Quantitative Trait Loci and Candidate Gene Mapping of Bud Set and Bud Flush in Populus

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Manuscript received April 1, 1999
Accepted for publication October 12, 1999

ABSTRACT

The genetic control of bud phenology in hybrid poplar was studied by mapping quantitative trait loci (QTL) affecting the timing of autumn bud set and spring bud flush. The founders of the mapping pedigree were collected from widely separated latitudes to maximize segregating variation for dormancy-related traits in the F_2 generation—the female *Populus trichocarpa* parent is from Washington State (48°N) and the male *P. deltoides* parent is from Texas (31°N). Bud set and bud flush timing were measured on the F_2 generation in a replicated clonal field trial. Using a linkage map constructed of AFLP and microsatellite markers, three QTL controlling bud set and six QTL controlling bud flush were detected. Additionally, five candidate genes believed to be involved in perception of photoperiod (*PHYB1*, *PHYB2*) or transduction of abscisic acid response signals (*ABI1B*, *ABI1D*, and *ABI3*) were placed on the QTL map. *PHYB2* and *ABI1B* were found to be coincident with QTL affecting bud set and bud flush.

WOODY perennial plants in the temperate zone undergo annual periods of dormancy. Growth cessation and dormancy induction are prerequisites for the development of substantial cold hardiness. The onset of the dormant period is marked by the cessation of stem elongation and the formation of terminal buds (autumn bud set). After dormancy release in the spring, bud flush occurs when buds swell and new leaves emerge.

The timing of bud set and bud flush represents a critical ecological and evolutionary tradeoff between survival and growth. Growth cessation, the formation of vegetative buds, cold acclimation, and the development of dormancy make plants more resistant to freezing and dehydration stress. Thus, if buds do not set soon enough and an adequate level of frost hardiness does not develop before the first frost of autumn, the tree will be damaged. Likewise, if bud flush occurs too early in spring, the growing tissues may be killed by a late frost. On the other hand, trees in which bud set occurs too early in the fall, or bud flush occurs too late in the spring, have a shortened growing season that reduces competitive ability and growth potential. The control of timing of bud set and bud flush thus has implications for adaptation of trees to their natural environment and for tree breeding.

Natural populations of forest trees show remarkably fine-scale adaptation to the length of the frost-free growing season characteristic of their local environment. Common garden studies reveal that the timing of autumn bud set and spring bud flush is correlated with the latitudinal and elevational origin of the trees. Tree ecotypes from high latitudes and elevations set bud and break bud early, relative to trees from lower latitudes and elevations, when grown in a common environment (Pauley and Perry 1954). Bud phenology often is found to be under exceptionally strong genetic control. In Populus (poplars, cottonwoods, and aspens), clonal field trials have shown that the timing of spring bud flush is among the most highly heritable traits measured in forest trees, with up to 98% of the total phenotypic variance explained by genetic factors and only 2% by environmental variables (Bradshaw and Stettler 1995).

Despite the evidence that bud phenology is under strong genetic control in forest trees, very little is known about the identity of the genes responsible for genetic variation in bud phenology traits. This lack of knowledge is largely due to two factors. First, trees are recalcitrant to classical genetic studies because of their long generation interval and outcrossing mating systems. Second, phenological traits generally are not inherited in a simple Mendelian fashion, but rather show the continuous phenotypic variation typical of a quantitative trait. Quantitative trait locus (QTL) mapping experiments have shown that major genes may be involved in the control of bud phenology. In Populus, as few as five QTL explain 85% of the genetic variation in the timing

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of spring bud flush (Bradshaw and Stettler 1995), but no specific genes have been shown to correspond to these QTL.

The goals of this study were to extend and refine our prior QTL analysis of bud phenology traits by (1) collecting phenotypic data on autumn bud set in addition to spring bud flush, (2) using a larger F_2 sample size ($N = 346$) to improve the power of QTL detection and the precision of QTL parameter estimates, (3) performing the mapping experiments in a separate three-generation pedigree to validate previously identified QTL affecting spring bud flush, and (4) mapping candidate genes with plausible connections to bud phenology to test for colocalization with mapped QTL.

MATERIALS AND METHODS

Pedigrees: A mapping pedigree was founded in 1980 by crossing a female *Populus trichocarpa* (clone 93-968) from western Washington (48°N latitude) with a male *P. deltoides* (clone S7C4) from Texas (31°N). Two of the resulting F_1 offspring were crossed in 1993 to produce the 346 F_2 progeny (family 822) used in this study.

