Theor Appl Genet (1994) 89: 1046–1054

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Quantitative genetics of growth and development in *Populus*. I. A three-generation comparison of tree architecture during the first 2 years of growth

Received: 20. May 1994 / Accepted: 6. July 1994

Abstract One approach to gain an insight into the genetics of tree architecture is to make use of morphologically divergent parents and study their segregating progeny in the F_2 and backcross (B_1) generations. This approach was chosen in the present study in which material of a three-generation pedigree growing side by side in a replicated plantation, was analyzed. The pedigree included *Populus trichocarpa* (T) and *P. deltoides* (D) parents, their F_1 and F_2 hybrids and their B_1 hybrids to the D parent. The trees were grown in the environment of the T parent and measured for the first 2 years of growth. Nine quantitative traits were studied at the stem, branch and leaf levels of tree architecture, in which the original parents differed. Strong F_1 hybrid vigor relative to the better parent (T) was expressed in growth and its components. Most quantitative traits in the F_2 and B_1 hybrids were intermediate between the T and D parents but displayed a wide range of variation due to segregation. The results from the analysis of variance indicated that all morphometric traits were significantly different among F_2 and B_1 clones, but the B_1 hybrids were more sensitive to replicates than the F_2. Broad-sense heritabilities (H^2) based on clonal means ranged from moderately high to high (0.50–0.90) for the traits studied, with H^2 values varying over age. The H^2 estimates reflected greater environmental "noise" in the B_1 than in the F_2, presumably due to the greater proportion of maladaptive D alleles in those hybrids. In both families, sylleptic branch number and length, and leaf size on the terminal, showed strong genetic correlations with stem growth. The large divergence between the two original parents in the traits studied, combined with the high chromosome number in *Populus* (2n=38), makes this pedigree well suited for the estimation of the number of quantitative trait loci (QTLs) underlying quantitative variation by Wright's biometric method (1968). Variation in several traits was found to be under the control of surprisingly few major QTLs: 3–4 in 2nd-year height and diameter growth, a single QTL in stem diameter/height ratio.

Key words Interspecific hybrid · Tree architecture Quantitative genetics · Quantitative trait loci · *Populus*

Communicated by P. M. A. Tigerstedt

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Introduction

The production of woody biomass for fiber or energy is an expanding crop system in which trees are grown under intensive culture in dense plantations (1500–8000 stems/ha), typically on agricultural land, and harvested in rotations of 2–10 years (Ranney et al. 1987). Poplars (*Populus* L.) are widely used for this purpose. Their rapid juvenile growth, ease of vegetative propagation, capacity to resprout after harvest, and diverse utilization options are all attractive to the grower. Much genetic diversity is available both among and within the approximately 30 species worldwide (Eckenwalder 1977) from which to select suitable crop genotypes. Many species can be hybridized and give rise to heterotic F_1 hybrids (reviewed by Zsuffa 1975), although the genetic basis of this heterosis is still poorly understood. Morphological, anatomical and physiological components of productivity have been identified in several species and interspecific hybrids (Hinckley et al. 1989), and ideotypes have been formulated for crop tree improvement (Dickmann 1985).

Tree architecture, i.e., the configuration and relative proportion of stem, branches, and leaves (Tomlinson 1983), is of central importance to both plantation productivity and utilization efficiency in short rotation culture. Pronounced clonal variation and moderately high broad-sense heritabilities (H^2=0.50–0.75) in key architectural and productivity traits have given prominence to clonal selection in poplar improvement (Stettler et al. 1992). Selected cultivars are typically grown in monoclonal stands, or as mosaics of monoclonal blocks (Zsuffa et al. 1993). Combined with the relatively uniform environment and the rel-

atively short time from planting to harvest, this makes for a silvicultural system in which individual-tree architecture and its underlying genetics assume major significance.

In 1991 a study was initiated to address the following questions: (1) How are stem and branch growth, leaf area, leaf distribution and phenology, and biomass allocation genetically controlled during the course of a 6-year rotation in hybrid poplars? (2) To what degree is superior stem growth, the object of pulpwood plantations, genetically associated with other attributes of the canopy? (3) How much genetic gain in yield can be obtained from direct selection as compared to indirect selection for morphometric traits? and (4) how do tree growth and architecture respond to environmental stress? Since both quantitative and molecular approaches were pursued, the study was based on a three-generation inbred hybrid pedigree (*Populus trichocarpa* × *P. deltoides*) which is also serving for the construction of a genetic linkage map for *Populus* (Bradshaw and Stettler 1993, 1994 a, b; Bradshaw et al. 1994) The material was chosen for its significant differences between the two parental species in many morphological, anatomical, and physiological traits (Hinckley et al. 1989) and their segregation in the F_2 and B_1 generations (Stettler et al. 1988). The objectives of the present paper are to describe differences in tree architecture among the female *P. trichocarpa* and male *P. deltoides* parents, two F_1 offspring, their F_2, and their B_1 hybrids to the *P. deltoides* parent, during the first 2 years in a replicated plantation; and, additionally, to estimate the numbers of effective quantitative trait loci (QTLs), broad-sense heritabilities, and genetic correlations for nine stem, branch and leaf traits in the F_2 and B_1 populations.

