

Molecular Genetics of Growth and Development in Populus. IV. Mapping QTLs With Large Effects on Growth, Form, and Phenology Traits in a Forest Tree

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ABSTRACT

We have mapped quantitative trait loci (QTLs) for commercially important traits (stem growth and form) and an adaptive trait (spring leaf flush) in a *Populus* F₂ generation derived from a cross between interspecific F₁ hybrids (*P. trichocarpa* × *P. deltoides*). Phenotypic data were collected over a 2-year period from a replicated clonal trial containing ramets of the parental, F₁, and F₂ trees. Contrary to the assumptions of simple polygenic models of quantitative trait inheritance, 1–5 QTLs of large effect are responsible for a large portion of the genetic variance in each of the traits measured. For example, 44.7% of the genetic variance in stem volume after 2 years of growth is controlled by just two QTLs. QTLs governing stem basal area were found clustered with QTLs for sylleptic branch leaf area, sharing similar chromosomal position and mode of action and suggesting a pleiotropic effect of QTLs ultimately responsible for stem diameter growth.

FOREST tree breeding traditionally has been viewed as an application of quantitative genetics (ZOBEL and TALBERT 1984). In some ways this approach has been unavoidable since most classical genetic studies depend upon resources generally unavailable to forest geneticists, such as inbred lines, collections of characterized mutants and marker stocks. The development of such resources is hampered by the long generation interval, outcrossing mating system and high genetic load typical of forest tree species. Further impetus for the application of quantitative genetics to forest tree breeding arises from the assumption that most commercially important traits, such as stem volume growth, are the result of cumulative, integrated physiological processes that necessarily involve many genes, each with a relatively small contribution to the phenotype.

The fundamental assumptions of the quantitative model in forest tree breeding may now be examined in greater detail using pedigrees that segregate for phenotypic traits of basic and commercial interest. By constructing a detailed linkage map within such a pedigree, quantitative trait loci (QTLs) governing the segregating traits may be located and counted, and their magnitude of effect and mode of gene action elucidated (LANDER and BOTSTEIN 1989).

The genus *Populus* (poplars, cottonwoods and aspens) has several advantages as a model system for forest tree genetics. Interspecific hybridization is usually straightforward (STETTLER *et al.* 1980) and often pro-

duces substantial heterosis for volume growth (above the high parent) in the F₁ (ZSUFFA 1975; HEILMAN and STETTLER 1985). The physiological and anatomical bases of this heterosis have been investigated in some depth (HINCKLEY *et al.* 1989, 1992; CEULEMANS *et al.* 1992). The F₂ of interspecific hybrids segregates for a wide variety of traits, including the growth, form and phenology characters that are the subject of this study. Since most *Populus* are readily propagated sexually and vegetatively, large segregating progenies may be produced easily and their phenotypes assessed in clonally replicated plantations containing side-by-side ramets of the parental, F₁ and F₂ generations.

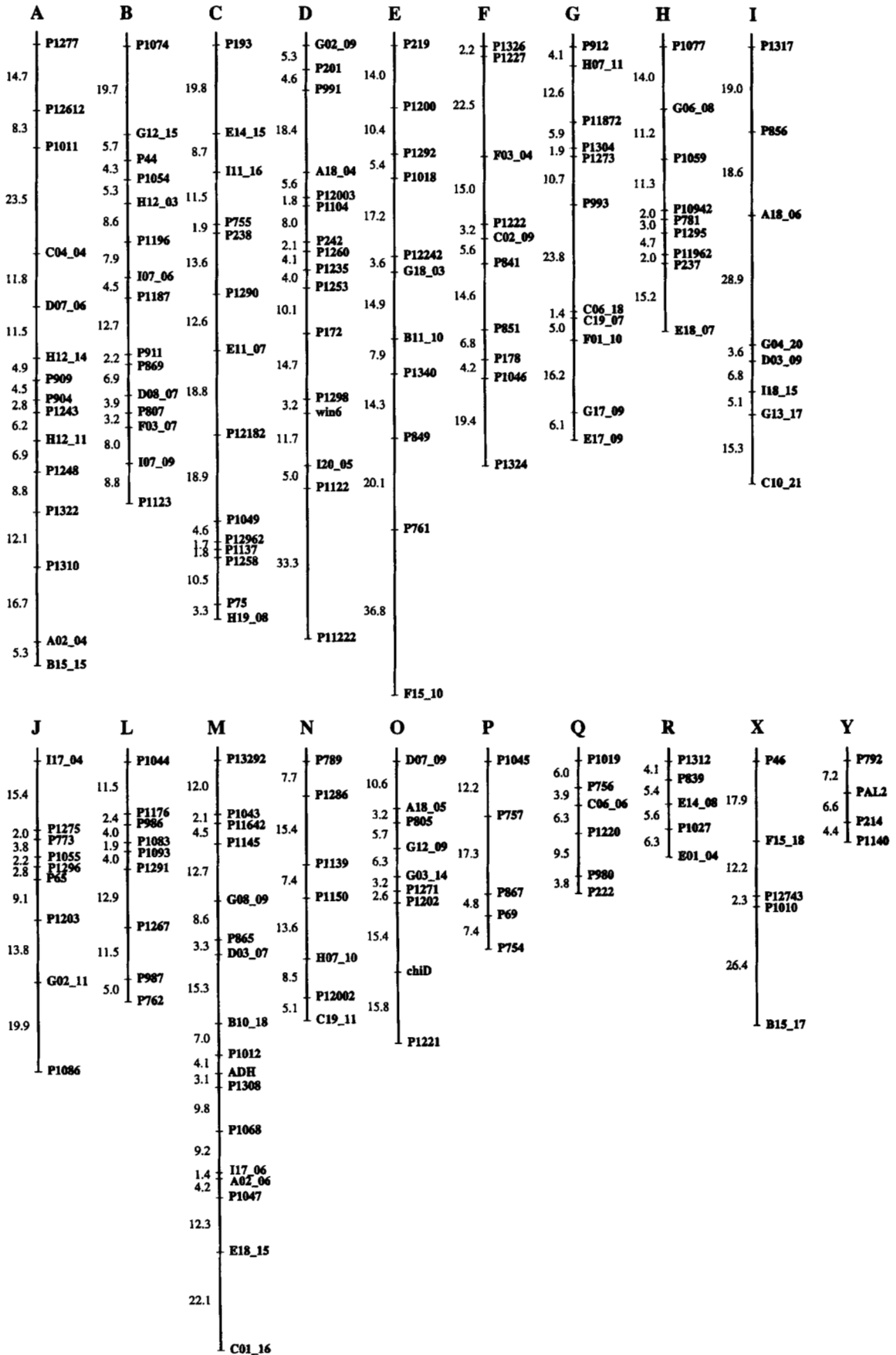
We have used a three-generation *Populus* pedigree founded by interspecific hybridization to construct a linkage map of the *Populus* genome (BRADSHAW *et al.* 1994). In conjunction with a replicated clonal trial we have searched for QTLs with major effects on adaptive and commercial traits: spring leaf phenology, stem proportion and height, basal area and volume growth.