A second, related F_2 pedigree (family 331) in which bud flush had already been measured (Bradshaw and Stettler 1995) was used for verification of QTL. Family 331 resulted from a cross between the same maternal *P. trichocarpa* clone (93-968) used in family 822 and a *P. deltoides* clone (ILL-129) from central Illinois (39°N). Parental, F_1, and F_2 ($N = 55$) clones of family 331 were planted in the spring of 1991 using a modified randomized complete block design in Puyallup, WA (47°N), with six ramets per clone. In the spring of 1993, clones were observed twice weekly and the date of bud flush was recorded as the number of days since 31 December 1992.

Phenotypic measurements: Four clonal replicates of each F_2 genotype from family 822 were grown in a randomized complete block design in a field in Corvallis, OR (44.5°N). Clones were planted every 1.5 m in rows spaced 2.6 m apart. The field was fertilized once with 50 kg/ha of N (urea) and irrigated by overhead sprinklers every 2 wk for 4 hr between June and September. All trees were scored weekly for the date of bud set and bud flush in autumn of 1997 and spring of 1998, respectively. Bud set date was recorded when all healthy apices on the tree had a terminal bud. A terminal bud was defined as the stage when the stipules of the foliage leaves covered the shoot apex and the youngest foliage leaf was offset from the central axis of the shoot apex. Bud flush was recorded when the first fully unfolded leaf was observed. The number of days from December 31 to the dates of bud set and bud flush were calculated and used in the analyses. Clone means were calculated using analyses of variance and the LSMEANS option of PROC GLM (Statistical Analysis System version 6.12, SAS Institute, Cary, NC). These least-squares means were used as the phenotypic descriptors for each clone in the QTL analyses.

Linkage map construction: Leaf tissue was collected from the parents, both F_1's, and all F_2 clones. DNA was extracted using a FastPrep homogenizer and FastDNA extraction kits (BIO 101, Vista, CA).

Amplified fragment length polymorphism (AFLP) marker analysis was performed essentially as described by Vos *et al.* (1995). The markers were named according to the restriction enzymes used, the three selective nucleotides used on the amplification primers, and the relative positions of the bands on the gels. A letter indicates either the *Eco*RI (E) or *Mse*I (M) adapter/primer and a number indicates the identity of the three selective nucleotides (Table 1). The polymorphic bands on each gel were numbered sequentially from top to bottom. A large number of AFLP primer pairs ($N = 192$) were screened for informative markers, from which 29 primer pairs with the greatest number of polymorphic bands were chosen. The AFLP genotype of all 346 F_2 trees was determined at 315 loci. Most ($N = 305$; 97%) AFLP markers were inherited in a dominant manner, while 10 markers were codominant.

TABLE 1

AFLP primers

		First selective nucleotide			
Second	Third	A	C	G	T
A	A	31	47	63	79
	C	32	48	64	80
	G	33	49	65	81
	T	34	50	66	82
C	A	35	51	67	83
	C	36	52	68	84
	G	37	53	69	85
	T	38	54	70	86
G	A	39	55	71	87
	C	40	56	72	88
	G	41	57	73	89
	T	42	58	74	90
T	A	43	59	75	91
	C	44	60	76	92
	G	45	61	77	93
	T	46	62	78	94

AFLP primers were named according to the three selective nucleotides they contained. A number was assigned to each combination of three selective nucleotides. For example, the number 49 is assigned to the combination CAG.

Microsatellite [simple sequence repeat (SSR)] marker analysis was performed using primers developed by the Poplar Molecular Genetics Cooperative (PMGC; Table 2). SSR markers have a PMGC prefix. The PCR conditions were as follows: 50 ng of DNA was used in 10-μl reactions containing 10 mm Tris-HCl, 50 mm KCl, 2 mm $MgCl_2$, 1.3 mg/ml bovine serum albumin, 200 μm of each dNTP, 76 μm of each primer, and 0.5 units of *Taq* polymerase (Perkin Elmer, Foster City, CA). Reaction conditions were 30 cycles of 94° for 5 sec, 50° for 15 sec, and 72° for 1 min. All F_2 clones were genotyped with two SSR markers, PMGC486 and PMGC573, to identify triploid individuals ($N = 10$), which were removed from subsequent analysis. SSR genotypes at seven additional loci were determined for 76 randomly chosen F_2 clones to determine synteny relationships between the repulsion phase linkage groups defined by dominant AFLP markers.