Materials and methods

Breeding and culture

The material used has been previously described (Bradshaw and Stettler 1993; Bradshaw et al. 1994) and was derived from an interspecific cross between a female *P. trichocarpa* Torr. & Gray (T; clone 93-968) and a male *P. deltoides* Bartr.(D; clone 59–129–17, designated as ILL129). From this hybrid family, two F_1 clones (T × D) with superior growth, female 53–246 and male 53–242, were crossed in 1988 to produce an inbred F_2 family 331 (TD × TD). Female 53–246 was also backcrossed to ILL129 to produce an inbred B_1 family 342 (TD × D). Seedlings from both families were raised in the greenhouse, then transferred outside. At the end of the first growing season, they were transplanted to a nursery site at Farm 5 of the Washington State University Research and Extension Center in Puyallup, Washington. Planted at a spacing of 1×1 m, they were cut back annually as stools and most of them developed multiple re-sprouts. A severe frost in February of 1989 caused some mortality, especially among the smallest of the F_2 plants.

In spring 1991, a replicated plantation was established next to the nursery site at Farm 5 with 20-cm unrooted cuttings from this material. The replicate contained six ramets of each of 55 and 53 genotypes of Families 331 and 342, respectively, both F_1 parents, and both original parents (93–968 and ILL 129). A triploid clone identified in Family 331 (Bradshaw and Stettler 1993) was excluded from all genetic analyses. The plantation was laid out in a randomized complete block design with three replicates and two-tree plots, at a spacing of 2.0 m × 2.0 m and with two border rows. To minimize competitive interactions among this highly varied material, each genotype was assigned to one of three size classes, based on its first 3 years' growth in the nursery. The three classes were laid out perpendicularly to the blocks in ascending and descending order.

Soils and climate at Farm 5 have been described previously (Weber et al. 1985). The plantation was irrigated as needed during the first growing season with an overhead sprinkler system. Weed control with glyphosate was conducted at appropriate intervals during both summers.

Morphometric measurements

Morphological measurements were taken for each tree in the first two growing seasons (1991 and 1992) at three levels of biological organization: stem, branch, and leaf. Stem height increment (HTI) and basal area increment (BAI) were measured and the ratio of total basal diameter to total height (DHR) calculated at the end of each year. Branch traits included number, size, and angle (see Honda 1971) of sylleptic branches for each of the two stem height increments. A sylleptic branch is the result of the continuous development of a lateral bud without an evident intervening period of rest (Hallé et al. 1978; Tomlinson 1983). Sylleptic branch number (SBN) was counted and two representative branches were measured to estimate average branch length (SBL) and adaxial angle to the stem (SBA). First-year sylleptic branch angle (SBA) averages were based on four branches spanning the range from the upper to lower positions along the stem. Single leaf area (SLA), width/length ratio of leafblade (WLR), and petiole length/leafblade-length (PLR) were determined on 2–4 mature leaves of the current terminal with a leaf plastochron index (LPI) of 8 to 12 (Larson and Isebrands 1971). SLA was measured with a Li-Cor 3100 area meter to an accurancy of 0.5 cm^2 for the 1991 leaves and estimated with an empirical regression equation ($SLA=-0.37+0.80\ LL \cdot LW$, $r_2=0.92$) of leaf area on leafblade length (LL) and width (LW) for the 1992 leaves.

Data analysis

The first analysis of variance (ANOVA) and covariance (ANCOVA), a three-way analysis model, was used to test the effects of clone, age, and replicate and their interactions on tree morphological development of F_2/B_1 hybrids. The second, a two-way ANOVA and ANCOVA model, which includes clone, replicate and clone replicate interaction effect, was used to estimate genetic parameters of morphometric traits at the two ages. The variance and covariance components in the two models were calculated by equating the mean squares with the expected mean squares derived from Type III sums of squares, PROC GLM (SAS institute Inc.). Broad-sense heritabilities on a clonal mean basis (H^2), coefficients of genetic variance (CGV), and genetic correlations (r_g) for the nine growth and morphological traits, were estimated according to Falconer (1989). A method for estimating the standard error of broad-sense heritability on an individual-tree basis, suggested by Singh et al. (1993), was modified to calculate the standard error of the clonal mean heritability. Tests for differences in heritability values between the two ages were based on bootstrapped confidence limits for pairwise differences (Mitchell-Olds 1986), using 500 bootstrap trials per test.

The method of estimating the minimum number (n_E) of QTLs that segregate for a trait in an F_2 generation was proposed by Wright (1968). The assumptions of Wright's method include additive gene action, unlinked loci, and equal allelic effects at all loci, with all increasing or decreasing QTLs to be fixed in the two inbred parental populations. Lande (1981) suggested that the same method could also be applied to two heterogeneous parent populations and their crosses. Carson and Lande (1984) extended Lande's suggestion to the utilization of variances within a full-sib family derived from variable parental populations. The standard error of the number of effective QTLs ($\tilde{n}_E$) was calculated by Lande's approximate formula (Lande 1981). The number of favorable loci affecting a trait contributed by the lower parent in the cross was estimated by using the formula suggested by Vega and Frey (1980). The number of favorable

loci in the higher-value parent is calculated by subtracting the number contributed to by the lower-value parent from the number estimated to be segregating in the cross. According to Lande (1981) and Zeng et al. (1990), Wright's method underestimates the number of true QTLs when any of the assumptions is violated. Because the mean of the F_1 hybrids deviates significantly from the midpoint of the two parental means for most of the traits studied (see also Stettler et al. 1988), the effects of gene interaction, such as dominance, need to be considered. For this reason, we used Serebrovsky's method of estimating QTL number to minimize the bias due to dominance by combining the B_1 and F_1 into the estimate program (Wright 1968; Cockerham 1986; Zeng 1992; Ollivier and Janss 1993). In addition, Zeng (1992) proposed a modified estimator for the number of QTLs controlling quantitative differences through a systematic examination of the influence of linkage and unequal effects of alleles on Wright's method (Zeng et al. 1990), which is expressed as

