Phenotypic variation in growth and biomass distribution for two advanced-generation pedigrees of hybrid poplar

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Abstract: To assess the genetic control of biomass distribution in trees, phenotypic variation in the distribution of dry mass to stems, branches, leaves, coarse roots, and fine roots was examined in two hybrid poplar (*Populus trichocarpa* Torr. & A. Gray (T) × *Populus deltoides* Bartr. ex Marsh. (D)) families grown under field conditions. Family 331 was an inbred F_2 (TD × TD) pedigree, whereas family 13 was an outbred backcross BC_1 (TD × D) pedigree. Fractional distribution of total whole-tree biomass to shoots and roots during their establishment year averaged (±SD) 0.62 ± 0.09 and 0.38 ± 0.09, respectively, across 247 genotypes in family 331, and 0.57 ± 0.06 and 0.43 ± 0.06, respectively, across 160 genotypes in family 13. In contrast, fractional distribution of total biomass in 2-year-old trees was 0.79 ± 0.04 to shoots and 0.21 ± 0.04 to roots. Allometric analysis indicated that as trees increased in age, biomass was preferentially distributed to stems and branches, whereas distribution to roots declined. Quantitative trait loci (QTL) analysis for family 13 indicated 31 QTL (likelihood of odds >2.5) for traits measured. The percent phenotypic variation explained by any single QTL ranged from 7.5% to 18.3% and averaged 11.2% across all QTL. These results show that aboveground and belowground patterns of biomass distribution are under genetic control. This finding has wide-ranging implications for carbon sequestration, phytoremediation, and basic biological research in trees.

Résumé : Les auteurs ont étudié la variabilité phénotypique de la distribution de la masse anhydre entre les tiges, les branches, les feuilles, les racines et les radicelles de deux descendances hybrides de peuplier (*Populus trichocarpa* Torr. & A. Gray (T) × *Populus deltoides* Bartr. ex Marsh. (D)) cultivées au champ afin de déterminer l'ampleur du contrôle génétique de la distribution de la biomasse chez les arbres. La famille 331 était une lignée endogame F_2 (TD × TD) alors que la famille 13 était une lignée exogame issue d'un rétrocroisement BC_1 (TD × D). La répartition de la biomasse totale de l'arbre entier entre la tige et les racines durant l'année de l'établissement des arbres était en moyenne (± erreur-type) de 0,62 ± 0,09 et 0,38 ± 0,09 pour les 247 génotypes de la famille 331 et de 0,57 ± 0,06 et 0,43 ± 0,06 pour les 160 génotypes de la famille 13. Par contre, chez les arbres âgés de deux ans, ces valeurs étaient de 0,79 ± 0,04 pour la tige et 0,21 ± 0,04 pour les racines. L'analyse allométrique a permis de démontrer que la biomasse était préférentiellement distribuée à la tige et aux branches alors que la distribution aux racines diminuait avec l'augmentation de l'âge des arbres. La détermination des loci quantitatifs (QTL) pour les caractères mesurés chez la famille 13 a produit 31 QTL (LOD >2,5). Le pourcentage de la variation phénotypique expliquée par chaque QTL variait de 7,5 à 18,3 %, avec une moyenne de 11,2 % pour tous les QTL. Ces résultats démontrent que les patrons de distribution de la biomasse entre les parties aériennes et souterraines sont sous contrôle génétique. Ces résultats ont plusieurs implications pour la séquestration du carbone, la phytoremédiation et la recherche biologique fondamentale chez les arbres.

[Traduit par la Rédaction]

Introduction

Trees, like all plants, demonstrate remarkable plasticity in the distribution of dry mass among leaves, stems, and roots (Körner 1994; Poorter and Nagel 2000). Most often, this plasticity is driven by resource availability, including light, soil water, and nutrients (Canham et al. 1996; Ibrahim et al. 1997; Wang et al. 1998). Such phenotypic plasticity, whether in the form of increased leaf mass produced in response to low light or increased root proliferation in response to drought, may promote the competitive ability of a given genotype over a range of resource availabilities, enabling such plants to capture those resources that most strongly limit growth and development (Aerts et al. 1991).

Received 28 September 2004. Accepted 2 May 2005. Published on the NRC Research Press Web site at http://cjfr.nrc.ca on 27 August 2005.

