lines of Pro_{HSP}:*FT1*/*FT2*-RNAi and controls for each variable. Means were separated as described above.

To test whether short-day conditions would regulate *FT2* and its downstream genes [*ETHYLENE RESPONSE FACTOR-APETALA2* (*ERF-AP2*; POPTR_0018s00700.1; forward primer: 5′-GCCATGACAGATCAGGTATTGTCTC-3′ and reverse primer: 5′-GCATTCTTCCATATTCTCTCCACCAC-3′), *JASMONATE-ZIM-DOMAIN PROTEIN 5* (*JAZ5*; POPTR_0003s06670.1; forward primer: 5′-CAATCTCAAGCTAATGCTTCAGATGTGC-3′ and reverse primer: 5′-GCTGTCTTCTTCAGATCTGGGAGGC-3′), *GLUTAREDOXIN 480 (GRX480)* (POPTR_0007s01400.1; forward primer: 5′-CTTTTCAACTTATTGCCCGCCACAAC-3′ and reverse primer: 5′-CTTACTCCCCTTTCCACCATCATTGCAG-3′), *JASMONATE-ZIM-DOMAIN PROTEIN 1* (*JAZ1*; POPTR_0006s14160.1; forward primer: 5′-GCTCAAATGCTCTTCCTAATTTTGGC-3′ and reverse primer: 5′-GAGCAGCCAAGCCGAGCCACGAGAAC-3′), *ZF14* (POPTR_0002s10770.1; forward primer: 5′-GATGATGATCAGGAAGAACAGTTACATG-3′ and reverse primer: 5′-CAGTGATGTTGGCGAAACCTATAGAG-3′), and *MAPK3* (POPTR_0009s07050.1; forward primer: 5′-GAGCTTTTCCCACTTGTTCACCCTC-3′ and reverse primer: 5′-CTAGCATGCATATTCTGGATTAAGTG-3′)], we reanalyzed samples from field-grown *P. deltoides* (for details, see *SI Materials and Methods*, "Genetic and Physiological Experiments to Determine Functions of *FT1* and *FT2*"). Six samples were reanalyzed (three shoots per tree × two treatments × one tree). A total of 162 qPCR reactions with three technical replications of each sample was performed [six samples × three technical replications × nine genes (six downstream genes, *FT1*, *FT2*, and *UBQ*); NTCs were not included in counting]. The average ratio (gene/*UBQ*) for each of the samples per tree was calculated and log2 transformed. A general linear model was used to analyze the effect of day length on fold change across the six trees, and pair-wise differences between the treatments were calculated.

To understand whether low light intensity would regulate *FT2* and the downstream genes listed above, 18 rooted cuttings were produced from three genotypes of *P. deltoides* (six ramets per genotype) and were planted in the field in 2009. Shade screens (three layers of black mesh) were placed over three trees of each genotype for 19 d in May 2010 to decrease the normal light intensity to 500 μmol s^{-1} m^{-2} at midday. Screens were placed 50–60 cm above the shoot tip with four sides open. Controls included three trees of each genotype that were allowed to grow under normal conditions with a light intensity of 1,700 μmol s^{-1} m^{-2} at midday. We selected May, because *FT2* is expressed abundantly during that month. Shoot growth and the number of leaves on all trees were recorded at the beginning and end of the treatment. Fully expanded leaves on each tree at nodes 9, 10, and 11 from the base of the main shoot were collected 2 h after sunrise on day 19. Leaves then were pooled within each tree. Six samples from the same genotype (three from each treatment group) were used to conduct qPCR analyses. A total of 162 qPCR reactions with three technical replications of each sample was performed [six samples × three technical replications × nine genes (six downstream genes, *FT1*, *FT2*, and *UBQ*); NTCs were not included in counting]. The average ratio (gene/*UBQ*) for each of the samples per tree was calculated and log2 transformed. A general linear model was used to analyze the effect of shade treatment on fold change across the six trees, and pair-wise differences between the treatments were calculated.

To determine whether heat stress regulates *FT2* and the downstream genes listed above, 12 actively growing potted rooted cuttings of a *P. deltoides* clone (*SI Materials and Methods*, "Regulation of *FT1* and *FT2* Transcription") were used for this experiment in May, when *FT2* transcripts normally are abundant. Six trees were treated at 25 °C, and the other six trees were treated at 38 °C under long-day conditions (16 h light) with a light intensity of 150 μmol s^{-1} m^{-2} for 14 d. Fully expanded leaves at nodes 9, 10, and 11 from the base of shoot were collected from each of three trees 2 h after the beginning of the light period on day 14. Thus, six samples were collected (one sample per tree × three trees × two environmental regimes). Leaves within a tree were pooled, and total RNAs were extracted. A total of 162 qPCR reactions with three technical replications of each sample was performed [six samples × three technical replications × nine genes (six downstream genes, *FT1*, *FT2*, and *18S* rRNA); NTCs were not included in counting]. The average ratio (gene/*18S* rRNA) for each of the samples per tree was calculated and log2 transformed. A general linear model was used to analyze the effect of temperature treatment on fold change across the six trees, and pair-wise differences between the treatments were calculated.

To identify whether water stress regulates *FT2* and the downstream genes listed above, 27 potted rooted cuttings of *P. deltoides* actively growing under ambient conditions (see details in *SI Materials and Methods*, "Regulation of *FT1* and *FT2* Transcription") were used for this experiment in May, when *FT2* transcripts normally are abundant. Twelve trees were watered with an automated irrigation system to soil saturation for 30 min two times per day, and 15 trees were water-stressed by withholding water for 19 d. Predawn (4:00–5:00 AM) leaf water potential was measured using a pressure chamber (Soilmoisture Equipment) on one leaf of each of four trees in each treatment group on days 14

and 19. Shoot growth and the number of leaves on all trees were recorded at the beginning and end of the treatment. Fully expanded leaves at nodes 9, 10, and 11 from the base of shoot were collected 2 h after sunrise from each of three trees for each treatment at days 14 and 19. Leaves within a tree were pooled. Six samples were collected at day 14 (three from controls and three from low water-stressed trees). Nine samples were collected at day 19 (three from controls, three from medium water-stressed trees, and three from severely water-stressed trees). A total of 405 qPCR reactions with three technical replications of each sample was performed [15 samples × three technical replications × nine genes (six downstream genes, *FT1*, *FT2*, and *18S* rRNA); NTCs were not included in counting]. The average ratio (gene/*18S* rRNA) for each of the samples per tree was calculated and log2 transformed. A general linear model was used to analyze the effect of water stress on fold change, and differences among treatments were calculated as described above.

Analysis of Expression Patterns of Paralogous Genes. To identify whether the pairs of paralogous genes on chromosomes VIII and X have diverged in expression pattern, we conducted Weighted Gene Coexpression Network Analysis (WGCNA) (30) using the third microarray data set (year-round leaf microarray). Paralogous gene pairs were identified as previously described (31). Briefly, distances corresponding to salicoid whole-genome duplication events were delineated based on discrete peaks in 4DTV distributions. 4DTV is a measure of the rate of transversions at fourfold degenerate coding sites in the gene. A maximum of 10 nonaligning genes between aligning genes within segment pairs was allowed, and at least five aligning genes per segment pair were required. The segments with 4DTV distances between 0.02 and 0.18 correspond to salicoid duplication and include 13,506 pairs of V1.1-annotated genes (including tandem repeats) or 7,701 pairs of paralogous genes of similar age arrayed in syntenic blocks across large regions of the genome. From this large set, we identified and extracted pairs of 1,565 paralogs located on chromosomes VIII and X.

To ensure that the network analysis was performed on genes with actual signal, we filtered the 61,413 affyIDs in the following manner. We removed affyIDs if they (*i*) corresponded to control sequences, (*ii*) were not called "present" on at least one array or "marginal" on two arrays of the 24 based on Affymetrix's Call Detection Algorithm (*GeneChip Expression Analysis Data Analysis Fundamentals Manual,* Affymetrix, www.affymetrix.com), or (*iii*) did not have at least one array with a GCRMA value >3.5. To remove the redundancy of multiple affyIDs mapping to the same gene, we further filtered the affyIDs using the mapping to Joint Genome Institute gene names provided in the custom annotation source array (K.-H. Han and J.-H. Ko, Michigan State University, East Lansing, MI, January 9, 2009). When more than one affyID mapped to the same gene, we selected the one with the lowest raw *P* value from the overall ANOVA *F*-test, which corresponded to the affyID showing the strongest year-round effect. The 26,031 affyIDs that passed all these filtering criteria represent unique genes with detectable year-round expression. The network then was constructed by first calculating Pearson correlation coefficients on all pair-wise comparisons of the unlogged GCRMA values for the 26,031 affyIDs. Next, the Pearson correlation matrix was transformed into a weighted network adjacency matrix using power $\beta = 6$, as calculated from the data using a scale-free topology criterion (32). This matrix of network connection strengths was used to calculate the topological overlap dissimilarity measure (1 − topological overlap), and the resulting values were subject to average linkage hierarchical clustering to produce a dendrogram. Modules of coexpressed genes were detected using the "Dynamic Hybrid" algorithm from the Dynamic TreeCut package (33) with deepSplit = 2 and minModuleSize = 30. More details on the methods of WGCNA network construction and module identification are given by Langfelder and Horvath (30), Zhang and Horvath (32), and Oldham et al. (34).