The linkage map was constructed using MAPMAKER, version 3.0 (Lander *et al.* 1987). Thresholds for linkage were set at LOD ≥5, maximum distance at 40 cM, and marker order at LOD ≥2. Any markers that could not be ordered unambiguously at LOD ≥2 were not included as framework markers in the map. The framework map consists of 276 AFLP markers, two microsatellite markers, and one candidate gene locus (*PHYB2*). Due to the high percentage of dominant markers used (266/279 markers; 95%) and the paucity of linkage infor-

TABLE 2

Primer sequences for microsatellite markers

Locus name	Linkage group	Forward primer sequence	Reverse primer sequence
PMGC61	C	GATCCCTCTGCACCGTTTAC	ACCCTAAATTTGCTGACAAC
PMGC223	BB	CGATGAGGTTGAAGAAGTCG	ATATATGTACCGGCACGCCAC
PMGC333	E	CTTAGTGGTGAAGTATTC	GAGTGGGTGCTGATTCATCC
PMGC409	C	ACGTATATGAAGTTCTTGATTGC	GACAGATCATTATGATTACTACAG
PMGC410A	AA	CCATTACCCGAGTCACG	CTATATTCACTGGAATAGTG
PMGC456	BB	TGTAGGAGATATCCACGTGG	AACAATATGCTTCATAGCACAG
PMGC486	F	AGAAGTTGTTGAACCCGATGGG	GCTACAAACTTTGTTGTACCC
PMGC573	?	GTCATAATCGCCTATACACAG	GATTGTGAACTCGATCTAAAGG
PMGC683	BB	CCAGCAATGATTGATTGCTCC	GAGCTTTAACTGTCCAGTAGC

Microsatellite marker primers were developed by Poplar Molecular Genetics Cooperative. The question mark signifies that the marker does not map to a known linkage group. Forward and reverse primer sequences are given from the 5′ to 3′ direction. Additional primer sequences are also available at http://poplar2.cfr.washington.edu/pmgc/ssr/pmgcssr.htm.

mation from dominant markers linked in repulsion (Ott 1985; Knapp *et al.* 1995), two linkage maps were constructed. These two maps, which represent the parental genomes of *P. trichocarpa* (T map) and *P. deltoides* (D map) consist mostly of dominant markers linked in coupling. Linkage groups labeled with the T subscript (*e.g.*, linkage group A_T) consist of dominant markers in which the recessive allele is derived from *P. trichocarpa*. Linkage groups labeled with the D subscript consist of dominant markers in which the recessive allele is derived from *P. deltoides*. Codominant markers were included in both data sets. Synteny between the two repulsion phase linkage groups was determined by the placement of codominant AFLPs, microsatellite markers, and candidate genes. Linkage groups were named to reflect homologies between the two family 822 maps as well as homologies with the previously published family 331 linkage map (Bradshaw *et al.* 1994). Linkage groups named with two different letters (*e.g.*, PY) were homologous to two linkage groups (P and Y) in the family 331 map. Linkage groups named with the same two letters (*e.g.*, AA) had no homologous linkage groups in the family 331 map. When two family 822 linkage groups were homologous to the same family 331 linkage group, the family 822 names were followed by a number (*e.g.*, DE1 and DE2).

Candidate genes: Five genes were placed on the linkage map using PCR-based markers. Genes (*PHYB1*, *PHYB2*) encoding phytochromes B1 and B2 were amplified using primers developed by Howe *et al.* (1998). DNA (10 ng) was used in 10-μl reactions containing 10 mm Tris-HCl, 50 mm KCl, 2 mm $MgCl_2$, 1.3 mg/ml bovine serum albumin, 200 μm of each dNTP, 0.5 μm of each primer, and 0.5 units of *Taq* polymerase (Perkin Elmer, Foster City, CA). The PCR reaction consisted of 35 cycles of 94° for 45 sec, 55° for 30 sec, and 72° for 1 min. The amplified *PHYB1* alleles can be distinguished by a length polymorphism resolvable on a 1% agarose gel. Amplified *PHYB2* alleles were digested with *Mse*I to reveal a restriction fragment length polymorphism (RFLP). Poplar homologs of an *Arabidopsis thaliana ab*scisic acid *i*nsensitivity gene (*ABI1*) were amplified by PCR with the following primers: 5′-GTCCGA GGAGATAGAATTTGT-3′ and 5′-GCCCTAGGAATAAACATA ACTT-3′. Constituents of the PCR reactions were identical to those for *PHYB1* and *PHYB2* except that 0.3 μm of each primer was used. The amplification conditions consisted of 30 cycles of 94° for 30 sec, 50° for 30 sec, and 72° for 2 min. Two Populus loci, designated *ABI1B* and *ABI1D*, were amplified, both of which had an informative banding pattern after digestion with *Ase*I. We determined that the shorter of the two amplification products (1237 bp) was *ABI1B* and the other (1937 bp) was *ABI1D*. The Populus homolog of the Arabidopsis *ABI3* gene was amplified under the same conditions as *ABI1* with the following primers: 5′-ATGGGTCCAAACGAATCA-3′ and 5′-GAGAGCCAGAGCCATTATTC-3′. Amplification products were digested with *Rsa*I to produce an RFLP. Genotypic data for the five candidate genes were collected for all F_2 offspring in family 822. Genotypic data for *ABI1B, ABI1D, ABI3*, and *PHYB2* were collected for 55 F_2 clones in family 331, for which linkage and QTL maps have been published (Bradshaw *et al.* 1994; Bradshaw and Stettler 1995).