MATERIALS AND METHODS

The mapping pedigree: The QTL mapping pedigree was founded in 1981 by interspecific hybridization between *Populus trichocarpa* (clone 93-968 from western Washington) and *P. deltoides* (clone ILL-129 from central Illinois). Two siblings from the resulting F₁ family (family 53), 53-246 and 53-242, were mated in 1988 to produce the F₂ family 331.

Linkage map construction: The *Populus* genetic map consists of 343 RFLP, STS and RAPD markers and has been described elsewhere (BRADSHAW *et al.* 1994). The map was made from the same pedigree used for phenotypic measurements. To increase the number of informative markers available for estimation of QTL genotypes, additional marker loci found

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on the linkage groups described previously (BRADSHAW *et al.* 1994) were added to the maps using the "place" command in MAPMAKER 3.0, and the most likely marker placement was accepted. These markers had originally been excluded from the published map because they could not be ordered with confidence exceeding a LOD score of 1.3 *vs.* alternative orders. The map used for QTL identification is shown in Figure 1.

Phenotypic measurements: Unrooted cuttings (30 cm) of *P. trichocarpa* 93–968, *P. deltoides* ILL-129, F₁ hybrids 53–246 and 53–242 and 55 F₂ trees from family 331 were planted at 2 × 2 m spacing in a modified randomized complete block design at Washington State University Farm 5 in Puyallup, Washington in the spring of 1991. All cutting material was taken from 1-year-old resprouts on 3-year-old rootstocks, thus minimizing possible position ("C") effects (DUNLAP *et al.* 1992). Each clone was represented by six ramets arranged in two-tree plots in each of three blocks. To minimize undue competition among the F₂ trees, which differ widely in growth potential, the clones within each block were grouped into three size classes according to their growth performance in the seedling bed. Height and diameter growth were measured after 1 and 2 years in the field, while branch and leaf traits were measured after the second year only. Basal area was estimated as $\pi * (\text{basal diameter}/2)^2$. Stem volume index was defined as height * basal area. Date of spring leaf flush was determined in the second year by twice-weekly inspection for the first fully expanded leaf on the main stem. Single leaf area was estimated separately for each branch type (first- and second-year sylleptics, proleptics), based on two sample leaves from each of two sample branches per tree; regression equations were calculated for leaf blade length and width measurements and actual leaf area as determined with a Li-Cor 3100 leaf area meter (R. WU and R. F. STETTLER, unpublished observations). Branch leaf number was estimated by counting leaf scars on two typical branches and multiplying by the total branch number (WU and STETTLER 1994). Broad-sense clone mean heritabilities (H^2) were estimated from the following model using the type III mean squares (MS) calculated by the SAS GLM procedure:

$$\sigma_{\text{trait}}^2 = \sigma_{\text{rep}}^2 + \sigma_{\text{clone}}^2 + \sigma_{\text{rep} \times \text{clone}}^2 + \sigma_{\text{error}}^2$$

$$H^2 = (\text{MS}_{\text{clone}} - \text{MS}_{\text{rep} \times \text{clone}}) / \text{MS}_{\text{clone}}$$

where σ_{trait}^2 is the total phenotypic variance, σ_{rep}^2 is the phenotypic variance among blocks, and σ_{clone}^2 is the phenotypic variance among clones.