To assign paralogs to affyIDs, all matches between the 1,564 paralogous genes and affyIDs were obtained from two annotation sources: the PopARRAY database (http://aspendb.uga.edu/poparray; July 14, 2009) and a custom annotation of the Affymetrix poplar array kindly provided by K.-H. Han and J.-H. Ko (Michigan State University, East Lansing, MI, (January 9, 2009). Then the entire set of affyIDs was reversed mapped using the PopARRAY database to identify those that mapped uniquely to only one gene and those that mapped to multiple genes. Preference was given to affyIDs that mapped uniquely to only one gene and to mapping found in both annotation sources. When more than one affyID met the criteria for a gene, the one with the lowest raw *P* value from the overall ANOVA *F*-test was used. Module assignments for each paralogous gene were based on the module number of the "best" affyID for those genes; not all affyIDs survived filtering and received module assignments, and therefore not all sets of paralogs had module assignments for both genes. We followed a similar approach to analyze all 7,701 pairs of paralogs in the *P. trichocarpa* genome.

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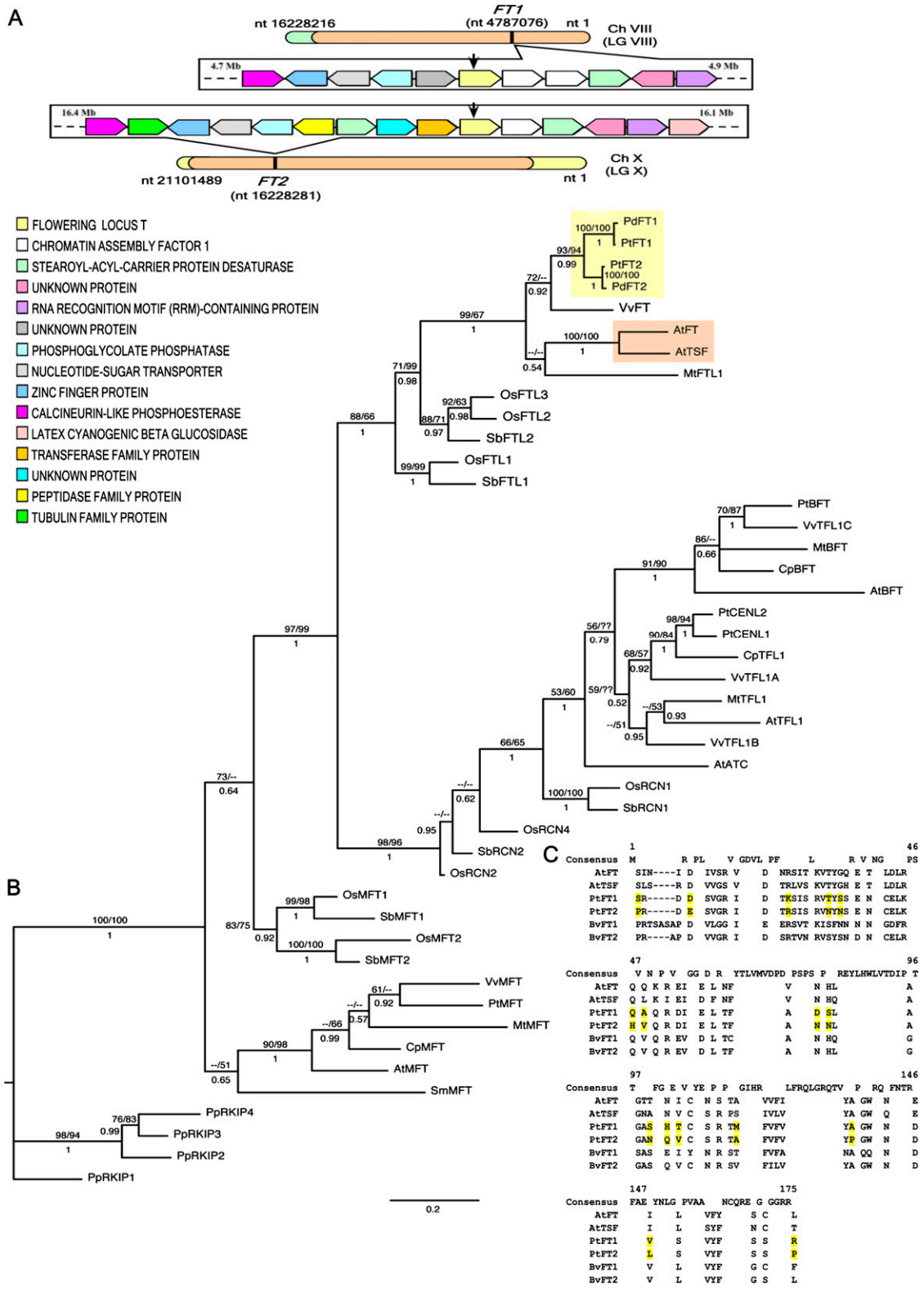

Fig. S1. Chromosomal locations of *FT1* and *FT2*, phylogeny of *FT*-like and *TERMINAL FLOWER 1*-like sequences and amino acid sequence of FT proteins. (*A*) In the poplar genome (*P. trichocarpa*), *FT1* and *FT2* are located on complete paralogous chromosomes VIII and X, respectively. Boxes of the same color indicate the paralogous genes. (*B*) Topology and branch lengths are derived from the consensus values of 4,900 sampled trees and parameters from a Bayesian analysis. Values below the branch indicate the Bayesian posterior probability for that node. Values above the branch are from maximum parsimony and maximum likelihood bootstrap analyses and are given as maximum parsimony/maximum likelihood. Values given as "–" indicate that there was no support >50% for that node in that analysis. At, *A. thaliana*; Cp, *C. papaya*; Mt, *Medicago truncatula*; Os. *O. sativa*; Pd, *Populus deltoides*; Pp, *P. patens*; Pt, *P. trichocarpa*; Sb, *S. bicolor*; Sm, *S, mollendorffi*; and Vv, *V. vinifera*. (*C*) Amino acid sequence of FT proteins in *Arabidopsis* (At), poplar (Pt), and sugar beet (Bv). Space under the consensus sequence indicates the identical sequence. Yellow highlights show differences between poplar *FT1* and *FT2*.