QTL analysis: The initial scan for QTL affecting the timing of bud set and bud flush was done with MAPMAKER/QTL 1.1 (Lincoln *et al.* 1992) using a detection threshold of LOD ≥2. These tentatively identified QTL were examined in detail using QTL Cartographer (Zeng 1994) model 6 to test for the presence of multiple QTL. Empirical statistical significance thresholds (experimentwise $P < 0.05$) for declaring the presence of a QTL were determined by performing 100 permutations of the data set (Churchill and Doerge 1994). The magnitude of QTL effect is expressed as the percentage of F_2 phenotypic variation explained (PVE). The QTL mode of action was determined by comparing three possible constrained models (additive, dominant, and recessive). For each QTL, the most likely model was reported. If one model was more likely than the alternatives by a LOD difference >1, it was considered to be strong evidence in favor of the proposed mode of action.

RESULTS

Phenotypic trait distributions: The range of segregating variation in family 822 for both phenology traits is large. For bud set, the earliest F_2 clones set bud on 19 August and the latest on 25 November 1997, a period of 98 days (Figure 1). Bud flush occurred over 40 days between 4 March and 13 April 1998. Clone mean heritabilities were estimated as $H^2 = 91\%$ for bud set and $H^2 = 94\%$ for bud flush, confirming that both traits are under very strong genetic control.

Linkage map construction: The T map spans a dis-

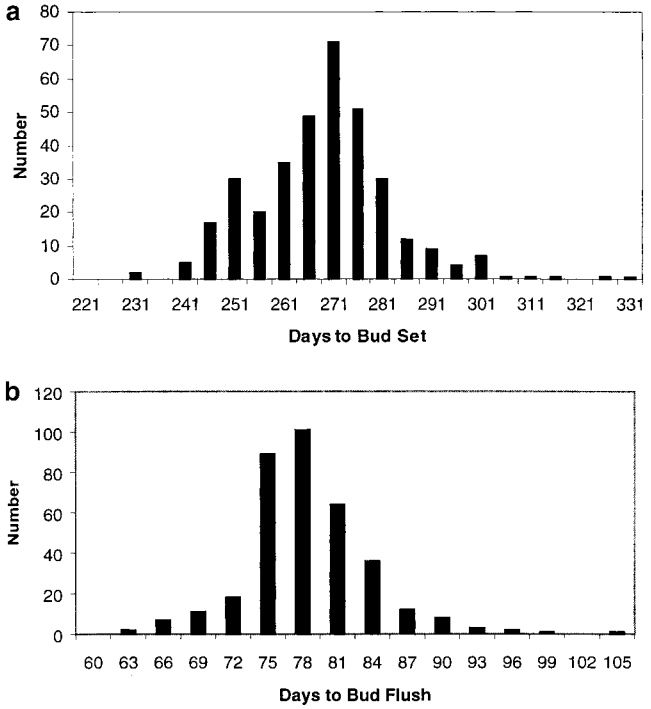

Figure 1.—The distribution of number of days to (a) bud set and (b) bud flush in family 822.

tance of 2002 cM, covering 77% of the estimated genome length (Bradshaw *et al.* 1994) with an average marker interval of 13.6 cM. The D map is 1778 cM in length, covering 68% of the genome with an average marker spacing of 12.3 cM. There were 26 linkage groups found in the T map and 24 in the D map. Because the haploid chromosome number is 19 in Populus, some of these linkage groups must represent different sections of the same chromosome.

QTL mapping: QTL for both bud set and bud flush were identified on the T and D maps (Figure 2). The number, magnitude of effect, and direction of effect of QTL identified are summarized in Table 3.

Four QTL affecting the timing of bud set were found, two on the T map and two on the D map. The QTL on homologs F_T and F_D likely represent the same locus, suggesting that a maximum of three unique bud set QTL were identified. Two bud set QTL are on linkage groups (J_T, PY_D) that have homologs (J_D, PY_T) but for which no homologous QTL was detected. All bud set QTL are on linkage groups that also contain bud flush QTL; however, none are located in the same marker interval. The estimated magnitude of effect of bud set QTL ranged from 6.0 to 12.2 PVE. The *P. trichocarpa* allele reduced the number of days to bud set at two of the three unique QTL. Differences in timing of bud set between homozygous classes at each QTL ranged between 6.1 and 14.1 days, which represents 6 and 14% of the period over which bud set occurred.