$$\tilde{n}_{E^*} = \frac{2r\tilde{n}_E + (z-1)(\tilde{n}_E - 1)}{1 - \tilde{n}_E(1-2r)} \quad (1)$$

where $\tilde{n}_E{}^*$ is the modified estimator for QTL number, considering linkage and unequal effects of alleles at all loci; $\tilde{n}_E$ is Wright's estimator; r is the mean frequency of recombination among the loci which can be estimated based on the number of haploid chromosomes and the genetic length of various chromosomes (Zeng 1992); z is the index of the differences of allelic frequency and effect at various loci. Given the total length of 19 poplar chromosomes to be 2600 cM in this material (Bradshaw et al. 1994), r is estimated as 0.4759 under the assumption of an equal length of all chromosomes. When all alleles are fixed in the two parents (this may be satisfied because of the pronounced divergence betwen the two parents for the traits used in this study) and individual allelic effects are normally distributed and also independent of each other, $z=/2=1.57$ (Zeng 1992). The standard error of $\tilde{n}_E{}^*$ was calculated using eq. 9 in Zeng (1992).

Results

Distribution of phenotypes

The two parentals, T and D, differed for all nine traits (Figs. 1, 2) by approximately two standard deviations or more (data not shown). Both height and radial growth were substantially greater in T trees than in D trees, but the latter had stouter stem proportions (Fig. 1 *c,f*). T trees had more, larger, and more widely angled sylleptics (Fig. 1 *g-l*), as well as more elongate leaves with shorter petioles, than D trees (Fig. 2 *a-f*). Strong hybrid vigor or heterosis was expressed in the F_1 hybrids for stem height (Fig. 1 *a, d*), basal area increment (Fig. 1 *b, e*), and other growth-related traits such as branch length (Fig. 1 *g, j*) and leaf size (Fig. 2 *a, d*). In all cases, F_2 and B_1 progeny means were intermediate between the two parentals but with the backcross being more skewed toward the values of its recurrent D parent for most traits (Figs. 1, 2).

All nine traits displayed continuous variation and approximately normal distribution in both F_2 and B_1 families. There were measurable differences in generation means and phenotypic distribution patterns between the 2 years (Figs. 1, 2). As expected with unrooted cuttings, 2nd-year stem growth increments were greater, particularly in the basal area, resulting in proportionately stouter stems (Fig. 1 *c, f*). Although Family 342 grew more slowly than Family 331 in the 1st year, the two families had virtually the same growth increment in the 2nd year (Table 1). In both families, sylleptic branches on the 2nd-year stem increment were more acutely angled, fewer and smaller in Family 331, but more frequent and larger in Family 342, than those on the 1st-year increment. Also for both families, 2nd-year leaves were much larger, slightly wider and held by a proportionately longer petiole, than 1st-year leaves.

Transgressive segregation, i.e., variation beyond the values of the original parents, was found in several traits. It was especially pronounced in such traits as stem proportion, sylleptic branch angle, and single leaf area, and suggests the presence of both positive and negative alleles in both parental genomes for these traits.

Sources of variation and covariation

The percentages and significance of variance components due to clone, replicate, and age, and their interactions, varied considerably for the nine traits as well as for the two families (Table 2). Although the clonal effect was highly significant for all traits, stem height increment and stem proportion had a higher clonal variance component than basal area increment. For sylleptic branch traits, the clonal effect was stronger in number than in angle and length in Family 331, whereas it was more uniform among the traits in Family 342. The clonal variance component was similar for leaf size in the two families, but differed in leaf shape and relative petiole length between the families. Variation was attributable to replicates in Family 342 but not in Family 331. As expected, tree age had a significant effect on virtually all traits, even proportions. The variance due to clone × age interaction was generally larger than that due to clone × replicate interaction; in fact, in Family 342, the latter was non-significant in six of the nine traits. There was no impact of age × replicate interaction on the variances of almost all traits, but clone × replicate × age interaction varied considerably, ranging from 0 to 13% in Family 331 and from 0 to 29% in family 342.

According to the ANCOVA model, stem height and radial growth were significantly associated with branch morphology and leaf dimension across clones and ages in both families, as well as across replicates in Family 342 (data not given). In both families, the influence of most branch and leaf traits on main stem growth rates was significantly determined by clone×replicate and clone × age interactions, showing considerable divergence among clones in their developmental sensitivity to age or site variation.

The effective number of QTLs and broad-sense heritabilities

Under the assumption of additive gene action, the number of effective loci estimated to segregate in Family 331 varied among the nine traits and between the 2 years (Table 3). For the 1st year, the number was highest for leaf shape