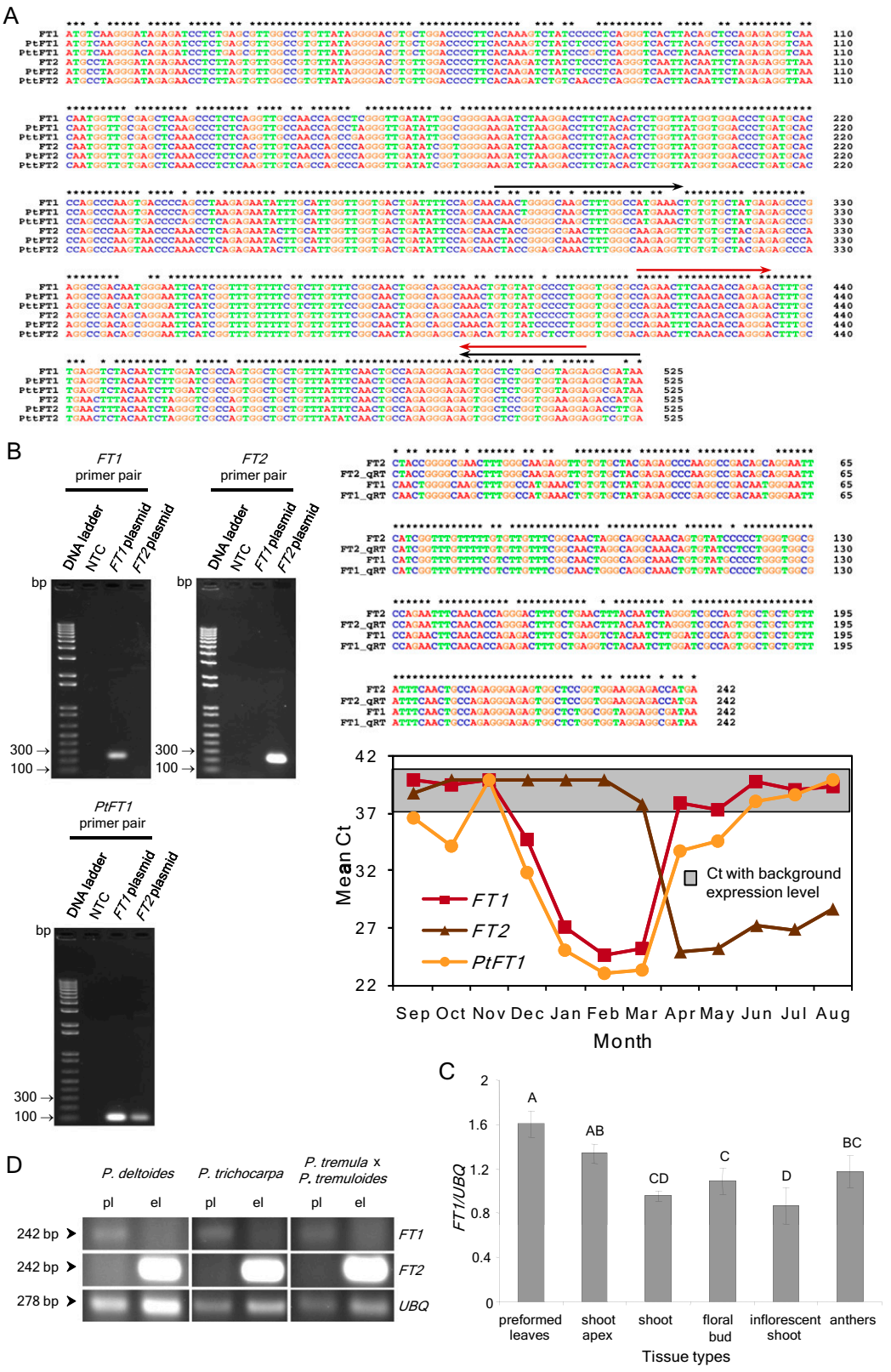

Fig. S2. Development of *FT1*- and *FT2*-specific primers and transcript analysis in poplar. (*A*) Transcript-specific primer pairs were designed by aligning nucleotide sequences from *P. deltoides*, *P. trichocarpa* (Pt), and *P. tremula* x *P. tremuloides* (Ptt). Black arrows show the locations of forward and reverse primers of *FT1* (forward, 28 nt, T_m 69 °C; reverse, 26 nt, T_m 64 °C) and *FT2* (forward, 28 nt, T_m 70 °C; reverse, 26 nt, T_m 68 °C). The forward primers for both genes were extended from exon 3 to exon 4; thus any genomic DNA with Intron 3 cannot be detected, whereas the reverse primers for both genes were located at the end of exon 4. Red arrows show the *PtFT1* primers used in Böhlenius et al. (7): forward, 20 nt, T_m 44 °C; reverse, 19 nt, T_m 49 °C. These primers are located in exon 4 and do not span an intron. Each *PtFT1* primer differs only by one nucleotide in the *FT1* and *FT2* sequences in *P. tremula* x *P. tremuloides* (Ptt), the species used by Böhlenius et al. (7). (*B*) PCR analyses using recombinant plasmids harboring *FT1* or *FT2* show that the *FT1* and *FT2* primer pairs are transcript specific, but

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PtFT1 primers are not, because they detect both *FT1* and *FT2* transcripts. The *FT1* and *FT2* amplicons from RT-PCR were cloned and sequenced. The alignment of sequences shows 100% match with their corresponding cDNA sequences. When we conducted year-round transcript analysis of *FT1* and *FT2* using leaf tissues from normally growing, sexually mature *P. deltoides* and qPCR (line graph), the *FT1* primer pair detected *FT1* transcripts only in winter, and the *FT2* primer pair detected *FT2* transcripts only during the growing season. In contrast, the *PtFT1* primer pair (7) not only detected *FT1* transcripts in winter but also detected *FT2* transcripts in the growing season, showing again that *PtFT1* primers were not *FT1* specific. (*C*) *FT1* transcripts expressed in all the tissues collected in February (winter) from a normally growing, sexually mature *P. deltoides* tree (n = 3 independent replications from a tree). qPCR was used to analyze transcript levels. Poplar *UBQ* was used as an internal control to normalize the expression data. Error bars show SD about the mean. Different letters designating tissue types indicate statistically significant differences (P = 0.0003). (*D*) *FT2* transcripts were more abundant than *FT1* transcripts when analyzed in fully expanded leaves (el) in May (*FT2*) and in preformed leaves (pl) in February (*FT1*) of two poplar species (*P. deltoides* and *P. trichocarpa*) and one hybrid poplar (*P. tremula* x *P. tremuloides*). Amplicon size is shown on the left. Poplar *UBQ* was used as an internal control.

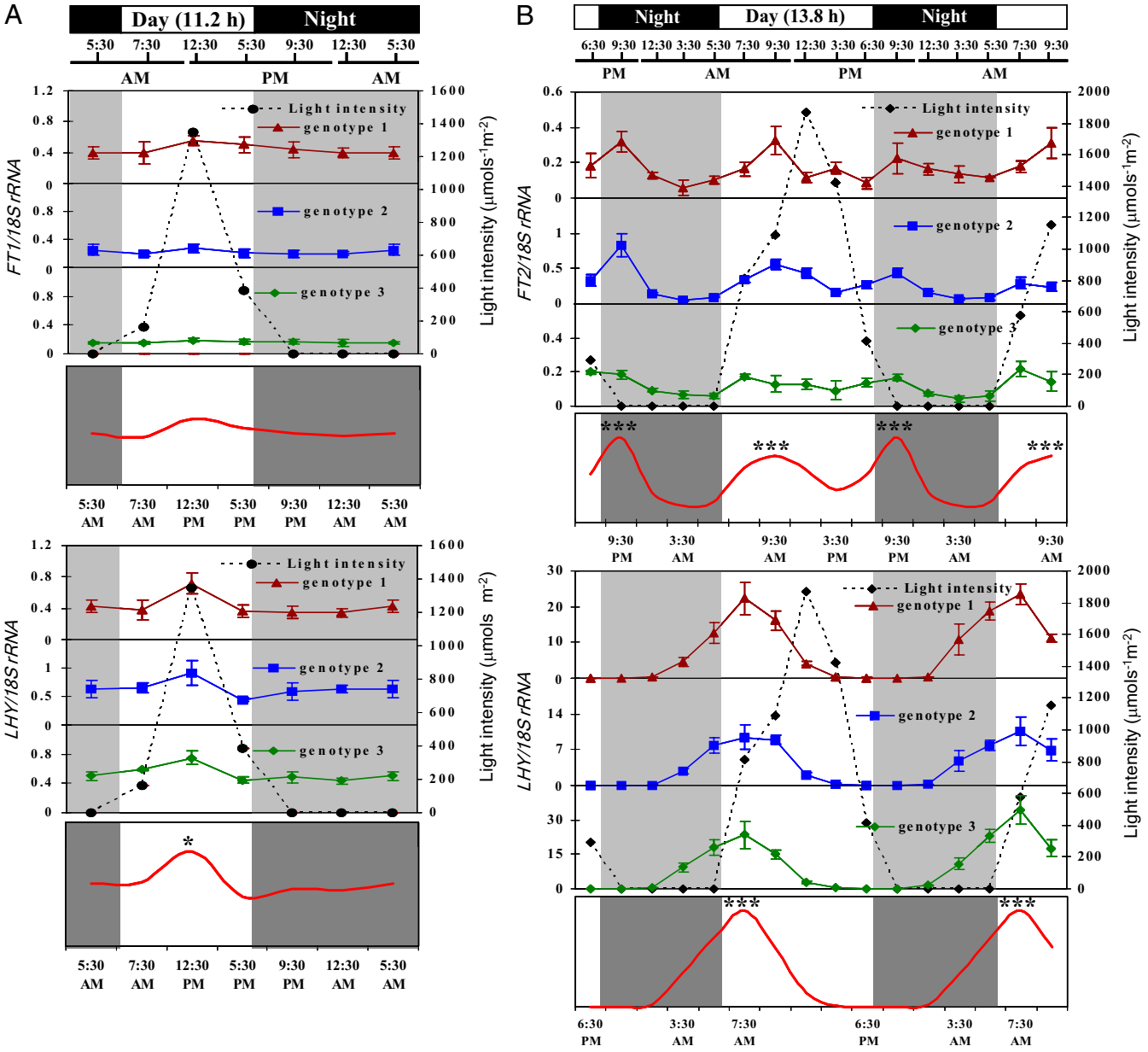

Fig. S3. Daily fluctuation of *FT1* and *FT2* abundance in normally growing mature *P. deltoides* at short intervals within a day in the field. *FT1* transcripts were analyzed in preformed leaves in February based on data shown in Fig. 1*B*, whereas *FT2* transcripts were analyzed in expanding leaves in May based on data shown in Fig. 1*C*. (*A*) There was no significant ($P > 0.2$) difference in the abundance of *FT1* transcripts within three genotypes when analyzed at six time points for 24 h. In contrast, there was a significant (*$P \leq 0.05$) difference in transcript abundance of positive control *LATE ELONGATED HYPOCOTYL* (*LHY*) at 12:30 PM in the same samples. In *Arabidopsis* and poplar, *LHY* shows a circadian expression pattern with a peak in the morning under long-day conditions (1, 2). (*B*) *FT2* expression showed a semidian rhythm with a periodicity of about 12 h. There were significant differences (***$P \leq 0.0001$) among the time points within three genotypes when analyzed for 16 time points over 48 h. For example, *FT2* transcripts were significantly ($P \leq 0.0001$) more abundant at 9:30 PM or 9:30 AM than at 3:30 PM and 3:30 AM, and *LHY* transcripts were significantly ($P \leq 0.0001$) abundant at 7:30 AM. Error bars show SD. Dotted lines indicate light intensity. The red curve shown at the bottom of each figure represents schematic drawing of consensus pattern of *FT1*, *FT2*, or *LHY* mRNA fluctuation out of three genotypes.