Nine bud flush QTL were identified, five on the T map and four on the D map. The QTL on F_T and F_D are likely the same locus, as are the two QTL on J_T and J_D and those on DE_T and $DE2_D$. Thus, a probable maximum of six unique bud flush QTL were detected. Two QTL occurred on linkage groups for which no homologous linkage group on the other family 822 linkage phase map has been identified (H_T, Z_D). The estimated magnitude of effect of bud flush QTL ranged from 5.9 to 16.8 PVE. The *P. trichocarpa* allele hastened the time to bud flush at four of the six unique QTL. The difference between the two homozygous classes for each bud flush QTL was between 3.4 and 5.7 days, 8–14% of the period over which bud flush occurred.

Candidate gene mapping: Five candidate genes putatively involved in the regulation of bud dormancy were mapped. *PHYB1*, *ABI1D*, and *ABI3* map to linkage groups on which there are no QTL affecting the timing of bud set or bud flush (Figure 2). *PHYB2* maps to linkage groups J_T and J_D, both of which contain a bud flush QTL in the same marker interval. On J_T, the QTL accounted for 9.6 PVE and on J_D the QTL accounted for 9.4 PVE. There is also a bud set QTL on J_T in an adjacent marker interval that accounts for 6.8 PVE. *ABI1B* maps to linkage group PY_T in an interval containing a bud flush QTL accounting for 9.1 PVE. On the D map, *ABI1B* maps to linkage group PY_D. Although there is not a significant bud flush QTL on this linkage group, the maximum LOD score for a bud flush QTL in the adjacent interval on PY_D is 3.00 while the threshold for significance ($P = 0.05$) determined by permutation test (Churchill and Doerge 1994) is 3.06. The interval to which *ABI1B* maps on linkage group PY_D contains a bud set QTL accounting for 6.0 PVE.

QTL and candidate gene validation in a related F_2 family: The timing of spring bud flush was measured previously in a related F_2 hybrid poplar pedigree, family 331 (Bradshaw and Stettler 1995). Five QTL on five linkage groups were detected (Bradshaw and Stettler 1995); of these, three linkage groups (E, F, and P) have known homologs in the family 822 maps. All three homologs also contain a bud flush QTL in family 822 (Table 3). Two QTL were found in family 822 that were not detected in family 331, on linkage groups H_T, J_T, and J_D. There is no linkage group in family 331 homologous to Z_D. *PHYB2* and *ABI1B* map to linkage groups J and P, respectively.

DISCUSSION

Inheritance of bud phenology: Traditional quantitative genetics studies suggest that autumn bud set and spring bud flush in forest trees are traits controlled by multiple genes (Pauley and Perry 1954; Eriksson *et al.* 1978). The detection of multiple QTL affecting bud set and bud flush in this study supports the quantitative genetic interpretation and in addition provides a detailed assessment of the minimum number of QTL in-