QTL mapping: The 19 largest linkage groups, representing approximately half the estimated length of the Populus genome (BRADSHAW *et al.* 1994), were scanned with MAPMAKER-QTL 1.1 (PATERSON *et al.* 1988; LINCOLN *et al.* 1992). Based on the scanned length (1527.3 cM) and the average spacing between mapped markers (6.7 cM), a LOD threshold of 2.9 was chosen from Figure 4 of LANDER and BOTSTEIN (1989) to reduce to ~5% the probability of falsely assigning a QTL. All genotypic data were encoded as though the pedigree were a cross between inbred lines; *i.e.*, only two alleles, derived either from *P. trichocarpa* (T) or *P. deltoides* (D), were recognized at any locus (BRADSHAW *et al.* 1994). The mode of QTL action was estimated by comparing LOD scores of the various

models (dominant, recessive, additive) in MAPMAKER/QTL and accepting the model with the highest score. Overdominance was inferred if the absolute value of the dominance/additivity ratio was >1 and the unconstrained "free" model had a LOD score more than one unit above any of the standard models (dominant, recessive, additive). Multiple QTL models for each trait were evaluated by MAPMAKER/QTL using the "sequence" function to specify QTL positions. Statistical tests for two-locus epistasis were performed using the SAS GLM procedure (SAS Institute) to test for significance of the interaction term. None of the phenotypic distributions differed significantly from normality as judged by the value of the Shapiro-Wilk statistic calculated using the SAS UNIVARIATE procedure (SAS Institute). A known triploid F₂, 331–1062, was excluded from the analysis (BRADSHAW and STETTLER 1993).

Estimate of heterozygosity: Codominant RFLP data were collected for each F₂ clone at a minimum of 25 loci. The proportion of loci heterozygous [TD/(TT + TD + DD)] was correlated with measures of growth using the SAS CORR procedure (SAS Institute).

RESULTS

Trait distributions: Five traits were chosen for detailed study, with emphasis on characteristics of potential adaptive and economic significance: date of spring leaf flush, stem height:diameter ratio and stem height, basal area, and volume growth. Each of these traits exhibits the continuous distribution of F₂ phenotypes often associated with quantitative traits (Figure 2, A–E). Various patterns of trait transmission are observed. For 2-year height, the mean of the F₁ is coincident with the taller of the two parental clones (T), while the F₂ phenotypes largely are bounded by the parental (T and D) values (Figure 2A). Two-year basal area and stem volume index show the F₁ heterosis (above the high parent) that is typical of many interspecific Populus hybrids and is the basis of most Populus genetic improvement programs (Figure 2, B and C). Height:diameter ratio is similar for the parental clones, lower for the F₁ and transgressive in both directions in the F₂ (Figure 2D). Spring bud flush in the F₂ reveals many transgressive segregants, as well, while the F₁ mean is very close to the "early" (T) parent (Figure 2E).

Stem growth QTLs: Twenty-four growth, branch and leaf traits were selected for initial QTL mapping, principally based upon previously discovered physiological and anatomical correlates of growth potential in hybrid Populus (Table 1) (HINCKLEY *et al.* 1989, 1992). We focused further attention on those regions of the genome with effects on stem basal area and height. Stem height and diameter (from which basal area was esti-

FIGURE 1.—Linkage map of the Populus genome used for QTL mapping. Only the 19 largest linkage groups were used. Marker loci beginning with P are RFLPs (*e.g.*, P1277), RAPD loci are named according to their Operon number and approximate fragment size (*e.g.*, C04_04 is a ~400-bp fragment from primer C4), and a few loci of known identity are named (*e.g.*, ADH is alcohol dehydrogenase). A more complete description of the map may be found in BRADSHAW *et al.* (1994).