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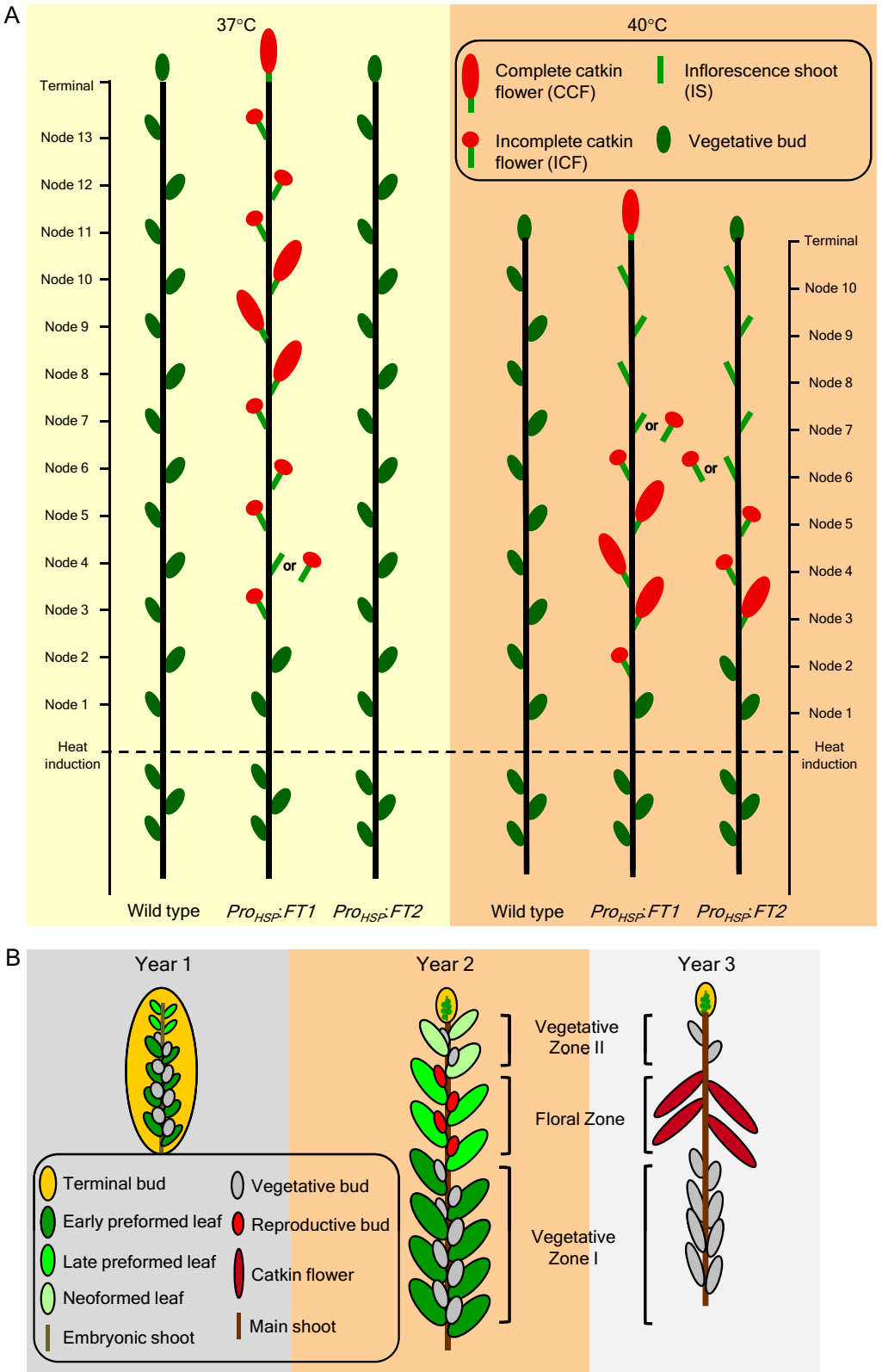

Fig. S4. Schematic drawing showing locations of inflorescences during heat treatment of *Pro$_{HSP}$:FT1* or *Pro$_{HSP}$:FT2* trees (*P. tremula* x *P. tremuloides*) and normal patterning of shoots. (*A*) Trees harboring *Pro$_{HSP}$:FT1* formed inflorescences with various morphology, including terminal complete inflorescences, on the new shoot growth after the initiation of heat treatment at 37 °C or 40 °C. In contrast, trees harboring *Pro$_{HSP}$:FT2* failed to induce inflorescences at 37 °C but produced a very few complete inflorescences at 40 °C under the same conditions. In trees harboring either construct, the axillary vegetative buds that formed before the heat treatment did not produce inflorescences or come out of dormancy. Wild-type controls produced only vegetative buds. This schematic drawing is based on observations on 10 trees. (*B*) Shoots of sexually mature *P. deltoides* possess a defined developmental pattern that includes three distinct leaf types

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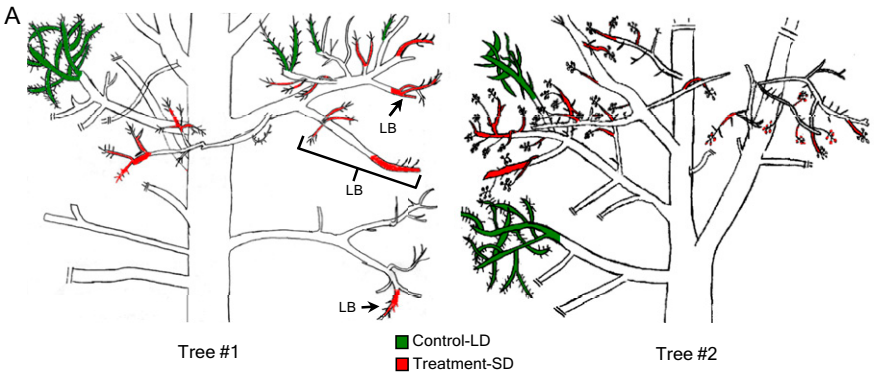
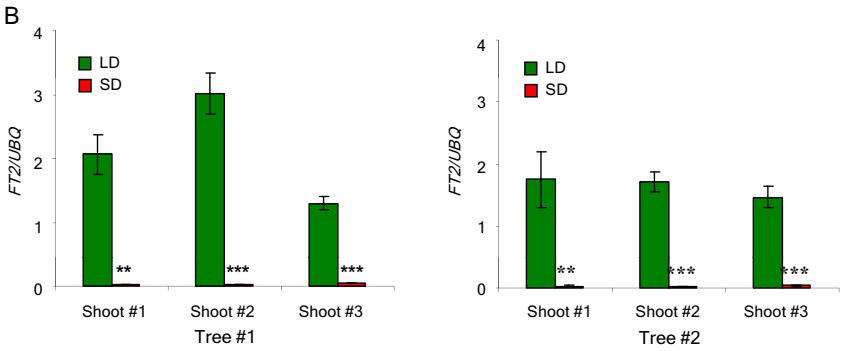
and specific locations of vegetative and reproductive buds. Shoots form early vegetative buds (Vegetative Zone I), reproductive buds (Floral Zone), and late vegetative buds (Vegetative Zone II) in a sequential manner. Leaf types associated with each bud type are the early preformed leaves, late preformed leaves, and neoformed leaves, respectively. The terminal vegetative bud forms during the first growing season (Year 1) and contains the early preformed leaves and the late preformed leaf primordia. Early preformed leaves are initiated early in the development of the terminal bud during Year 1 and have a long developmental period, which is interrupted by a cold period before expansion in the second growing season (Year 2). The preformed buds that form in axils of the early preformed leaves (Vegetative Zone I) normally do not develop into reproductive buds but rather into vegetative shoots with true leaf primordia. These buds form before *FT1* activation during a warm period (e.g., July to November). Late preformed leaf primordia develop during the advanced stage of terminal bud development during the dormancy period and stay in a primordial stage in winter. The buds that develop in axils of these leaves are all reproductive. Our data show that the fate of reproductive buds is determined during this cold period via *FT1* up-regulation. Following the development of reproductive buds in Year 2, male and female inflorescences emerge from reproductive buds in March of Year 3. Proximal to the shoot apical meristem, neoformed leaves (Vegetative Zone II) with vegetative buds in axils form under warm temperatures during the growing season in Year 2. This figure is modified from Yuceer et al., 2003 (1).