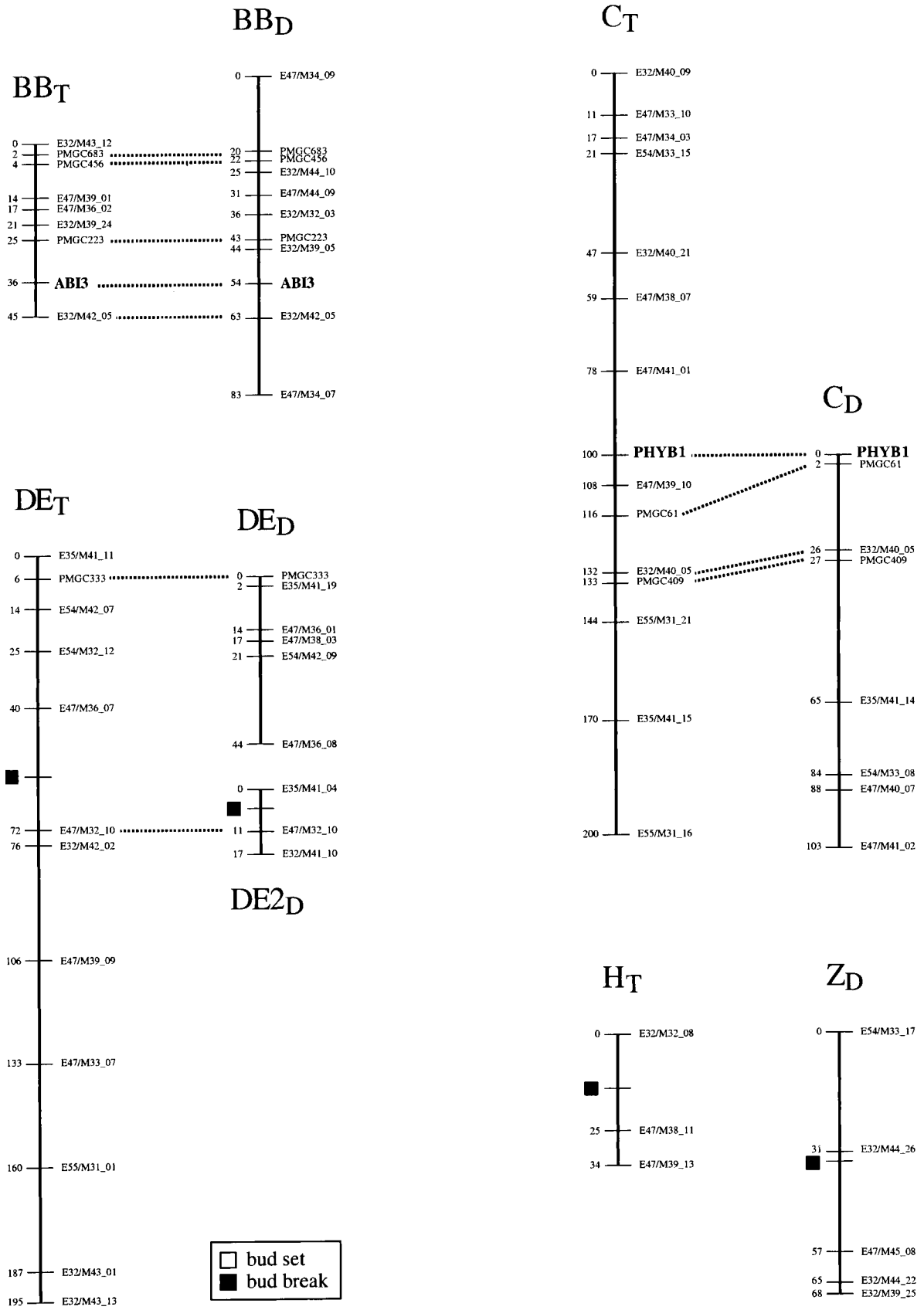

Figure 2.—Linkage map of hybrid poplar family 822. Homologous linkage groups are shown side by side. Lines between homologs indicate the position of codominant AFLP and microsatellite markers used to align the T and D maps (see materials and methods). Candidate genes are shown in boldface.