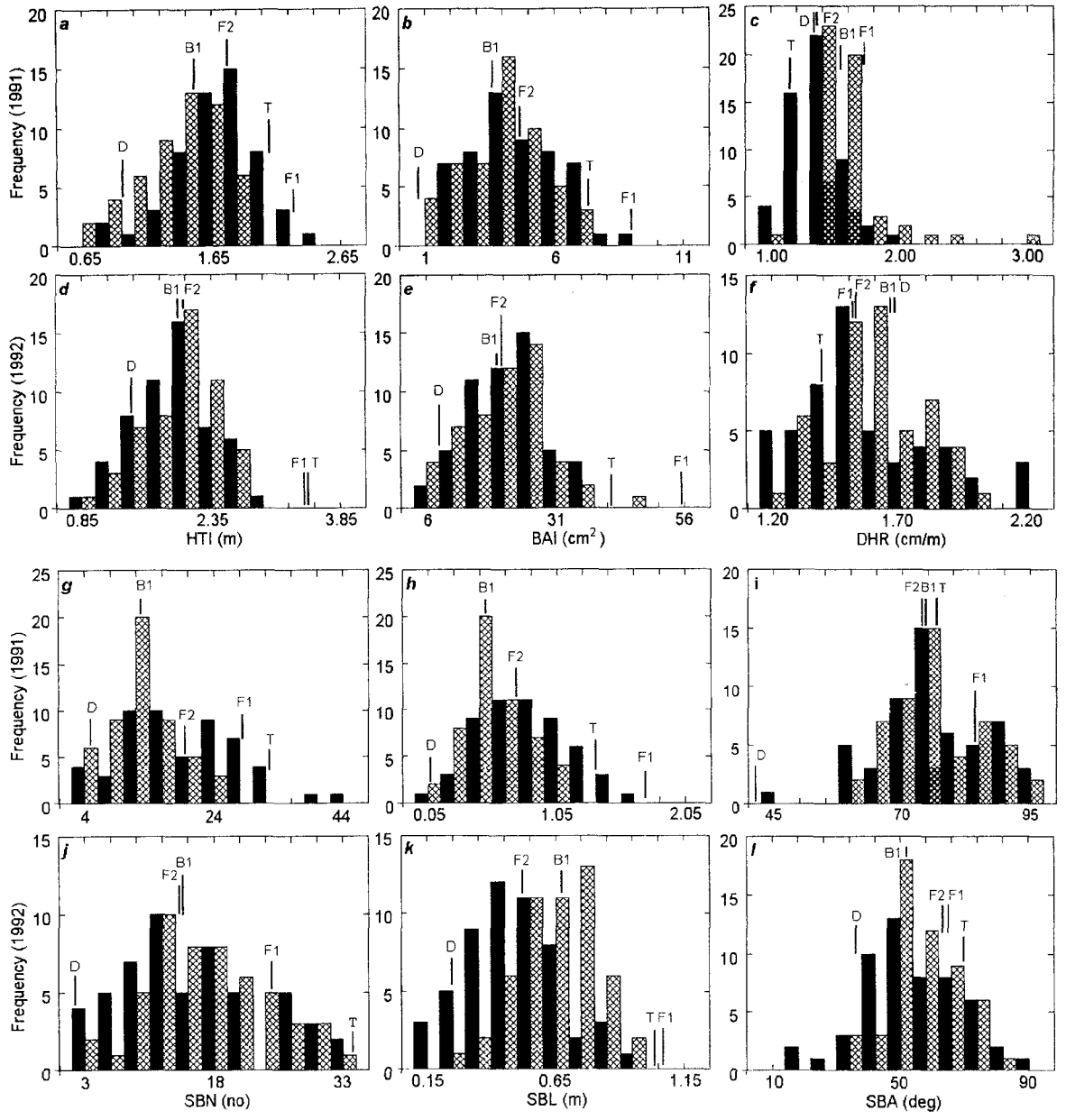

Fig. 1 Distribution of phenotypes for six stem and branch traits measured in the 1st (1991) and 2nd year (1992) in Family 331 (F_2, *black bars*) and Family 342 (B_1, *cross-hatched bars*). Means for the two original parentals (*T and D*), the F_1 parents and the two families are indicated

(4), lower (2–3) for height and radial growth, branch length and number, and relative petiole length, and at the level of a single locus for the variation in stem proportion, branch angle, and leaf size. For the first six traits, the parents contributed unequal numbers of favorable alleles to their progeny. For example, the T parent contributed more favorable alleles to boost stem height, branch number and branch length than the D parent; whereas the reverse was true for radial growth, leaf shape, and relative petiole length. Families 342 (B_1) and 53 (F_1) were used to estimate the number of loci having both additive and dominance gene action (Table 3). In seven out of nine traits, the numbers of QTLs estimated from the additive-dominance model were larger than those from the additive model. In addition, when linkage and unequal effect of alleles at all QTLs were considered, the estimates of QTL numbers were substantially increased for most traits. Finally, there was a general increase in the number of loci estimated for the 2nd year.

Broad-sense heritabilities based on clonal means (H^2) were moderately high to high, with similar values for Families 331 and 342 in most traits (Table 4). Low standard errors for the heritabilities indicated reasonable estimates for them in this study. In Family 331 in the 1st year, H^2 values showed a descending series in the order of relative petiole length (0.90)>branch number and leaf size (0.80) >branch length and angle and stem growth (0.70)>stem proportion (0.50)>leaf shape (0.40). In the 2nd year, these values increased significantly for stem proportion, leaf