1. Yuceer C, Land SB, Jr., Kubiske ME, Harkess RL (2003) Shoot morphogenesis associated with flowering in *Populus deltoides* (*Salicaceae*). *Am J Bot* 90:196–206.





C

	LD	SD
Shoot length (cm)	19.2 ^{A}	12.8 ^{B}
Leaf length (cm)	18.3 ^{A}	18.8 ^{A}
# of vegetative buds	3.0 ^{A}	2.6 ^{B}
# of floral buds	1.6 ^{A}	1.3 ^{B}
n	128	40

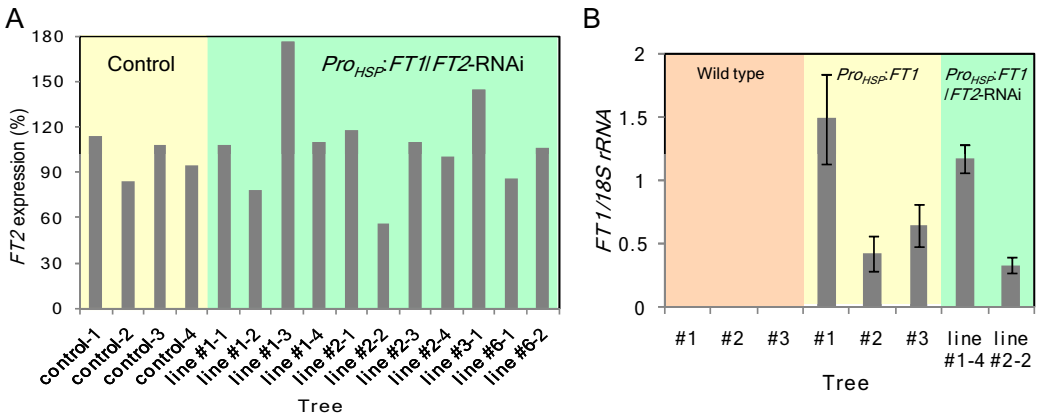
D

	LD	SD
shoot length (cm)	14.7 ^{A}	10.9 ^{B}
leaf length (cm)	16.8 ^{A}	16.9 ^{A}
# of vegetative buds	2.6 ^{A}	2.2 ^{B}
# of floral buds	0.9 ^{A}	0.5 ^{B}
n	224	103

E

	Small bag	Large bag (LB)	Range
Shoot length (cm)	13.0 ^{A}	12.7 ^{A}	
Leaf length (cm)	18.6 ^{A}	18.8 ^{A}	
# of vegetative buds	2.7 ^{A}	2.5 ^{A}	
# of floral buds	1.4 ^{A}	1.3 ^{A}	1 - 4
n	12	28	

Fig. S5. Suppression of *FT2* transcription during its normal expression in two normally growing, sexually mature *P. deltoides* trees. (*A*) Individual shoots and large branches were covered daily with small bags (individual shoots) and large bags (LB, large branch units) to create a short-day (8 h light) environment from March 25 to May 31, 2009. Red shows short-day–treated shoot/branch units, and green shows controls under ambient long days (12–14 h). The large branch units were used to take into account the possibility that *FT2* could be a short-range diffusible signal and might move to the neighboring shoots from controls. (*B*) Abundance of *FT2* transcripts in leaves from three shoots each of Tree #1 and Tree #2 was determined using qPCR. Poplar *UBQ* was used as an internal control to normalize the expression data. $^{**}P \leq 0.005$ and $^{***}P \leq 0.0005$ between short-day (SD) and long-day (LD) treatments for each shoot. (*C*) Response variables [shoot length (cm), leaf length (cm), and number (#) of vegetative and reproductive buds] were compared among treated shoots that at least had one reproductive bud. Superscript letters for response variables indicate a statistically significant ($P \leq 0.005$) difference between short-day and long-day treatments. We observed a small, but significant ($P \leq 0.005$), difference in the number of vegetative buds between the treatments. The early cessation of shoot growth under short days appears to have prevented the production of a number of vegetative buds that normally form proximal to the shoot tip in Vegetative Zone II. (*D*) Comparison of response variables between all shoots treated in long-day and short-day conditions, regardless of presence of a reproductive bud on a shoot. (*E*) Comparison of response variables in small-bag– and large-bag–treated shoots. No significant ($P > 0.2$) difference was observed between the treatments in all measured variables, implying lack of influence of diffusible signals from the neighboring shoots. Superscript letters for response variables indicate statistically significant ($P \leq 0.0001$) differences between short-day and long-day treatments.



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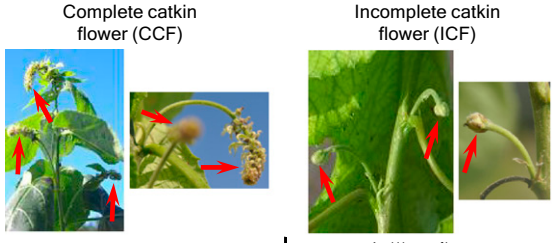

Transgenic lines	Axillary flowers # of CCF	# of ICF	# of IS	Total	Terminal flowers # of CCF	# of ICF	# of IS	Total
Pro $_{HSP}$:FT1/FT2-RNAi line #1-1	0	0	6	6	1	0	0	1
Pro $_{HSP}$:FT1/FT2-RNAi line #1-2	2	3	5	10	1	0	0	1
Pro $_{HSP}$:FT1/FT2-RNAi line #1-3	2	1	5	8	1	0	0	1
Pro $_{HSP}$:FT1/FT2-RNAi line #1-4	0	0	2	2	1	0	0	1
Pro $_{HSP}$:FT1/FT2-RNAi line #2-1	3	0	5	8	1	0	0	1
Pro $_{HSP}$:FT1/FT2-RNAi line #2-2	0	0	0	0	0	0	0	0
Pro $_{HSP}$:FT1/FT2-RNAi line #2-3	1	1	5	7	1	0	0	1
Pro $_{HSP}$:FT1/FT2-RNAi line #2-4	0	2	7	9	1	0	0	1
Pro $_{HSP}$:FT1/FT2-RNAi line #3-1	4	2	3	9	1	0	0	1
Pro $_{HSP}$:FT1/FT2-RNAi line #6-1	2	3	3	8	1	0	0	1
Pro $_{HSP}$:FT1/FT2-RNAi line #6-2	2	1	6	9	1	0	0	1
Control-wild type (n = 10)	0	0	0	0	0	0	0	0

D

		Control	Pro $_{HSP}$:FT1
Terminal Axillary flowers	# of CCF	0	0
	# of ICF	0	0.3 ± 0.7
	# of IS	0	3.3 ± 1.5
Terminal flowers	# of CCF	0	0.6 ± 0.5
	# of ICF	0	0
	# of IS	0	0
	shoot length (cm)	2.8 ± 2.7	3.4 ± 1.3
	# of leaves	3.6 ± 2.1	5.0 ± 1.1
	n	9	9

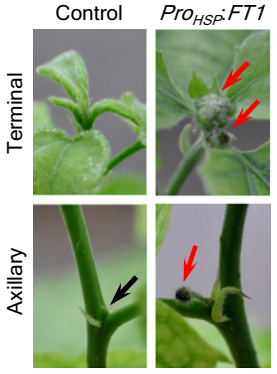

Fig. S6. Functional analysis of *FT1* and *FT2* in poplar (*P. tremula* x *P. tremuloides* 353). (*A*) To increase *FT1* but reduce *FT2* transcript levels, *Pro$_{HSP}$:FT1* and *FT2*-RNAi constructs were mobilized into the same trees. The graph shows percentage of *FT2* transcripts remaining in *Pro$_{HSP}$:FT1/FT2*-RNAi lines compared with wild-type controls. The lowest *FT2*-expressing *Pro$_{HSP}$:FT1/FT2*-RNAi line is 2–2, followed by 1–2. (*B*) Abundance of *FT1* transcripts was low during heat treatment at 37 °C in line 2–2. Error bars show SD about the mean. (*C*) When 11 *Pro$_{HSP}$:FT1/FT2*-RNAi lines and 10 wild-type controls were heat-treated at 37 °C, all lines except2–2 and controls formed axillary and terminal inflorescences. Pictures show inflorescence morphologies (red arrows) following the heat treatment. "Complete catkin" (CCF) refers to a fully developed normal poplar inflorescence. "Incomplete catkin" (ICF) refers to an inflorescence shoot with a few flowers. "Inflorescence shoot" (IS) refers to a developmentally arrested inflorescence that was unable to form flowers. (*D*) Heat treatment of *Pro$_{HSP}$:FT1* trees at 40 °C induced inflorescences under short-day conditions (8 h light). *FT2* normally is not expressed under short-day conditions. Black arrow shows a dormant axillary vegetative bud in a control tree; red arrows show inflorescences in *Pro$_{HSP}$:FT1* trees. Data are given as mean ± SD.