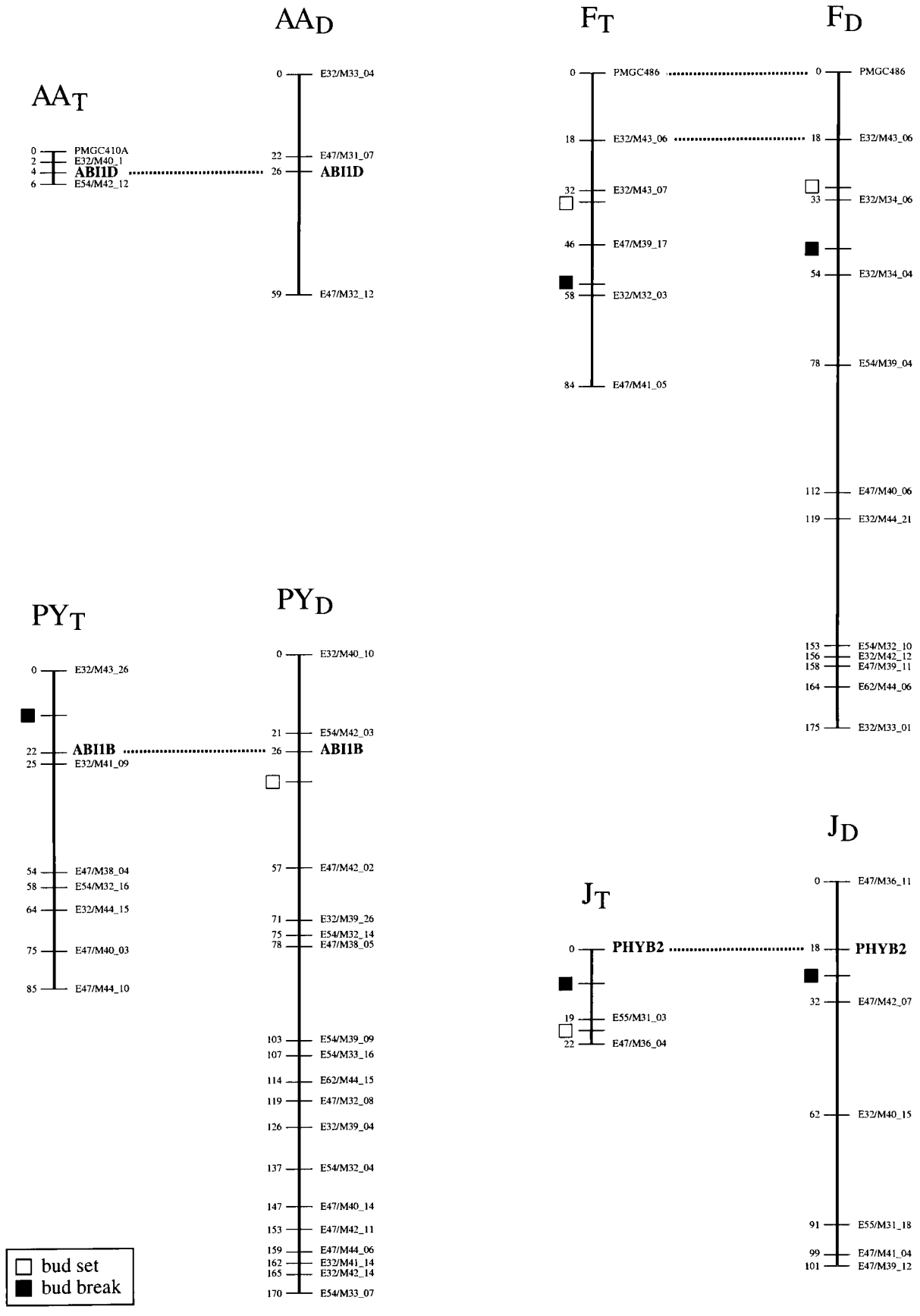

Figure 2.—*Continued.*

TABLE 3

Bud phenology QTL mapped in Family 822

Trait	Linkage group	PVE (%)	LOD	Significance threshold	*P. trichocarpa* homozygote mean at QTL	*P. deltoides* homozygote mean at QTL	Mode of action
Bud set	F_D	12.2	8.8	3.1	266.66	252.58	R
	F_T	11.6	4.6	3.9	267.07	255.97	R
	J_T	6.8	4.8	3.9	260.57	266.67	d
	PY_D	6.0	3.7	3.1	263.82	272.7	r
Bud flush	F_T	16.8	4.5	3.5	75.65	81.33	r
	F_D	9.7	5.7	3.1	76.75	80.95	R
	J_T	9.6	6.6	3.5	79.78	75.54	d
	J_D	9.4	7.2	3.1	79.74	74.724	d
	PY_T	9.1	3.5	3.5	74.05	77.73	d
	DE_T	8.6	5.7	3.5	74.85	79.19	a
	$DE2_D$	6.0	5.2	3.1	75.1	78.638	a
	Z_D	7.2	4.0	3.1	74.98	78.82	a
	H_T	5.9	3.5	3.5	79.33	75.97	d

"Linkage group" refers to the map in Figure 2. PVE, the percentage of F_2 phenotypic variance explained as calculated by interval mapping in MAPMAKER/QTL; LOD, the likelihood ratio (LR) taken from the QTL Cartographer analysis and converted to be equivalent to a LOD score (LR*0.2171 = LOD). Significance threshold was calculated by permutation test (see materials and methods). Phenotypic means of alternative QTL allele homozygotes are shown. The most likely mode of action of each QTL is shown with reference to the *P. trichocarpa* allele. R indicates that the *P. trichocarpa* allele is recessive to the *P. deltoides* allele; a and d stand for additive and dominant, respectively. Uppercase letters are used when the statistical support for the most likely model is LOD ≥1 higher than the alternative models.

volved, their genetic map positions, magnitude of effect, and direction of action.

The F_2 mapping population (family 822) consists of 346 trees, which is adequate for identifying QTL with moderately large effects (van Ooijen 1992; Beavis 1998). However, the three bud set and six bud flush QTL we detected are necessarily a minimum estimate of the total number of QTL controlling bud phenology traits in Populus. Compared with previously reported "major" QTL magnitudes (28.7–55.1 PVE) for bud flush in Populus F_2 family 331 (Bradshaw and Stettler 1995), the QTL we detected in family 822 have notably smaller effects (5.9–16.8 PVE). This is undoubtedly due to an upward bias in magnitude estimates resulting from the small F_2 sample sizes (Beavis 1998) in the original QTL mapping experiment (Bradshaw and Stettler 1995). The estimates of QTL number and magnitude made in family 822 are likely to be more accurate.

There is considerable coincidence in map position among QTL affecting bud set and bud flush, despite the fact that these physiological processes are separated temporally and that the genetic correlation between them is low in family 822 ($r_g = -0.12$). All three unique bud set QTL are found on linkage groups that also contain bud flush QTL, suggesting that pleiotropic effects of single QTL on both traits could result from sharing components of a biochemical pathway.