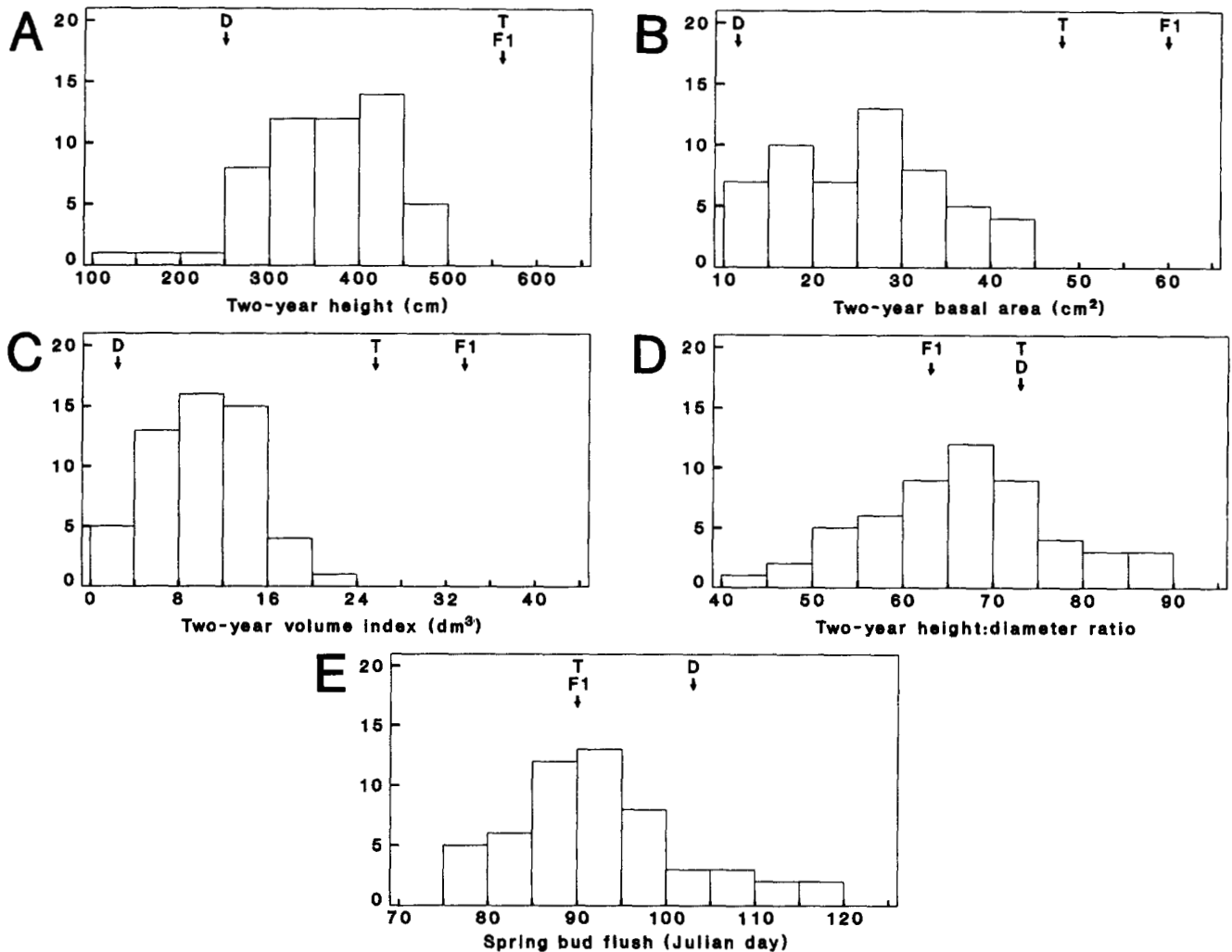


FIGURE 2.—Phenotypic trait distributions based on clonal means in the F₂ generation, with clonal means of the *Populus trichocarpa* parent (T), *P. deltoides* parent (D) and F₁ generation (F₁) shown.

mated) were measured after one and two seasons of growth in the field. Broad-sense heritabilities for height and basal area were fairly high, ranging from 63.5 to 81.7% (Table 2). For first-year stem height, no QTLs with LOD scores > 2.9 were found. A first-year basal area QTL was found on linkage group E (Table 2). It shows a dominant negative effect of the D allele and accounts for 27.2% of the phenotypic variance and 40.5% of the genetic variance in this trait. For second-year height, a QTL was found on linkage group D (Table 2). This QTL accounts for 25.9% of the phenotypic variance and 31.7% of the genetic variance in 2-year height, with the T allele having a positive effect dominant to the D allele. Second-year basal area is partly controlled by a QTL on linkage group O, explaining 24.4% of the phenotypic variance, 38.4% of the genetic variance and having the D allele dominant with a positive effect (Table 2).

We attempted to map QTLs directly affecting second-year stem volume index, which is an excellent predictor of subsequent growth and yield in hybrid poplar for which rotation (harvest) age is 6–15 years. No LOD scores \geq 2.9 were found when 2-year stem volume index was used as the trait variable, but a locus corresponding to the second-year basal area QTL on linkage group O gave a LOD score of 2.15, yielding a probability of 0.38 of detecting a QTL somewhere in the *Populus* genome using the “sparse map” estimator of LANDER and BOTSTEIN (1989). To carry the analysis of the genetic control of stem volume growth further, a model was constructed using the two QTLs identified for the components of second-year volume index: stem height and basal area. The two-QTL model explains 29.4% of the phenotypic variance and 44.7% of the genetic variance in stem volume index (LOD = 3.86). Genotypic data from the marker nearest each QTL were used to test

for epistasis, but no significant interaction term ($P < 0.05$) was found.

Marker heterozygosity as a predictor of growth: Because of the heterosis for growth in the F_1 and the inbreeding depression commonly observed in F_2 progenies (R. F. STETTLER and R. WU, unpublished observations), the relationship between marker heterozygosity and growth was investigated in the F_2 . The range of marker heterozygosity in the F_2 mapping population ($n = 60$, not all of which are represented in the clonal field trial) was 25–69%, with a mean \pm SD of $47 \pm 9.7\%$. Neither height nor basal area in either year was significantly ($P < 0.05$) correlated with heterozygosity as determined by RFLP marker inheritance, and none of the correlation coefficients exceeded ± 0.16 .

Stem form QTL: Height:diameter ratio (HDR; a surrogate for stem taper) was assessed after 2 years of growth. There is a single QTL for HDR on linkage group M (Table 2). The T allele increases the HDR and is dominant to the D allele, as was found for the 2-year height QTL on the same linkage group. This single QTL accounts for 39.0% of the genetic variance in HDR.

Spring leaf phenology: Spring flush had the highest broad-sense heritability of any trait measured ($H^2 = 98.0\%$; Table 2). Five QTLs with major effects on the timing of spring bud break were identified. The QTLs were distributed among five different linkage groups each accounting for 28.7–51.5% of the phenotypic variance (Table 2). Three of the QTLs showed a dominant mode of action, with early flush dominant to late flush regardless of the origin of the allele (T or D) (Table 2). The two remaining QTLs apparently are additive in effect (Table 2). When all five QTLs are combined in a single model, 84.7% of the genetic variance is explained (LOD = 16.63).

Clustering of QTLs affecting growth: Both the first- and second-year basal area QTLs, while on different linkage groups, were found in a cluster of QTLs for branch and leaf traits (Table 3). For this analysis, QTLs associated with radial growth were included if the LOD score exceeded 2.0. Since the region of the genome searched for associated traits was restricted to the linkage groups harboring basal area QTLs, the probability of falsely declaring a QTL remains below 0.05 using the “sparse map” estimator of LANDER and BOTSTEIN (1989). The second-year basal area QTL found on linkage group O is flanked by likely QTLs for sylleptic branch total leaf area (sylleptic branches arise from buds that have not overwintered) and possible QTLs for first-year basal area, basal area increment (from year 1 to year 2), sylleptic branch number and sylleptic branch leaf number (Table 3). At each of the six QTLs the D allele has a dominant positive effect on the phenotype (Table 3).