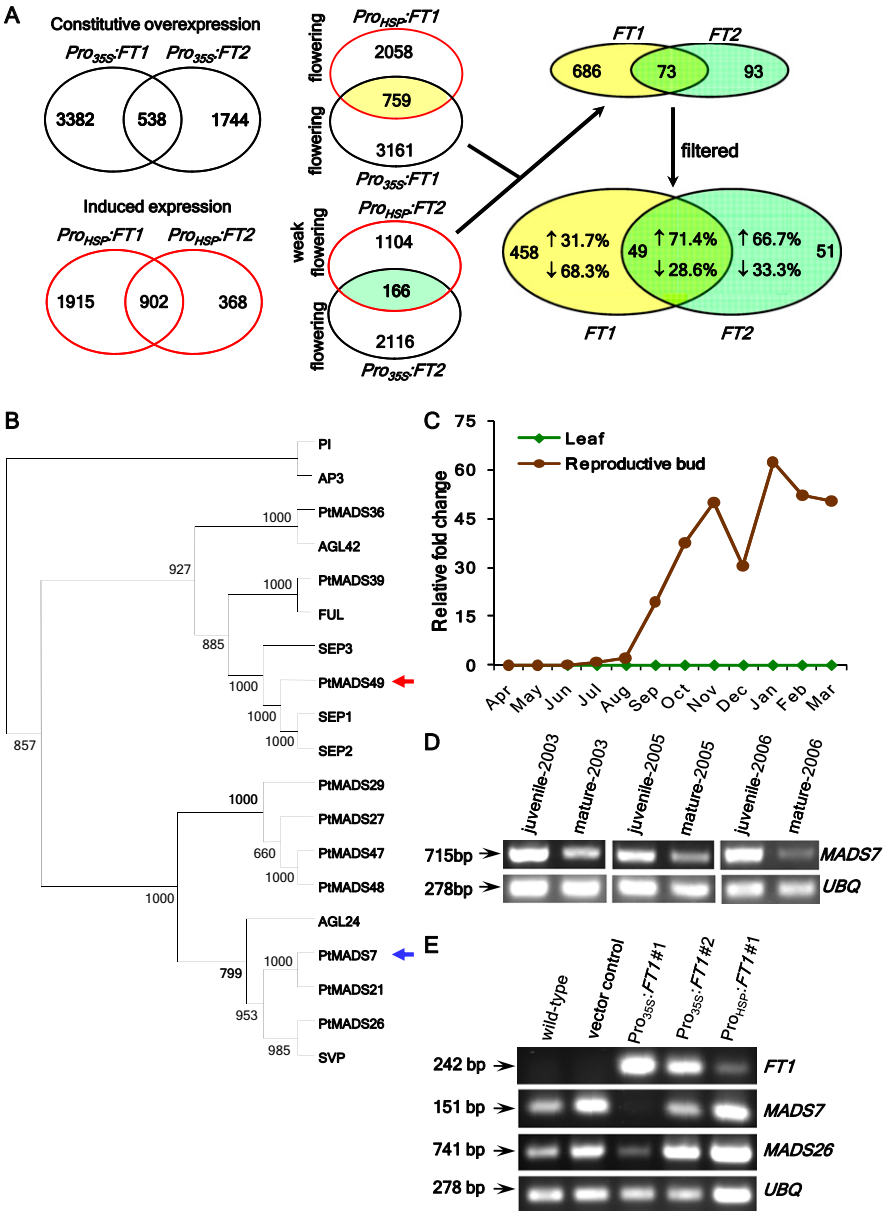

Fig. S7. *FT1* and *FT2* genetic networks in poplar and evolutionary and transcript analyses of *MADS49* and *MADS7*, two genes downstream of *FT1*. (*A*) Genes downstream of *FT1* and *FT2* were identified via microarrays using trees harboring the constitutive (Pro_{35S}:*FT1* and Pro_{35S}:*FT2*) and inducible (Pro_{HSP}:*FT1* and Pro_{HSP}:*FT2*) constructs. Data were filtered to remove the probe sets in opposite directions such as a probe set that shows up-regulation in Pro_{HSP}:*FT1*, but down-regulation in Pro_{35S}:*FT1*. Eventually, 458 *FT1*-specifc transcripts, 51 *FT2*-specific transcripts, and 49 *FT1*/*FT2*-common transcripts were identified. Arrows in the Venn diagrams indicate up-regulation (↑) and down-regulation (↓). (*B*) Phylogenetic relationship of MADS49 and MADS7 proteins (arrow) in poplar [*P. trichocarpa* (*Pt*)] with *A. thaliana* MADS-box proteins PISTILLATA (PI) (At5g20240), APETALA3 (AP3) (At3g54340), AGAMOUS-LIKE 42 (AGL42) (At5g62165), FRUITFULL (FUL) (At5g60910), SEPALLATA 3 (SEP3) (At1g24260), SEP1 (At5g15800), SEP2 (At3g02310), AGL24 (At4g24540), and SHORT VEGETATIVE PHASE (SVP) (At2g22540). PtMADS36 (POPTR_0012s13980.1), PtMADS39 (POPTR_0012s05960.1), PtMADS49 (POPTR_0004s11440.1), PtMADS29 (POPTR_0007s03300.1), PtMADS27 (POPTR_0007s03260.1), PtMADS47 (POPTR_0017s07160.1), PtMADS48 (POPTR_0017s07200.1), PtMADS7 (POPTR_0002s10580.1), PtMADS21 (POPTR_0005s17950.1), and PtMADS26 (POPTR_0007s14310.1) are all from poplar. Gene naming is after Leseberg et al. (1). PI and AP3 were used as outgroups. (*C*) Year-round transcript abundance of *MADS49* (a homolog of *Arabidopsis SEP*; forward primer, 5′-CATATGTTGCTGGAAGCAAACAGAGC-3′; reverse primer: 5′-GCAGTTATCTGGTCTGAACCAACAGAG-3′) in normally growing, sexually mature *P. deltoides* leaves and reproductive buds. Three independent replications of tissues from a tree were sampled. (*D*) *MADS7* transcripts (forward primer, 5′-AATGGCTAGAGAGAAGATCAAGATCAAG-3′; reverse primer: 5′-CTACTGGATCAGTTCATTTTCCTCCAG-3′) were abundant in juvenile (1-y-old) wild-type trees when measured in 2003, 2005, and 2006 but were scarce at the sexually mature (12 y old) stage. The poplar *UBQ* transcripts were amplified to verify that similar amounts of cDNA were used in the RT-PCR. Amplicon size is shown on the left. (*E*) *FT1* transcripts were undetectable in leaves of juvenile (3-mo-old) wild-type (control) trees and juvenile (3-mo-old) vector control trees (with no 35S promoter), whereas *MADS7* transcripts (forward primer, 5′-GCAATTGGAGAAGGCGCTTGAAGTAG-3′; reverse primer, 5′-GTAAATGGTTGCGATCTTCTGTTTTAGTTG-3′) were abundant. *FT1* transcripts were abundant in Pro_{35S}:*FT1* (lines #1 and 2) with a 3-mo-old early-flowering phenotype, but *MADS7* transcripts were at background levels. *MADS7* transcript levels increased in Pro_{HSP}:*FT1* trees with less *FT1* expression. *MADS26* (forward primer, 5′-CCTGTTTTCTTAGCTTAAAATGGCAAGAG-3′; reverse primer: 5′-GCATTTGCTGGTGAAGAAATCTCCCTG-3′) clustered closely with *MADS7* and *SVP* and did not consistently show change in expression pattern under the same conditions.