The direction of QTL allele effects was not always predictable. The simplest expectation is that all *P. trichocarpa* QTL alleles should tend to make buds set and flush earlier, because the geographic origin of the female *P. trichocarpa* parent is 17° further north than that of the *P. deltoides* parent of F_2 family 822 and 9° further north than the *P. deltoides* parent of F_2 family 331. Two of the three bud set QTL (those on linkage groups J and PY) act in this expected direction, as do four of the bud flush QTL (those on linkage groups F, PY, DE, and Z). However, three QTL act in a direction opposite to the simple expectation. There are at least two plausible explanations for the fact that the high-latitude parent is not fixed for QTL alleles that hasten bud set and bud flush. First, because each QTL affects only a modest proportion of the segregating genetic variance, it is possible that directional selection has not been sufficiently strong to fix all of the QTL alleles with the expected effects. A second explanation for the QTL alleles acting in the "wrong" direction is that there may be QTL allelic differences between the *P. trichocarpa* and *P. deltoides* species that override the within-species components of genetic variation. The two species may have arrived at the same adaptive solution to the timing of bud set and bud flush, but via different evolutionary trajectories in which mutations in different sets of QTL were fixed in each species. Only artificial hybridization has brought these historically distinct QTL alleles into the pool of segregating genetic variation. It remains to be seen

whether the same QTL responsible for variation among interspecific F_2 progeny are also relevant within populations of the pure species.

Candidate genes: Once a QTL is identified by linkage mapping, there are relatively few options for determining the identity of the gene or genes that actually are responsible for variation in the phenotype. The method most applicable to tree species is the placement of candidate genes onto QTL maps in an attempt to look for coincidence of map position. This approach is limited by our understanding of the physiology and biochemistry of the trait of interest and by the previous identification of genes potentially involved in trait expression. We had access to a small set of genes ($N = 5$) to test as candidates for bud dormancy-related QTL.

PHYB2: Photoperiod is an important environmental cue in the induction of bud set in Populus. Short days (SD) either induce or accelerate bud set whereas long days (LD) delay or prevent bud set (Vince-Prue 1975; Nooden and Weber 1978). On the basis of the red/far-red reversibility exhibited by SD-induced bud set, it is inferred that SD-induced bud set is controlled by one or more phytochromes in many species, including Populus (Sharrock 1992; Smith 1995; Howe *et al.* 1996). Although there is evidence that different phytochromes control distinct responses (Quail *et al.* 1995; Smith 1995; Furuya and Schafer 1996), little is known about the relative importance of different phytochromes in the control of bud set. The control of growth cessation and bud set by *PHYA* was demonstrated by the overexpression of the oat *PHYA* gene in a hybrid aspen (*P. tremula* × *P. tremuloides*; Olsen *et al.* 1997).

ABI1B: Arabidopsis *ABI* genes were identified as abscisic acid (ABA) insensitive mutants whose seed germination is not inhibited by ABA (Koornneef *et al.* 1984). During seed dormancy the concerted action of *ABI1* and *ABI3* is required for the establishment of dormancy (Koornneef *et al.* 1989). Both proteins are involved in signal transduction; ABI1 is a serine/threonine protein phosphatase 2C and ABI3 is a transcription factor (Giraudat *et al.* 1992; Leung *et al.* 1994, 1997; Meyer *et al.* 1994).

To ask whether seed and bud dormancy may involve similar molecular mechanisms, the Populus homologs of these two Arabidopsis seed dormancy genes were isolated and characterized. Populus *ABI1B* and *ABI3* transcripts were detected in autumn buds of *P. trichocarpa* at the time of vegetative growth arrest. *ABI1B* showed additional expression at the time of bud flush (A. Rohde, unpublished results).

Conclusion: Since the F_2 populations used for these QTL and candidate gene mapping experiments are in strong linkage disequilibrium, associations between candidate genes and dormancy phenotypes are only suggestive and will require more rigorous validation within populations expected to be in linkage equilibrium. Such studies are in progress for *PHYB2* and *ABI1B.* If allelic variants of *PHYB* or *ABI1B* are found to be associated with variation in the timing of dormancy in natural populations of Populus, verification that *PHYB2* and *ABI1B* are QTL may be accomplished by creating a series of transgenic trees with alternative QTL alleles in a common genetic background and observing the phenotypic effect of each allele. Because we are dealing with true QTL—*i.e.*, each candidate gene is expected to have only a modest contribution to the total genetic variance—the phenotypic effects of transgenes are likely to be subtle and will demand large numbers of independent transformations to provide a statistically acceptable sample size.

This work was supported by a postdoctoral fellowship to A.R. from the Vlaams Instituut voor de Bevordering van het Wetenschappelijk-Technologisch Onderzoek in de Industrie (Brussels, Belgium), a grant to G.H. from the National Science Foundation (IBN9421420), a grant to T.H.H.C., H.D.B., and G.H. from the U.S. Department of Agriculture National Research Initiative Competitive Grants Program (9600678), and by the members of the Poplar Molecular Genetics Cooperative.

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Communicating editor: Z-B. Zeng