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 doi: 10.1139/X05-101

Genetics also plays a role in determining the distribution of dry mass to leaves, stems, and roots (Ericsson et al. 1996; Zobel 1996; Poorter et al. 2005). In *Populus* — a genus containing more than 30 species worldwide — a number of studies have reported significant clonal variation in biomass distribution (Dickson et al. 1998; Heilman et al. 1994; Karim and Hawkins 1999; Pregitzer et al. 1990; Scarascia-Mugnozza et al. 1997), providing circumstantial evidence that a genetic basis exists for aboveground and belowground dry mass distribution. One of the classic studies in this regard compared patterns of carbon allocation and biomass distribution for two *Populus* clones with contrasting morphology and phenology (Isebrands and Nelson 1983; Michael et al. 1988; Pregitzer et al. 1990). These studies demonstrated that *Populus* × *euramericana* (Dode) Guinier 'Eugenei' consistently allocated more carbon to leaves, branches, and stems, and less to roots than did the clone *Populus tristis* Fisch. × *Populus balsamifera* L. 'Tristis' (Isebrands and Nelson 1983; Pregitzer et al. 1990). However, although these and other early studies helped characterize morphological plasticity in hybrid poplars, most studies have evaluated phenotypic variation in biomass distribution for only a small number of *Populus* clones. As a result, inferences are limited, and there is a need to examine growth and biomass distribution using a larger number of clones, preferably from structured pedigrees (Stettler and Bradshaw 1994), focusing not only on phenotypic variation among clones, but also on identifying loci responsible for quantitative traits such as the total and fractional distribution of dry mass to leaves, stems, and roots (Poorter and Nagel 2000).

Interspecific hybridization and quantitative trait loci (QTL) analysis are techniques used widely in *Populus* to better understand the genetic basis of complex phenotypes (Bradshaw and Stettler 1995; Wu et al. 1998). QTL analysis, along with the recent sequencing of the *Populus* genome (Tuskan et al. 2004; Wullschleger et al. 2002), provides a means not only to verify a genetic basis for biomass distribution in this genus, but also allows initial steps to be taken towards identifying the genes responsible for such traits. In this paper, we report results from a 3-year investigation during which growth and biomass distribution to stems, branches, leaves, coarse roots, and fine roots were determined for 1- and 2-year-old hybrid poplar trees. Since our primary goal was to identify QTL associated with aboveground and belowground traits, two advanced-generation pedigrees suitable for use in QTL mapping were established from cuttings in the Pacific Northwest. Specific objectives were to (1) characterize the extent of phenotypic variation in growth and biomass distribution for 1- and 2-year-old hybrid poplar pedigrees, (2) derive allometric relationships among individual plant components and total tree biomass, and (3) identify QTL associated with the fractional distribution of biomass to leaves, stems, branches, and roots as a means to revealing a genetic basis for growth and biomass distribution in young hybrid poplar trees during the initial 2 years of stand establishment.

Material and methods

Site description and establishment of field plantings

Field plantings were established in 2000 and 2001 at a site near Wallula, Washington. Mean annual precipitation is 220 mm. Trees were grown under drip irrigation and received ca. 360 mm of supplemental irrigation per year. Irrigation lines were placed within rows with an emitter at each planting location. All trees were fertilized with a macro- nutrient and micronutrient solution that was injected into the irrigation lines. Trees received ca. 80 $kg \cdot ha^{-1}$ nitrogen per year. Soils are classified as active dune associated with the Quincy soil series. Mean annual temperature is 12.1 °C.

Two populations of hybrid poplars (*Populus trichocarpa* Torr. & A. Gray (T) × *Populus deltoides* Bartr. ex Marsh. (D)) were used to study phenotypic variation in growth and biomass distribution. Family 331 is an inbred F_2 (TD × TD) pedigree (Bradshaw and Stettler 1993), whereas family 13 is an outbred backcross BC_1 (TD × D) pedigree (Yin et al. 2004*a*). Dormant cuttings were collected from either 8-year-old trees near Clatskanie, Oregon (family 331), in late 1999 or from 5-year-old trees outside Thief River Falls, Minnesota (family 13), in late 2000. Eight cuttings were collected from each of 328 genotypes in family 331 and 171 genotypes in family 13. Cuttings were 15 to 20 cm in length with each having four to five dormant buds. Cuttings were planted the following spring in three blocks at a 1.1 m × 3 m spacing (ca. 3050 $plants \cdot ha^{-1}$) and in a fourth block at a 2.2 m × 3 m spacing (ca. 1525 $plants \cdot ha^{-1}$). Two cuttings were planted at each position. In the case that both cuttings survived and produced sprouts, one was randomly removed early in the season. Plants from the narrow spacing and wide spacing were harvested as 1- and 2-year-old trees, respectively. Genotypes from family 331 were harvested after one growing season (2000), whereas genotypes from family 13 were harvested after one (2001) and two (2002) seasons of growth.