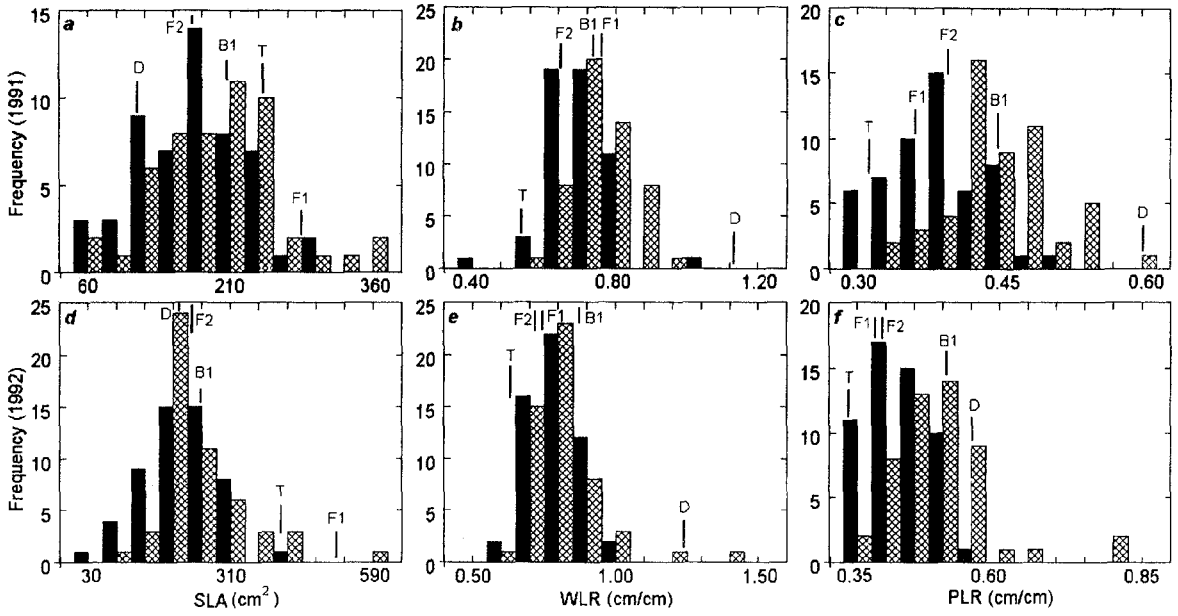

Fig. 2 Distribution of phenotypes for three leaf traits in the 1st (1991) and 2nd year (1992) in Family 331 (F_2, *black bars*) and Family 342 (B_1, *cross-hatched bars*). Means for the two original parentals (*T* and *D*), the F_1 parents and the two families are indicated

shape, and stem height increment, but decreased for branch length, branch angle, and relative petiole length. Stem basal area increment, branch number, and leaf size were relatively stable over the 2 years. More changes in heritability values over the 2 years were found in Family 342.

Coefficients of genetic variation were consistent between families and years, showing the highest values for branch number, branch length, leaf size, and basal area increment, followed by stem height increment and branch angle (Table 4). The three ratios, describing stem proportion, leaf shape, and relative petiole length, had the lowest genetic variabilities in both families.

Genetic correlations

Stem height increment shared about 35% of its genetic variance between the 2 years in both families, whereas stem radial increment had a lower common variance in the 2 years (39%) in Family 331 than in Family 342 (63%) (see diagonal values in Table 5). Conversely, the 1st-year stem proportion was a better indicator of its 2nd-year value in Family 331 than in Family 342. Branch traits, except for branch angle, had higher year-to-year genetic correlations than leaf traits in both families.

The two growth traits were genetically associated with each other, having a tighter relationship in Family 342 than in Family 331 (Table 5). In both families, the correlation between the two traits was much lower in the 2nd year than in the 1st year. Branch traits were less strongly correlated with basal area growth in the 2nd year than in the 1st year, whereas their correlations with height growth were quite consistent between the 2 years. Of the three branch traits, branch length showed the strongest correlation with stem

Table 1 Mean values of nine stem, branch and leaf traits in Families 331 (F_2) and 342 (B_1). HTI stem height increment, BAI stem basal area increment, DHR the ratio of stem basal diameter to total height, SBN sylleptic branch number, SBL average length of sylleptic branches, SBA average angle of sylleptic branches on the current terminal, SLA single leaf area, WLR leafblade width to length ratio, PLR petiole length to leafblade length ratio on current terminal. 1 first growing season (1991); 2 second growing season (1992)

Trait	Family 331 (F_2)		Family 342 (B_1)	
	1	2	1	2
Stem				
HTI (m)	1.72±0.33	1.93±0.48	1.39±0.31	1.97±0.43
BAI (cm^2)	4.60±1.75	21.92±7.56	3.92±1.54	20.65±7.90
DHR (cm/m)	1.38±0.19	1.56±0.27	1.59±0.38	1.59±0.19
Branch				
SBN (no)	19.26±8.97	15.52±8.35	12.49±5.34	16.31±7.02
SBL (cm)	0.83±0.33	0.50±0.18	0.56±0.24	0.64±0.15
SBA (°)	74.79±10.09	53.08±14.11	74.06±8.92	56.61±10.41
Leaf				
SLA (cm^2)	174.08±54.31	213.48±74.63	198.38±64.54	251.78±86.26
WLR (cm/cm)	0.70±0.09	0.79±0.08	0.76±0.08	0.81±0.13
PLR (cm/cm)	0.38±0.05	0.42±0.06	0.45±0.06	0.49±0.09

Table 2 Variance components (in percentages) of nine stem, branch and leaf traits in Families 331 (F_2) and 342 (B_1). See explanation of all traits in **Table 1**