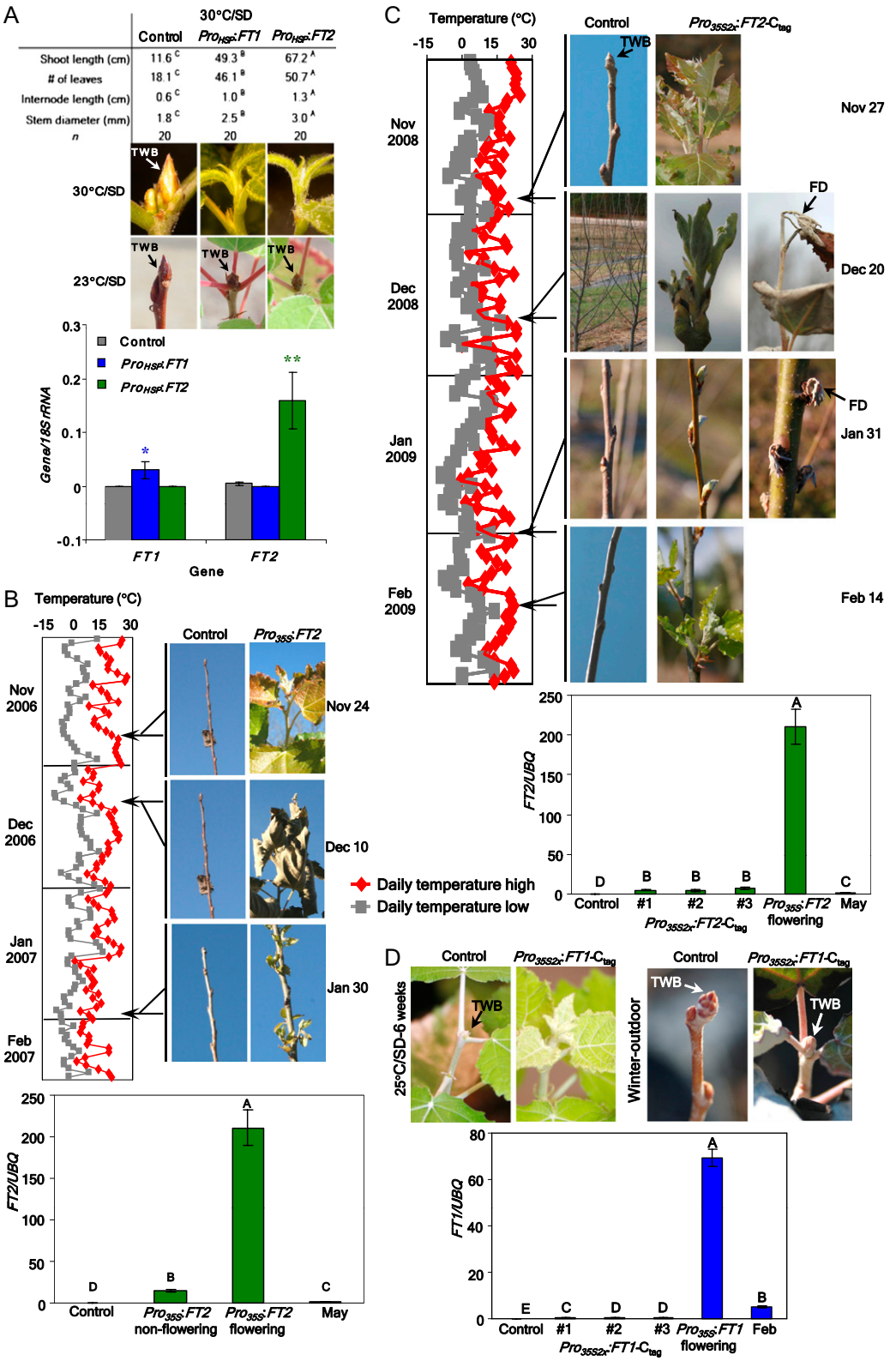

Fig. S8. Increased expression of *FT2* promotes shoot growth in poplar. (*A*) Trees (*P. tremula* x *P. tremuloides*) harboring Pro_{HSP}:*FT1* or Pro_{HSP}:*FT2* with controls were grown at 30 °C in short-day conditions (SD). *FT1* and *FT2* transcript abundance was determined in leaves. Plants were returned to 23 °C in short-day conditions following the experiment. TWB, terminal bud. Superscript letters indicate a statistically significant difference ($P \leq 0.001$). *$P \leq 0.05$; **$P \leq 0.005$. Error bars indicate SD about the mean. (*B*) *P. tremula* x *P. alba* trees ($n = 25$) harboring Pro_{35S}:*FT2* and showing relatively less *FT2* expression with no early flowering did not cease shoot growth under short-day conditions and/or cold temperatures in the field. A frost killed growing parts in December (winter). When temperatures were warmer in January, undamaged dormant axillary buds came out of dormancy and grew. Control trees ($n = 36$) remained dormant throughout fall and winter until the resumption of normal growth in the spring. Temperature data were collected in 2006 and 2007 when observations on

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trees were made. Trees harboring Pro_{35S}:*FT2* had significantly ($P \leq 0001$) more *FT2* transcripts than controls (three independent replications of leaf tissues from each tree). For comparison purposes, an early-flowering tree harboring Pro_{35S}:*FT2* was included in qPCR experiments (three independent replications of leaf tissues). Also, for comparison purposes, the amount of *FT2* transcripts was determined in fully expanded leaves (three independent replications) of a normally growing, sexually mature *P. deltoides* tree in May when *FT2* transcripts normally are abundant. Letters above the bars showing the abundance of *FT2* transcripts indicate statistically significant ($P \leq 0.0001$) differences. Error bars indicate SD about the mean. (*C*) *P. tremula* x *P. alba* trees ($n = 107$) harboring $Pro_{35S2\times}$:*FT2*-C_{tag} did not induce early flowering in the field after two growing seasons. However, these trees grew as long as the air temperature stayed above freezing and frost did not kill them. When the weather was warmer for a few weeks in the winter after a frost event (e.g., January and February), undamaged axillary buds began growing until another frost killed them. Controls ($n = 74$) were dormant in the fall and winter until early spring. Temperature data were collected in 2008 and 2009 when observations on trees were made. Trees #1, #2, and #3 harboring $Pro_{35S2\times}$:*FT2*-C_{tag} had significantly ($P \leq 0001$) more *FT2* transcripts than controls (three independent replications of leaf tissues from each tree). An early-flowering tree harboring Pro_{35S}:*FT2* was included in qPCR experiments (three independent replications of leaf tissues). The amount of *FT2* transcripts also was determined in fully expanded leaves (three independent replications) of a normally growing, sexually mature *P. deltoides* tree in May when *FT2* transcripts normally are abundant. Letters above the bars indicate statistically significant ($P \leq 0.0001$) differences. Error bars indicate SD about the mean. TWB, terminal buds. FD, frost damage. (*D*) *P. tremula* x *P. alba* trees ($n = 6$) harboring the constitutive $Pro_{35S2\times}$:*FT1*-C_{tag} construct did not induce early flowering in the field or growth rooms after two growing seasons. These trees set terminal buds 8 wk later than wild-type controls ($n = 12$) both in a growth room [25 °C, short-day conditions (8 h light)] and in the field. Trees #1, #2, and #3 harboring $Pro_{35S2\times}$:*FT1*-C_{tag} had significantly ($P \leq 0001$) more *FT1* transcripts than controls (three independent replications of leaf tissues from each tree). An early-flowering tree harboring Pro_{35S}:*FT1* was included in qPCR experiments (three independent replications of leaf tissues). Also, the amount of *FT1* transcripts was determined in preformed leaves (three independent replications) of a normally growing, sexually mature *P. deltoides* tree in February, when *FT1* normally is expressed. Letters above the bars indicate statistically significant ($P \leq 0.0001$) differences. Error bars show SD about the mean.