Determination of growth and biomass distribution

Harvesting the aboveground portion of the plants entailed cutting the stem at ground level and separating the plant into stems, branches (if there were any), and leaves. Below- ground portions of each tree, including the original cutting, coarse roots (>2 mm), and fine roots (<2 mm) were carefully excavated and sampled by hand. Roots were removed from a conical volume of soil having a radius of 0.5 m centered on the tree stem and nominally 0.5 m in depth. A hole this size was more than adequate, allowing access to coarse and fine roots in 1-year-old trees. Coarse roots and fine roots were recovered at the time of excavation by sieving soils through 3 mm × 3 mm mesh screens. In the case of 2-year-old trees, the size of the hole was sufficient to enable large coarse roots to be identified. These roots were completely extracted from the soil, which in some instances required following roots for several metres both laterally and vertically until root diameter was less than 1 cm. Belowground biomass resulting from the original cutting was separated from other belowground fractions and then processed along with the rest of the sampled and subsampled biomass.

Fresh mass of all plant components was determined in the field with a battery-operated digital balance (Intercomp, Inc., Minneapolis, Minnesota). A random subsample of stems, branches, leaves, coarse roots, and fine roots was taken, transported to Oak Ridge National Laboratory via overnight express, dried in forced-ventilation ovens at 70 °C for at least 3 days, and then weighed. Dry mass to fresh mass ratios for each sample were used to calculate the total dry

mass of each component. The relative distribution of biomass to leaves, stems, branches, cutting, coarse roots, and fine roots was estimated as a fraction of total dry mass for each tree.

Allometric analysis

Since quantitative metrics that describe the relationship of one plant component to another (i.e., mass fraction and root/shoot ratio) are subject to ontogenetic and environmental influences, biomass data were also analyzed using the following equation:

$$[1] \qquad \log(Y) = \log(\beta) + \alpha \log(X)$$

where X and Y are interdependent variables of plant biomass, and the parameters β and α are regression coefficients. Estimates of β and α obtained in this manner could then be inserted into an allometric equation of the form

$$[2] \qquad Y = \beta X^{\alpha}$$

where β is the allometric constant, and α is the exponential or scaling coefficient. All allometric relationships involving eq. 1 were analyzed using model type I least-square regressions fitted to log–log transformed data (Systat Software, Inc., Point Richmond, California).

Statistical analysis

All growth and biomass distribution parameters were analyzed statistically by analysis of variance (ANOVA) to test for significant ($P \leq 0.05$) differences among genotypes. Analyses were restricted to 1-year-old trees in families 331 and 13, since genotypes were not replicated in 2-year-old trees. Potential shifts in the distribution of total biomass among leaves, stems, branches, and roots due to either pedigree or age were identified by testing for significantly different slopes in the relationship between whole-tree biomass and each plant component. In addition to testing allometric relationships for leaves, stems, branches, and roots plotted against tree biomass, correlation matrices among all plant components were also examined. Box plots were used to graphically depict variation among genotypes for total and fractional distribution of biomass by percentiles. Lower and upper portions of the box indicate the 25th and 75th percentiles, respectively. The values of the bars are the 5th and 95th percentile, and the 50th percentile (median) is given by the horizontal line within the box.

Construction of a genetic linkage map for family 13

Ninety-two microsatellite (simple sequence repeat) and 24 amplified fragment length polymorphism primer pairs generated 556 markers that were used to construct a genetic linkage map from 171 genotypes in family 13. GenScan and Genotyper software (Applied Biosystems, Foster City, California) were used to extract data and score the electro- phoragrams, and processed by PERL scripts. Markers were assigned to linkage groups at a minimum likelihood of odds (LOD) threshold of 10.0 and a maximum recombination fraction of 0.30, with map distances corrected for segregation distortion (Lorieux et al. 1995). The resulting linkage map includes 544 molecular markers mapped onto 19 linkage groups (LG), equivalent to the *Populus* chromosome number, with all markers displaying internally consistent linkage patterns. Complete details of genetic map construction can be found in Yin et al. (2004*a*).