TABLE 1
Traits mapped

Trait	H^2 (%)	QTL number (LOD \geq 2.9)
Height		
Year 1	68.9	0
Year 2	81.7	1
Height increment		
Year 2	75.0	1
Basal area		
Year 1	67.2	1
Year 2	63.5	1
Basal area increment		
Year 2	62.1	0
Volume index		
Year 1	67.2	0
Year 2	65.7	0
Volume index increment		
Year 2	65.4	0
Height:diameter ratio		
Year 2	85.7	1
Sylleptic branch		
Year 1 number	77.3	0
Year 2 number	80.4	0
Proleptic branch		
Year 1 number	68.3	1
Sylleptic branch		
Year 1 leaf number	76.5	0
Year 2 leaf number	82.8	0
Proleptic branch		
Year 1 leaf number	66.3	1
Sylleptic branch		
Year 1 single leaf area	70.5	1
Year 2 single leaf area	73.0	0
Proleptic branch		
Year 1 single leaf area	78.0	1
Sylleptic branch		
Year 1 total leaf area	58.5	2
Year 2 total leaf area	75.3	2
Proleptic branch		
Year 1 total leaf area	55.2	0
Year 1 origin angle	79.3	0
Spring bud flush	98.0	5

The first-year basal area QTL on linkage group E coincides with QTLs for spring flush, first-year height, second-year basal area, sylleptic branch number and sylleptic branch leaf number (Table 3). The position and mode of QTL action appear similar for first-year basal area, sylleptic branch number and sylleptic branch leaf number with the D allele having a negative effect on both parameters dominant to the T allele (Table 3). The second-year basal area QTL, some distance away, shows the same negative effect of the D allele, but additivity is suggested as the most probable mode of action. However, additivity is only slightly more likely

TABLE 2
Growth, form and phenology QTLs

Trait/linkage group	LOD	Phen. (%)	H ² (%)	Gen. (%)	Mode	Mean TT	Add D	Dom D	d:a ratio
Height year 2 (cm)									
D	3.43	25.9	81.7	31.7	T+ > D-	388.92	-39.06	41.35	-1.06
Height increment year 2 (cm)									
D	3.43	26.1	75.0	34.8	T+ > D-	204.47	-23.46	32.46	-1.38
Basal area									
Year 1 (cm ²)									
E	3.01	27.2	67.2	40.5	D- > T+	5.74	-0.61	-1.39	2.28
Year 2 (cm ²)									
O	2.94	24.4	63.5	38.4	D+ > T-	20.32	3.27	6.86	2.10
Height:diameter ratio year 2									
M	4.04	33.4	85.7	39.0	T+ > D-	66.06	-4.02	9.88	-2.46
Spring bud flush (Julian day)									
G	4.71	41.0	98.0	41.8	T- > D+	88.50	8.26	-5.90	-0.71
M	4.12	34.7	98.0	35.4	D+ (additive)	84.44	8.50	-0.95	-0.11
F	3.96	51.5	98.0	52.6	D- > T+	108.72	-9.01	-9.99	1.11
E	3.21	30.3	98.0	30.9	T- > D+	91.25	7.31	-7.84	-1.07
P	3.18	28.7	98.0	29.3	D+ (additive)	86.00	6.82	-0.18	-0.03

Linkage group refers to the map in Figure 1. Phen., the proportion of the total phenotypic variance explained by the QTL; Gen., the proportion of the total genotypic variance explained by the QTL ($\text{Gen.} = \text{Phen.}/H^2$); >, dominant to; + and -, the direction of effect on the phenotype; Mean TT, the clonal mean phenotype for T/T (*Populus trichocarpa*) homozygotes at the QTL; Add D, the additive effect of the D (*P. deltoides*) allele; Dom D, the dominance effect of the D allele; d:a, the dominance: additivity ratio. Increments (e.g., basal area increment) are calculated by subtracting year one growth from total growth measured in year 2.

(LOD = 2.03) than dominance (LOD = 1.97). At the QTLs on linkage group E for first-year height and spring flush, the effect of the D allele is recessive to the T allele, perhaps implying that these QTLs are distinct from the cluster of QTLs with similar modes of action (Table 3). This argument is further supported for the first-year height QTL, which has its most likely placement at some distance from the basal area QTL (Table 3).

The QTL for 2-year height on linkage group D was not closely coincident with any branch and leaf trait QTLs, although a possible QTL for sylleptic branch number was located >88 cM from the height QTL.

DISCUSSION

Commercially important quantitative traits are not necessarily polygenic in forest trees: Growth and adaptive characters, such as height, basal area, stem proportion and leaf phenology, are generally normally distributed in forest tree populations, and the F₂ of our three-generation *Populus* pedigree is no exception (Figure 2, A-E). Such distributions could be due to genetic control by a large number of unlinked genes each with a small effect on the phenotype, as is assumed in the conventional quantitative "polygenic" model. Alternatively, these traits could be controlled by a few loci with relatively large effects (the "oligogenic" model), with

nongenetic (environmental) variation masking any discrete phenotypes (ALLARD 1960).