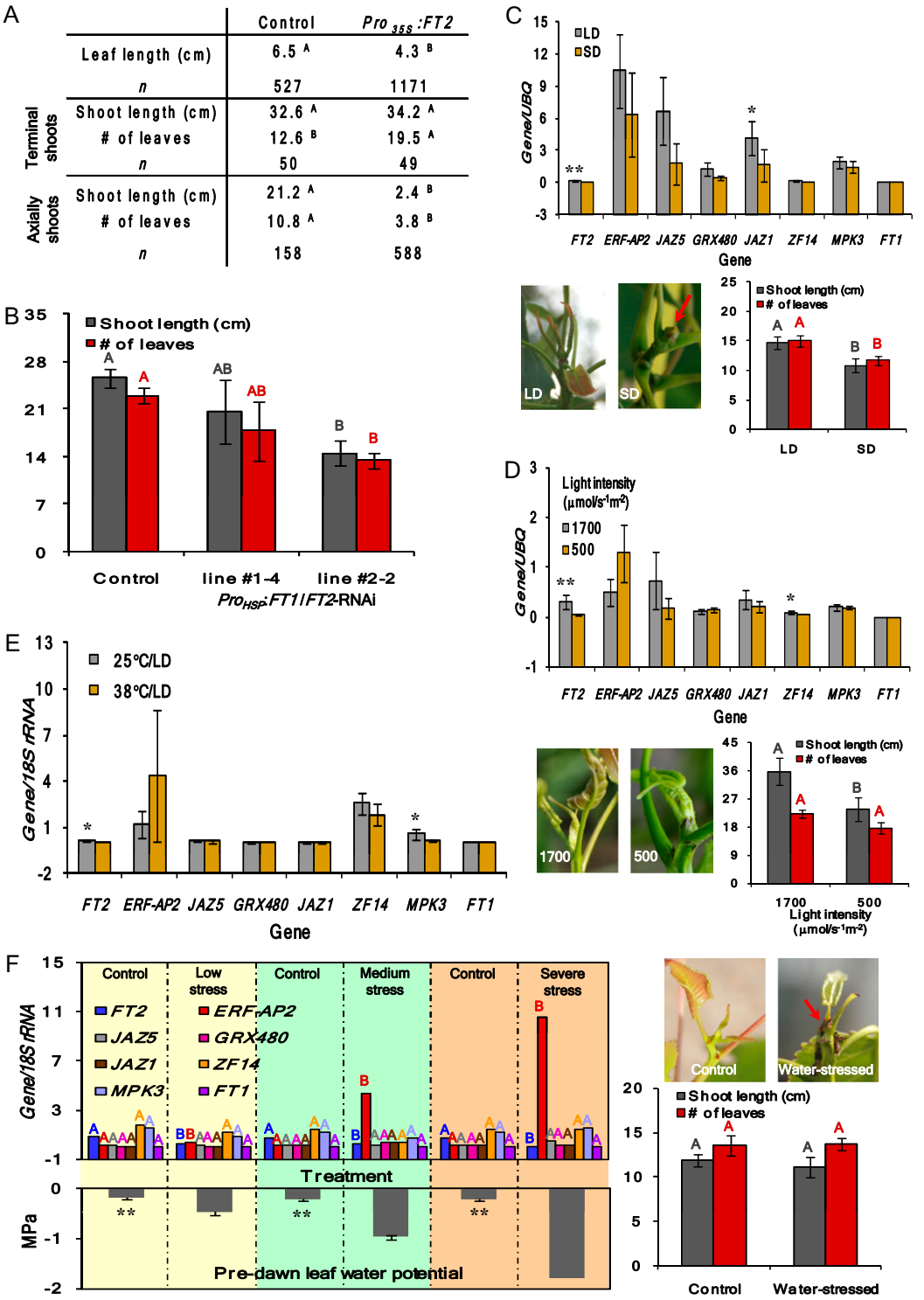

Fig. S9. *FT2* controls vegetative growth in poplar. (*A*) Comparison of axillary and terminal shoot growth in control and Pro_{35S}:*FT2* (not early flowering) *P. tremula* x *P. alba* trees grown in the field. Two wild-type controls and two Pro_{35S}:*FT2* (line 47) trees at age 4 y were used for this purpose. Superscript letters indicate statistically significant ($P \leq 0.001$) differences. (*B*) Lines 1–4 and 2–2 of trees (*P. tremula* x *P. tremuloides*) harboring Pro_{HSP}:*FT1*/*FT2*-RNAi produced less vegetative growth at 30 °C under long-day conditions. Compared with 1–4, 2–2 showed lower expression of both *FT1* and *FT2* (Fig. S6 *A* and *B*). Letters above bars for response variables (shoot length or number of leaves) indicate statistically significant ($P \leq 0.05$) differences. Error bars indicate SD about the mean. (*C*) *FT2* and *JAZ1* transcripts were less abundant under short-day conditions (SD) in *P. deltoides* leaves. *FT1* transcripts were undetectable in the identical tissues. Short-day conditions induced cessation of shoot growth (arrow), resulting in shorter shoots. LD, long day. $*P \leq 0.05$; $**P \leq 0.005$. Letters above bars for response variables (shoot length or number of leaves) indicate statistically significant ($P \leq 0.05$) differences. Error bars indicate SD about the mean. (*D*) When ambient light intensity (1,700 μmol s^{-1} m^{-2}) was reduced to 500 μmol s^{-1} m^{-2}, the abundance of *FT2* and *ZF14* transcripts was decreased in *P. deltoides* leaves. *FT1* transcripts were undetectable in the identical tissues. Low light intensity produced shorter shoots. $*P \leq 0.05$; $**P \leq 0.005$. Letters above bars for response variables (shoot length or number of leaves) indicate statistically significant ($P \leq 0.05$) differences. Error bars indicate SD about the mean. (*E*) Compared with normal temperature (25 °C), heat stress (38 °C) decreased transcript abundance of *FT2* and *MAPK3* under long-day conditions (LD). *FT1* transcripts were un-

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detectable in the identical tissues. *$P \leq 0.05$. Error bars indicate SD about the mean. (*F*) Abundance of *FT2* transcripts was reduced, whereas the abundance of *ERF-AP2* was increased under low, medium, and severe water stress. *FT1* transcripts were undetectable in the identical tissues. Water stress caused cessation of shoot growth (arrow). Letters above bars for response variables (transcript) indicate statistically significant ($P \leq 0.05$) differences among treatments for each tested gene. **$P \leq 0.005$. Error bars represent SD about the mean. Letters above bars for response variables (shoot length or number of leaves) indicate statistically significant ($P \leq 0.05$) differences.

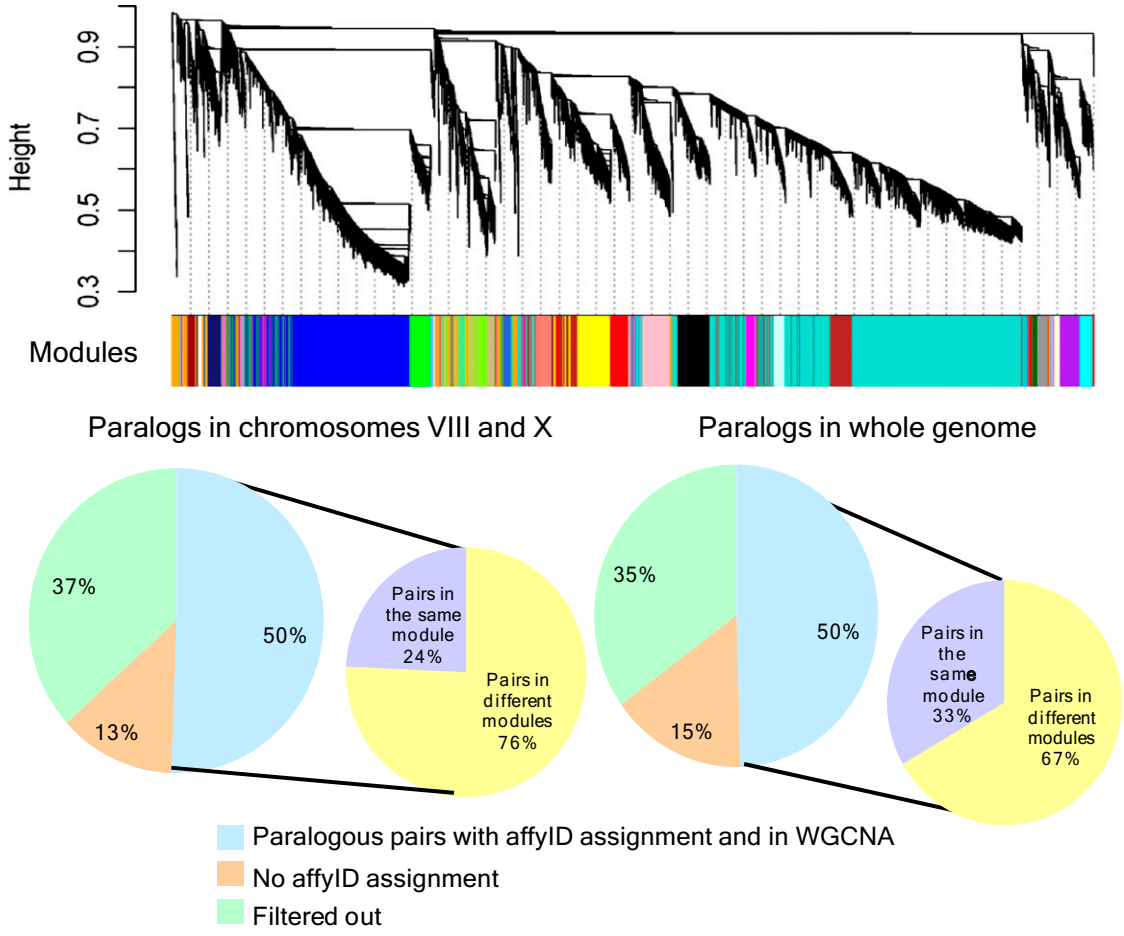

Fig. S10. Weighted Gene Coexpression Network Analysis (WGCNA) resulted in 42 modules (coexpressed genes groups) using the year-round leaf microarray data from normally growing mature trees. Following a strict filtering process, of the remaining 791 paralogous pairs out of 1,564 paralogous genes on poplar (*P. trichocarpa*) chromosomes VIII and X, both members of 192 pairs (24%) fell into the same modules (similar expression patterns), whereas members of 574 pairs (76%) fell into different modules (diverged expression patterns). After filtering, of the remaining 3,815 paralogous pairs out of all the pairs of 7,701 paralogous genes in the whole genome, both members of 1,271 pairs (33%) fell into the same modules, whereas pairs of 2,544 (67%) fell into different modules.

Other Supporting Information Files

Dataset S1 (XLSX)
Dataset S2 (XLSX)