QTL analysis

Phenotypic data were plotted by QTL Cartographer Windows version 2.0. The normality of the trait data was tested by the statistic S:

$$[3] \qquad S = \frac{nk_3^2}{6} + \frac{nk_4^2}{24}$$

where k_3 is the coefficient of skewness, and k_4 is the coefficient of kurtosis. The S statistic is distributed as a χ^2 value with two degrees of freedom, and the critical value for rejection of normality is 5.99 at the 5% level. In the case that raw data for a particular trait did not follow a normal distribution, a log transformation was performed. For traits that could not be normalized in this manner, we removed outlying data points (outside of $\mu \pm 2\sigma$) and retested normality. QTL analysis was performed using Mapmaker/QTL 1.1 software (Lincoln and Lander 1992), setting a LOD score threshold of 2.5 for accepting the presence of a QTL in the mapped interval. Detected QTL were then examined using QTL Cartographer to test for the presence of multiple QTL. Empirical statistical thresholds were set by permutations at significance level $P \leq 0.05$ repeated 300 times (Churchill and Doerge 1994). The QTL effect was expressed as the percentage of phenotypic variation explained (PVE). Only additive effects of the detected QTL are reported because of the nature of the family 13 pedigree (i.e., a "pseudotestcross" from a backcross pedigree).

Results

Phenotypic analysis

Frequency distributions showed considerable variation among genotypes for total biomass and the distribution of biomass to shoots and roots (Fig. 1). The magnitude of this variation was similar between families 331 and 13, with approximately a 55-fold difference between the smallest and largest genotypes within a pedigree. The number of genotypes in a particular size class (i.e., grouped according to dry mass), however, was skewed in family 331, whereas total, shoot, and root biomass in family 13 were more normally distributed. One-year-old trees with a total biomass <200 g dry mass represented 65% of all trees harvested in family 331, but only 20% of the genotypes harvested in family 13 (Fig. 1).

Total biomass averaged (±SD) across 1-year-old genotypes was 197 ± 167 g dry mass in family 331 and 367 ± 224 g dry mass in family 13 (Fig. 2). Shoot and root biomass were similarly larger in family 13. Root-to-shoot ratio averaged 0.65 ± 0.27 in family 331 and 0.77 ± 0.19 in family 13. Fractional distribution of total biomass (i.e., mass fraction) to shoots and roots averaged 0.62 ± 0.09 and 0.38 ± 0.09, respectively, in family 331 and 0.57 ± 0.06 and 0.43 ± 0.06, respectively, in family 13 (Fig. 3). Phenotypic variation in the fractional distribution of biomass to roots was considerable, ranging from 0.13 to 0.64 for trees during their establishment year. Total biomass distributed to aboveground components averaged across the two pedigrees (i.e., 406 genotypes) was 0.29 ± 0.07 for leaves, 0.21 ± 0.06 for stems, and 0.10 ±

Fig. 1. Frequency distributions of total biomass (A and B), shoot biomass (C and D), and root biomass (E and F) for 1-year-old trees from two hybrid poplar pedigrees.