Genetic improvement strategies in hybrid *Populus* differ markedly depending upon which of the above models is more nearly correct. Should growth and adaptation be treated as quantitative traits controlled by a large number of unlinked genes and the phenotypes handled as statistical abstractions, or should the breeding strategy focus on identifying, further characterizing and manipulating key QTLs of known number, map position, magnitude of effect and mode of action?

Stem volume is a yield trait, the cumulative result of carbon assimilation and allocation over multiple seasons of growth. Since both assimilation and allocation are influenced by many biochemical pathways, physiological processes and anatomical traits, as well as environmental variables such as water and nutrient status, light intensity and duration, air and soil temperature, daylength and heat sum and perhaps pest and pathogen pressure, it is intuitive that the integration of growth with these variables must be genetically complex. Our QTL mapping results suggest that, for 2-year stem volume growth, ~30% of the phenotypic variance and 45% of the genetic variance are accounted for by just two QTLs in the F₂. This implies that the simplest version of the polygenic model (*i.e.*, many unlinked QTLs of small effect) is not applicable to this F₂ family in the

TABLE 3
QTL clusters associated with stem growth

Trait	QTL position (cM)	Mode	LOD	Gen. (%)
Linkage group O				
Basal area year 1 (cm ²) ^a	6	D+ > T-	2.83	39.9
Basal area year 2 (cm ²) ^a	10	D+ > T-	2.94	38.4
Basal area increment year 2 (cm ²) ^a	11	D+ > T-	2.60	34.1
Sylleptic branch year 1 total leaf area ^b (cm ²)	20	D+ > T-	3.87	48.5
Sylleptic branch year 1 leaf number ^b	20	D+ > T-	2.60	26.8
Sylleptic branch year 1 number ^b	20	D+ > T-	2.00	21.6
Linkage group E				
Basal area year 1 (cm ²) ^a	24	D- > T-	3.01	40.5
Spring bud flush (Julian day)	30	T- > D+	3.21	30.9
Sylleptic branch year 1 leaf number ^b	36	D- > T+	2.63	45.9
Sylleptic branch year 1 number ^b	42	D- > T+	2.11	37.9
Basal area year 2 (cm ²) ^a	73	D- (additive)	2.16	27.4
Height year 1 (cm) ^a	134	T+ > D-	2.38	55.3

Linkage group refers to the map in Figure 1. QTL position the distance from the "top" of the linkage group shown in Figure 1; >, dominant to; + and -, indicate the direction of effect on the phenotype; Gen., the proportion of the total genotypic variance explained by the QTL (as in Table 1).

^a Key growth traits.

^b Measured in year 2.

environment tested. It is an open question whether each of the mapped QTLs represents many tightly linked genes each with a relatively small effect on the phenotype or single genes governing rate-limiting steps in key biochemical pathways.

Height:diameter ratio is a surrogate measure for stem taper and indirectly affects yield and straightness of the bole. Fast-growing trees with low stem taper often have sinuous trunks undesirable for many purposes. Stem straightness is known to be highly heritable in several tree species (ZOBEL and TALBERT 1984, p. 446). As expected, in our Populus mapping pedigree the T allele has the effect of increasing HDR, creating the tall, tapered form characteristic of *P. trichocarpa* (Table 2).

Spring bud flush was studied for three principal reasons: it is an adaptive trait in forest trees (correlated with tolerance of late spring frosts), it segregates transgressively in the mapping pedigree (Figure 2E) and it is known to be under very strong genetic control in Populus (WILCOX and FARMER 1967; YING and BAGLEY 1976; DUNLAP 1991) and other tree species, including conifers such as Douglas fir (ADAMS *et al.* 1994). The two parental trees are from very different climates, with the *P. trichocarpa* female originating in maritime Washington west of the Cascades and the *P. deltoides* male from continental Illinois in the midwestern United States. Unlike the QTL alleles involved in growth and form traits, we did not expect the parental species to

be substantially fixed for the alleles giving rise to phenotypic segregation in the F₂. Since the QTL mapping only considered two alleles (T and D) at any locus, although as many as four RFLP alleles (T₁, T₂, D₁, D₂) could be segregating depending upon the allelic constitution of the F₁s, we anticipated a reduced power to identify QTLs for spring flush. Nevertheless, a large proportion (84.7%) of the genetic variance in spring flush date was accounted for by the simple two-allele model, with contributions from five QTLs. As expected, both T and D alleles were capable of producing "earliness" and "lateness" in spring flush, although "earliness" was dominant to "lateness" regardless of the source of the allele when dominance was the mode of action (Table 2). The D allele at the five QTLs, with one exception, delayed the onset of bud burst, probably due to its northern continental origin and perhaps explaining the success of the oversimplified two-allele model.

Heterosis and inbreeding depression in the Populus mapping population: It is likely that interspecific hybridization and subsequent inbreeding to produce the F₂ have intensified the effects of the dominant QTL alleles contributing to volume growth in this pedigree. The two parental species belong to different sections of the genus (ECKENWALDER 1977) and are adapted to essentially nonoverlapping environments. The heterosis for stem volume growth above the high parent seen

in F_1 hybrids indicates that dominance, overdominance and/or epistasis are more important contributors to phenotypic variance in the F_2 than is simple additive gene action. The phenotypic variation seen in the relatively small F_2 family we have tested is, in fact, much more extreme than one could reasonably expect from an intraspecific F_2 of similar size, facilitating the search for QTLs of large effect. Similarly, close inbreeding is rarely used in operational tree breeding, but simplifies some aspects of QTL mapping by reducing the number of potential QTL alleles segregating in the F_2 . Inbreeding could lead to the identification of "growth" QTLs that are merely expression of the mutational load carried by the parental trees, rather than "growth" QTLs that are generally reflective of all F_2 s derived from the two *Populus* species, and this possibility is discussed below.