Family/Source of variance	Main Stem			Branches			Leaves		
	HTI	BAI	DHR	SBN	SBL	SBA	SLA	WLR	PLR
Family 331 (F_2)									
Clone (C)	30***	4***	25***	33***	15***	20***	39***	16***	45***
Replicate (R)	0^{ns}	0^{ns}	0^{ns}	0^{ns}	0^{ns}	0^{ns}	0^{ns}	0^{ns}	0^{ns}
Age (A)	10***	66***	12***	3***	22***	11***	26***	17***	3***
C×R	3*	3**	9**	1^{ns}	5*	5*	7***	7*	5*
C×A	14***	11***	5*	15***	16***	19***	8***	12***	7**
R×A	2^{ns}	1*	0^{ns}	2*	1^{ns}	1^{ns}	0^{ns}	0^{ns}	0^{ns}
C×R×A	13***	2*	9**	12***	5*	7*	4**	0^{ns}	3^{ns}
Error	30	13	39	35	36	37	16	48	36
Family 342 (B_1)									
Clone (C)	25***	6***	13***	26***	21***	25***	35***	52***	30***
Replicate (R)	3**	1***	0^{ns}	3***	2***	3***	1***	1***	1***
Age (A)	36***	65***	0^{ns}	7***	3***	8***	32***	7***	10***
C×R	3^{ns}	1^{ns}	6*	0^{ns}	6*	3^{ns}	2^{ns}	3^{ns}	10**
C×A	8**	12***	15***	3^{ns}	13**	10**	9***	6**	7*
R×A	0^{ns}	1**	1^{ns}	0^{ns}	0^{ns}	0^{ns}	0^{ns}	0^{ns}	0^{ns}
C×R×A	7**	1^{ns}	29***	29***	5^{ns}	9*	3*	0^{ns}	9*
Error	18	13	36	32	51	42	18	30	35

*** Significant at $P< 0.001$; ** Significant at $P< 0.01$; * Significant at $P< 0.05$; ^{ns} Non-significant

Table 3 The number of segregating loci governing variation in nine quantitative traits, estimated for each of the first two growing seasons from the additive gene action model (Family 331, F_2), and from the additive dominance gene action model (Family 342, B_1). The number of "favorable" alleles (i.e., causing an increase in a trait) contributed to by the *P. trichocarpa* (left) and *P. deltoides* parentals (right) are given in parenthesis. See explanation of all traits in **Table 1**. $\tilde{n}_E$, Wright's estimator (Wright 1968); $\tilde{n}_E^*$=modified estimator, considering linkage and unequal but normally distributed effects of alleles over the genome (Zeng 1992)

Trait	Family 331 (F_2): Additive				Family 342 (B_1): Additive-dominance			
	$\tilde{n}_E \pm$ SE		$\tilde{n}_E^* \pm$ SE		$\tilde{n}_E \pm$ SE		$\tilde{n}_E^* \pm$ SE	
	1	2	1	2	1	2	1	2
Stem								
HTI	2 ± 0.6 (1 2)	3 ± 0.8 (2 1)	3 ± 1.5	5 ± 2.3	4 ± 1.2	5 ± 1.9	7 ± 1.8	10 ± 3.4
BAI	2 ± 0.6 (2 1)	4 ± 0.7 (1 3)	3 ± 1.5	7 ± 2.2	7 ± 2.3	8 ± 3.4	15 ± 3.5	19 ± 5.1
DHR	1 ± 0.4 (1 1)	1 ± 0.5 (1 1)	1 ± 0.6	1 ± 0.8	1 ± 0.5	1 ± 0.6	1 ± 0.8	1 ± 0.9
Branches								
SBN	2 ± 0.5 (2 1)	2 ± 0.9 (2 1)	3 ± 1.2	3 ± 2.2	3 ± 1.1	3 ± 1.3	5 ± 1.7	5 ± 1.9
SBL	2 ± 0.6 (2 1)	4 ± 1.2 (2 1)	3 ± 1.5	7 ± 3.7	4 ± 2.0	6 ± 2.7	7 ± 3.0	12 ± 4.1
SBA	1 ± 0.5 (1 1)	1 ± 0.6 (1 1)	1 ± 0.7	1 ± 0.9	2 ± 1.0	3 ± 1.2	3 ± 1.5	5 ± 1.8
Leaves								
SLA	1 ± 0.6 (1 1)	2 ± 0.8 (1 1)	1 ± 0.9	3 ± 1.9	3 ± 1.2	5 ± 1.7	5 ± 1.9	10 ± 3.1
WLR	4 ± 1.3 (1 3)	5 ± 2.2 (2 3)	7 ± 2.1	10 ± 3.6	5 ± 2.1	6 ± 3.2	10 ± 3.6	12 ± 5.0
PLR	3 ± 0.8 (1 2)	4 ± 0.7 (1 3)	5 ± 2.3	7 ± 2.3	4 ± 0.7	4 ± 0.8	7 ± 2.1	7 ± 2.4

growth, branch angle the weakest. Leaf size on the current stem was more closely correlated with height increment than with basal area increment in both years in Family 331, as well as in the 2nd year in Family 342. Furthermore, its correlation with radial growth showed a more pronounced decrease in the second year than that with height growth. Leaf shape had moderately low associations with growth traits, whereas relative petiole length had none.

Branch number was strongly and positively correlated with branch length, with a far higher correlation in the 1st than in the 2nd year (Table 5). Yet, these two traits were less tightly associated with branch angle, especially in the second year. Non-significant correlations existed among leaf traits, except between leaf shape and relative petiole length in Family 342. Branch traits had variable, but generally non-significant, relationships with leaf dimensions.

Discussion

This comparison of clonal derivatives from a three-generation hybrid pedigree, grown side by side in a replicated plantation, confirms several earlier findings on poplar growth, while offering additional insight into the underlying genetics. As expected, F_1 hybrids displayed hybrid vigor in growth-related traits, whereas F_2 and B_1 family means were intermediate between T and D parents, with B_1 distributions often skewed to the recurrent parent (D). Allowance has to be made in both families, in terms of missing genotypes and reduced growth for some inbreeding depression. Bradshaw and Stettler (1994 a) found that a recessive lethal allele, inherited from the T parent, caused embryo and seedling mortality in the inbred F_2 Family 331.

Table 4 Broad-sense heritabilities (H^2±SE) based on clonal means and coefficients of genetic variation (CGV, %) of nine stem, branch and leaf traits during each of the first two growing seasons in Families 331 (F_2) and 342 (B_1). See explanation of all traits in **Table 1.** Heritability values not significantly diffferent between two ages were denoted by the same letter

Trait	Family 331 (F_2)				Family 342 (B_1			
	H^2 ± SE		CGV		H^2 ± SE		CGV	
	1	2	1	2	1	2	1	2
Stem								
HTI	0.691 ± 0.023a	0.753 ± 0.024b	16.2	18.0	0.665 ± 0.015a	0.765 ± 0.030b	17.8	17.3
BAI	0.670 ± 0.018a	0.642 ± 0.013a	31.6	26.5	0.602 ± 0.013a	0.704 ± 0.025b	30.2	31.8
DHR	0.530 ± 0.028a	0.874 ± 0.043b	10.4	15.7	0.524 ± 0.012a	0.724 ± 0.026b	13.6	10.6
Branches								
SBN	0.810 ± 0.024a	0.806 ± 0.038a	42.6	47.4	0.641 ± 0.026b	0.574 ± 0.025a	33.6	32.8
SBL	0.735 ± 0.013b	0.666 ± 0.022a	35.0	30.6	0.638 ± 0.015b	0.596 ± 0.011a	34.6	28.9
SBA	0.762 ± 0.012b	0.419 ± 0.012a	11.2	21.9	0.714 ± 0.016b	0.600 ± 0.010a	9.1	15.2
Leaves								
SLA	0.820 ± 0.054a	0.786 ± 0.048a	28.3	31.8	0.784 ± 0.026a	0.877 ± 0.038b	28.3	31.9
WLR	0.425 ± 0.014a	0.791 ± 0.030b	8.6	9.3	0.904 ± 0.043b	0.507 ± 0.021a	10.2	12.8
PLR	0.914 ± 0.047b	0.828 ± 0.039a	12.4	12.5	0.916 ± 0.035b	0.521 ± 0.008a	12.6	14.8

Table 5 Coefficients of genetic correlation between (on the diagonal, in boldface) and within (off the diagonal) the two years for nine stem, branch and leaf traits in Families 331 (F_2, above and including the higher diagonal) and 342 (B_1, below and including the lower diagonal). Values greater than 0.27 (F_2) or 0.28 (B_1) are significant at $P< 0.05$. See explanation of all traits in **Table 1**

Family/ Trait/Age		Stem			Branches			Leaves		
		HTI	BAI	DHR	SBN	SBL	SBA	SLA	WLR	PLR
HTI	1	**0.595**	0.724	–0.345	0.368	0.685	0.521	0.696	0.259	–0.101
	2	**0.608**	0.590	–0.515	0.449	0.627	0.383	0.515	0.328	–0.013
BAI	1	0.835	**0.626**	0.339	0.609	0.762	0.340	0.592	0.161	–0.141
	2	0.678	**0.796**	0.254	0.489	0.613	0.166	0.299	0.173	0.177
DHR	1	–0.421	0.028	**0.015**	0.294	0.092	–0.181	–0.089	–0.083	–0.059
	2	–0.281	0.375	**–0.180**	0.014	–0.086	–0.293	–0.334	–0.144	0.158
SBN	1	0.592	0.644	–0.025	**0.493**	0.870	0.164	0.018	0.001	–0.025
	2	0.629	0.563	–0.149	**0.464**	0.653	0.330	0.045	0.063	0.109
SBL	1	0.749	0.786	–0.063	0.852	**0.423**	0.330	0.352	0.162	–0.027
	2	0.775	0.589	–0.068	0.596	**0.394**	0.374	0.167	0.276	0.233
SBA	1	0.365	0.351	–0.129	0.331	0.247	**0.674**	0.555	0.178	–0.325
	2	0.382	0.195	–0.154	0.260	0.353	**0.502**	0.413	–0.021	–0.181
SLA	1	0.677	0.696	–0.081	0.277	0.432	0.360	**0.684**	0.328	–0.251
	2	0.546	0.234	–0.251	0.186	0.416	0.362	**0.693**	0.139	–0.375
WLR	1	0.339	0.355	0.001	0.121	0.209	0.188	0.185	**0.632**	0.168
	2	0.176	0.227	–0.079	0.176	0.009	0.015	–0.113	**0.628**	0.144
PLR	1	0.081	–0.081	–0.113	0.156	0.043	–0.009	–0.279	0.351	**0.608**
	2	0.045	0.184	0.021	0.224	0.029	0.035	–0.312	0.774	**0.593**

Relative to two outcrossed F_2 families sharing the T parent with Family 331, the inbred F_2 family 331 showed 27.2% inbreeding depression for 3rd-year resprout stem volume index in a coppiced seedling bed (R.F. Stettler and R. Wu, unpublished data). Inbreeding depression may in part be responsible for negative transgressive variation in such traits as stem growth, leaf size and branch length. But transgression in both directions was observed in branch angle, stem proportion and relative petiole length, showing the possibility of expanding the envelope of variation through interspecific recombination.

According to the ANOVA, clone, age, and clone×age interaction were the dominant variance components in both families. Family 342 also showed a significant replicate effect, whereas Family 331 displayed significant clone × replicate interaction in all but one trait. This divergence is likely to reflect the different representation and distribution of *P. deltoides* alleles in the two families. In an earlier study, conducted on the same site, *P. deltoides* had manifested greater sensitivity to site variation and poorer rooting ability than *P. trichocarpa* and F_1 hybrids (Ceulemans et al. 1992). A backcross to *P. deltoides* should share in these properties more, and more uniformly, than an F_2 family.