P. trichocarpa, native to the geographic area in which the F_2 progeny was tested, is characterized by its excellent height growth potential and prolific rooting from hardwood cuttings. That the major positive QTL allele for 2-year height growth is dominant and derived from the *P. trichocarpa* parent (Table 2) is in concordance with previous expectations resulting from the observation that the T parent of T \times D hybrids is generally as tall as the F_1 (Figure 2A). The heterosis for 2-year stem volume in the F_1 is due entirely to the superior basal area component of growth. By exclusion, this increased basal area must be derived from a net positive contribution of the *P. deltoides* parent. QTL mapping shows that the QTL allele on linkage group O with a positive effect on 2-year basal area comes from *P. deltoides* and is dominant to the T allele. The positive contribution of *P. deltoides* to stem growth could not have been deduced from its poor performance in the testing environment (Figure 2, A–C) but is revealed clearly in the F_2 (see also WU and STETTLER 1994), analogous to the unexpected direction of some QTL allele effects in wide crosses of *Lycopersicon* (DEVICENTE and TANKSLEY 1993).

The control of a large proportion of stem growth by relatively few QTLs and the prevalence of dominant allelic interactions at QTLs with effects on growth traits potentially could be explained by the common mechanism of inbreeding depression. There is the possibility that the dominant "growth" QTLs we have mapped are simply masking deleterious recessives with effects on some component of growth and reflect the genetic load carried by each of the original *P. trichocarpa* and *P. deltoides* parents. This possibility remains to be tested in outcrossed F_2 progenies, but neither of the regions containing a QTL for 2-year height and 2-year basal area shows significant segregation distortion (BRADSHAW and STETTLER 1994). The observed oligo-QTL control of volume growth in the F_2 is unlikely to be due simply to inbreeding depression for another reason:

the parental species contributed QTL alleles beneficial for volume growth in the way expected from traditional genetic tests; *i.e.*, increased height growth from the *P. trichocarpa* parent and net increased basal area from the *P. deltoides* parent. If inbreeding depression resulting from homozygosity of deleterious alleles were the only cause of growth reduction in the F_2 , we might expect that either parent would be equally likely as the source of a beneficial QTL.

The role of dominance in stem growth is perhaps to be expected given the wide interspecific hybrid pedigree we have used for QTL mapping (STRAUSS *et al.* 1992). However, nonadditive (dominance, overdominance and/or epistatic) variance also plays a large role even in intraspecific progenies of *P. deltoides*, where additive genetic variance was estimated at nearly zero while nonadditive variance accounted for 24% of the phenotypic variance in 4-year stem volume index (FOSTER 1985). Nonadditive genetic control of stem growth is also prominent in conifers such as loblolly pine, exceeding the contribution of additive variance for the first 10–12 years of stand development (BALOCCHI *et al.* 1993).

The low and insignificant correlation between marker heterozygosity and growth reinforces the notion that stem growth is not polygenic in this pedigree. If the polygenic model of many unlinked QTLs, each with small effect, were valid for describing growth in this F_2 family, and heterosis above the high parent is due to dominance and/or overdominance, it might be expected that those F_2 offspring approaching the 100% heterozygosity characteristic of the F_1 generation would grow faster than clones with lower heterozygosity. The superiority of F_2 s with high heterozygosity should be further enhanced if inbreeding depression in the F_2 is due to quantitatively distributed deleterious recessive alleles. The lack of correlation between overall marker heterozygosity and growth implies that neither growth nor inbreeding depression in the F_2 are polygenic. The situation in our *Populus* pedigree is quite different from what is seen in maize, where grain yield is highly correlated ($r = 0.68$) with heterozygosity (STUBER *et al.* 1992).

Clustering of QTLs: In forest tree breeding, molecular markers may be less useful for marker-aided selection (STRAUSS *et al.* 1992) than for helping to elucidate the connection between specific genetic loci and the anatomical, physiological and biochemical mechanisms that produce desirable phenotypes (but see WILLIAMS and NEALE 1992). Traditional morphological and physiological predictors of growth have relied upon trait correlations whose genetic basis is unknown or described only in statistical terms. Such studies typically cannot accurately estimate the number of loci underlying the trait, determine the mode of action of the QTLs or

distinguish between linkage and pleiotropy. On the other hand, QTL mapping results may be applicable mainly within the pedigrees in which the experiments are performed and not easily extended to the general population because of presumed linkage equilibrium between markers and the QTLs themselves (STRAUSS *et al.* 1992). A synthesis of the two approaches, using carefully defined genetic materials to understand the processes that govern tree growth and the genetics of those processes, may be a viable long-term strategy to complement traditional tree breeding and selection.

The clustering of QTLs controlling stem basal area growth with those governing sylleptic branch traits (Table 3) is an example of such a synthesis of information. From patterns of carbohydrate allocation determined by tree physiologists, the critical role of sylleptic branch leaf area to radial growth corroborated previous statistical correlations between the traits (HINCKLEY *et al.* 1989, 1992; CEULEMANS *et al.* 1992). Now we have added a genetic description of the anatomical trait. Both QTLs (linkage groups E and O) involved in basal area growth are approximately coincident with QTLs for sylleptic branch and leaf area traits, with remarkable congruence in mode of QTL action and effect of allelic substitution as determined by the sign of the additive and dominance components of the phenotypic variance (Table 3). For example, near the 2-year basal area QTL on linkage group O all the associated sylleptic branch and leaf traits have the same mode of action as the basal area QTL itself (Table 3). The combination of position and mode of action information suggests that pleiotropy of a single QTL is probably responsible for the QTL "clustering" and is more likely than simple linkage to explain the correlations between radial growth and sylleptic branch leaf area observed at the phenotypic level.