Estimation of the number of QTLs underlying quantitative trait expression, and of the magnitude and nature of

their effects, can be pursued via biometric (Wright 1968; Lande 1981; Carson and Lande 1984; Zeng 1992) and molecular approaches (Thompson 1975; Doebley et al. 1990; Paterson et al. 1991; Stuber et al. 1992). For both approaches, this interspecific poplar pedigree offers several advantages, including large divergence between the parental species, high chromosome number (2n=38), adequate fertility, and clonal replication of genotypes (see Paterson et al. 1991; Zeng 1992). However, for the biometric treatment the modest sample size in both families calls for caution in the interpretation of estimates (Lande 1981). For the estimation of QTL numbers, modified estimators, obtained by relaxing unlinked and unequal allelic effect and additive gene action, obtain higher values than Wright's method, by which only 'minimum' or 'effective' factors are estimated (Wright 1968; Cockerham 1968; Lande 1981; Zeng et al. 1990; Zeng 1992). Thus, linked QTLs with unequal and/or dominance effects may play an important role in the growth and morphological development of poplar hybrids. It should be noted that even the modified estimators may give underestimates because the material may not satisfy the assumptions of uniform distribution of QTLs over the 19 chromosomes (of uniform length), uniform allelic frequencies, dominance relationships at all QTLs, and normal distribution of allelic effects on phenotypes (Zeng 1992).

Not surprisingly, the analysis revealed that, for most traits, 'favorable' alleles segregating in the F_2 were contributed differentially by the two parents. The *P. trichocarpa* parent provided more favorable alleles for height growth and for the number and length of sylleptic branches than the *P. deltoides* parent, whereas the reverse was true for basal area. These findings, which have been confirmed and extended by molecular analysis (Bradshaw and Stettler 1994 b), help explain the commonly observed heterosis in *P. trichocarpa* × *P. deltoides* F_1 hybrids (Stettler et al. 1988) and implicate functional complementation by the two species as a contributing factor, as earlier suggested by Hinckley et al. (1989). The trait with the (apparently) simplest genetics was stem proportion, which seems to be governed by a single QTL explaining 55–87% of the phenotypic variation. This estimate held for both years and even when the modified estimator was used. The continuous distribution and transgressive segregation of this trait may result from the action of minor QTLs each with positive or negative effects and subject to environmental factors (Anderson et al. 1993; deVicente and Tanksley 1993). More QTLs are implicated for the control of stem growth (3–7) and branching (2–7), depending on the estimators, and accounting for a moderately high to high fraction (64–78%) of phenotypic variation. The lower estimates (2–3) suggest the likelihood of oligogenic control with QTLs as 'leading' factors (e.g., Doebley and Stec 1991; Paterson et al. 1991; 1993; Zeng 1992), i.e., with larger effects on trait variation than assumed by the polygenic model (Coyne and Lande 1985). However, for the higher estimates (5–7), purely quantitative genetic analysis does not permit a reliable discrimination between a model involving some QTLs with major effects, the remainder with minor effects and the polygenic model (all QTLs with equal and small effects). For this purpose, the genetic linkage map of molecular markers constructed for this pedigree (Bradshaw et al. 1994), and the associated QTL analysis (Bradshaw and Stettler 1994 b), offer higher genetic resolution.

One may well expect changes in the genetic control of a quantitative trait over age in a perennial, such as a tree (e.g., Balocchi et al. 1993). For many traits under study, the numbers of effective QTLs (Table 3) and the associated broad-sense heritabilities (H^2, Table 4) were found to change over the 2 years, although the change of the H^2 values was more obvious in Family 342 than in Family 331. Differential QTL expression in 1st- and 2nd-year growth traits was observed by the molecular genetic analysis of the same material (Bradshaw and Stettler 1994 b).

Genetic correlations between traits also showed a recognizable change over the 2 years. Our data confirm the close phenotypic relationships between leaf size, sylleptic branch length and number, and growth rate, as previously found in other studies (Scarascia-Mugnozza 1991;Ceulemans et al. 1992; Hinckley et al. 1992), and show them to have a strong genetic basis, but with the degree changing over the 2 years. Leaf size on the terminal contributes more substantially to growth in the 1st year than in the 2nd year; this indicates that total leaf area (and therefore growth) in the 2nd year is more strongly affected by the number and size of branches. A separate analysis revealed that in 2-year-old trees the sylleptics on the 1st-year height increment were important contributors to basal area growth through branch length and number as well as leaf size and number. By contrast, these components of the sylleptics on the 2nd-year height increment were more strongly correlated with height growth (data not shown).

The first 2 years of growth in a hybrid poplar plantation represent a third of a typical commercial rotation. During this period, canopy closure is reached and the competitive fate among individual genotypes in a stand is decided. Gaining insight into the determinants of tree architecture during this early growth phase is not only of scientific interest but of practical significance, as it bears on the opportunity and criteria for early selection and parental evaluation. This will be further discussed in a second paper in which additional features of canopy development in this material will be presented.

Acknowledgements We thank P. Glackin and S. Wolpow for technical assistance, G. Ekuan and D. Fogle for plantation maintenance, and H. Bradshaw and D. Riemenschneider for helpful comments on the manuscript. Support from the US Department of Energy (19X-43382C), the USDA NRICGP (91–37300–6458), and the Washington Technology Center (09–1022) is gratefully acknowledged.

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