Populus as a model for QTL mapping in forest trees: Populus has proven to be a useful choice for QTL mapping in a forest tree, primarily because it is straightforward to generate large amounts of segregating genetic and phenotypic variation by interspecific hybridization. For the same reasons, interspecific hybridization has been used for QTL mapping in crop plants such as tomato (PATERSON *et al.* 1988, 1991) and soybean (KEIM *et al.* 1990a,b). Most conifer breeding does not involve interspecific hybridization, which may limit the applicability of our results; but, among fast-growing hardwoods, such as Populus, Salix and Eucalyptus, interspecific hybridization is perhaps the most promising and widely used method for genetic improvement (ZSUFFA 1975; ZOBEL and TALBERT 1984, p. 349).

The use of interspecific hybrids also permits the Populus mapping pedigree to be treated as a cross between two inbred lines, with the caveat that only those QTLs substantially fixed for alternate alleles in the pa-

rental species will segregate in all such interspecific F_2 families. Furthermore, it is conceivable that within our mapping pedigree QTL effects were downwardly biased from their maximum values because heterozygosity within the parental species was ignored. For example, the *P. trichocarpa* parent could be heterozygous at a QTL, Q_1/Q_2 , and the F_1 offspring could have inherited alternative alleles. Yet, because we have treated the F_1 as the product of a cross between inbred lines, and the sib-mating as a self-fertilization, F_2 offspring scored as *P. trichocarpa* homozygotes at marker loci (T/T) actually would be heterozygous at the linked QTL (TQ_1/TQ_2). Heterozygotes (T/D) at marker loci would represent at least two QTL genotypes, TQ_1/DQ_n and TQ_2/DQ_n . Thus, the phenotype of T/D marker heterozygotes in the F_2 depends on the magnitude of effect and allelic interaction of Q_1 and Q_2 as well as the allelic constitution inherited from the *P. deltoides* parent. In the absence of complete dominance, the estimated effect of the QTL linked to the T marker allele will be lower than the maximum possible effect, which occurs when the QTL allele of largest magnitude is inherited by both F_1 s. The ability of MAPMAKER and MAPMAKER/QTL to infer haplotypes from dominant markers (RAPDs) linked to codominant markers (RFLPs) made possible the use of a large set of RAPD marker genotypes in the F_2 (LINCOLN *et al.* 1992). The prevalence of dominance as a mode of QTL action in the Populus F_2 makes the dominant inheritance of RAPDs less problematic, as well. We have assumed that the allelic variation at QTLs for the traits we have studied is much less within than between the parental species, an assumption that natural selection has produced parents fixed for alternate QTL alleles in the same way that artificial selection is used to produce divergent parents for QTL mapping pedigrees in crops (LANDER and BOTSTEIN 1989). Thus, no distinction was made between T alleles when more than one was segregating in the F_2 , nor between D alleles in the same situation. The reproducible heterosis in the F_1 of all *P. trichocarpa* \times *P. deltoides* crosses (STETTLER *et al.* 1988) suggests that the QTL alleles responsible for heterosis are largely fixed between the two species, but the critical breeding and QTL analysis experiments remain to be performed. In such an analysis, as in more conventional tree breeding programs not making use of interspecific hybrids and close inbreeding, it will be advantageous to follow all parental alleles whenever possible, rather than making assumptions of QTL allele homozygosity in organisms as genetically diverse as forest trees.

Implications of QTL mapping for breeding Populus hybrids: Commercial plantation culture of Populus is distinct from traditional forestry because of the reliance on interspecific hybridization and F_1 heterosis, high productivity (up to 27 Mg/ha/yr; HEILMAN and STET-

TLER 1985), relatively short rotation (time from planting to harvest, typically 4–8 years for a biomass planting and 15–20 years for veneer or solid wood), deployment of clonal material and intensive cultural practices. The benefits from a more detailed understanding of the genetic basis of heterosis in hybrid *Populus* may be realized quickly because of these factors (ABELSON 1991).

Populus breeding programs around the world have relied upon interspecific hybridization and F_1 heterosis to make genetic gains in stem volume growth (ZSUFFA 1975). There has been relatively little work on F_2 and/or backcross breeding (but see JOHNSON 1947; STETTTLER *et al.* 1988), perhaps because the mean performance of F_2 and backcross families is disappointingly low compared to that of F_1 families (Figure 2, A–C). Since *Populus* plantations are typically clonal, however, the mean family performance is relatively unimportant, and the F_2 and backcross generations are richer than the F_1 in variation for many traits of basic and commercial interest. As long as sufficiently large F_2 family sizes can be produced, which is usually not difficult in *Populus*, there is a potential opportunity to recover transgressive segregants above the F_1 phenotypic values. Further, advanced generation breeding, as opposed to recurrent F_1 breeding, could take advantage of useful germplasm from many other *Populus* species, such as *P. nigra* and *P. maximowiczii*, known to contribute desirable alleles in hybrid combination with *P. trichocarpa* and *P. deltoides* (HANSEN *et al.* 1992). These species, distributed across temperate latitudes in the northern hemisphere and adapted to many different growing environments, could be treated as a single gene pool.

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