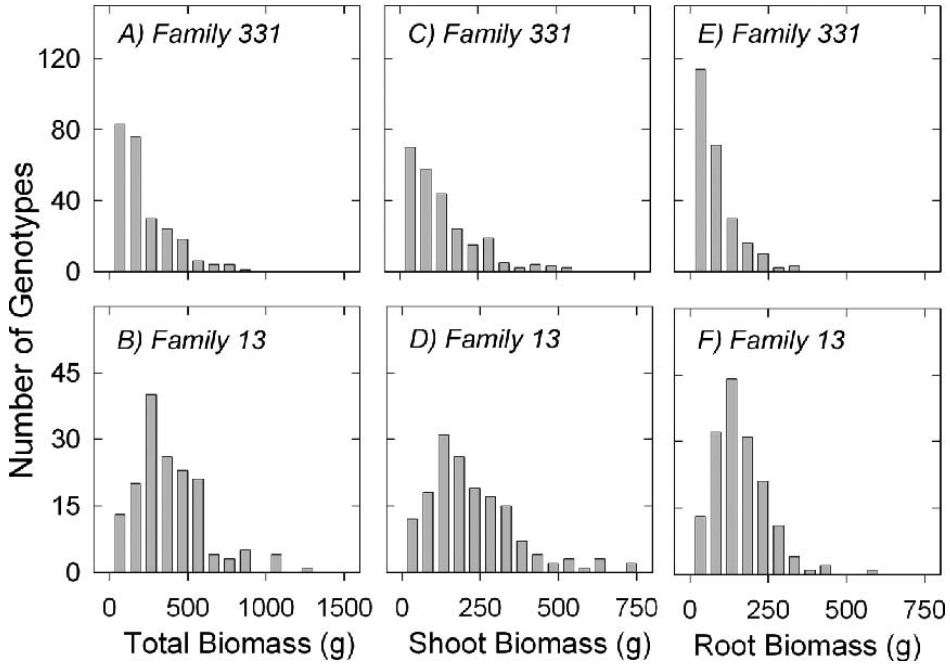
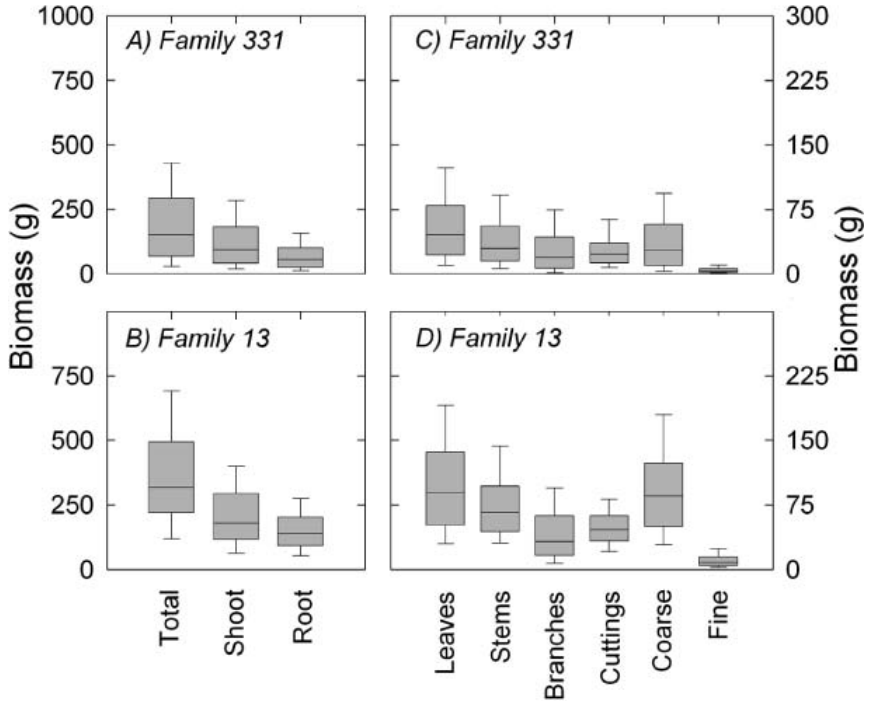


Fig. 2. Aboveground and belowground biomass for two hybrid poplar pedigrees. Data for both family 331 (A and C) and family 13 (B and D) are for 1-year-old trees.



0.07 for branches. Likewise, distribution of whole-tree biomass to belowground components averaged 0.20 ± 0.08 for coarse roots, 0.17 ± 0.07 for cuttings, and 0.03 ± 0.03 for fine roots (Fig. 3).

Strength of correlations among the various aboveground and belowground components of total biomass was variable in both families (Table 1). Nonetheless, all correlations were significant. The strongest correlations were among leaves, stems, branches, cuttings, and coarse roots. Correlations among these individual plant components and fine roots, however, while still significant, were noticeably weaker (Table 1). Allometric analysis of the log–log transformed data indicated significant relationships between shoot, root, and all plant components and total biomass (Fig. 4). There were no differences between families 331 and 13 in either the slope or intercept of these relationships. The most significant re-

Fig. 3. Relative distribution of total biomass (i.e., mass fraction) to shoots, roots, and individual plant components for 1-year-old trees harvested from two hybrid poplar pedigrees.

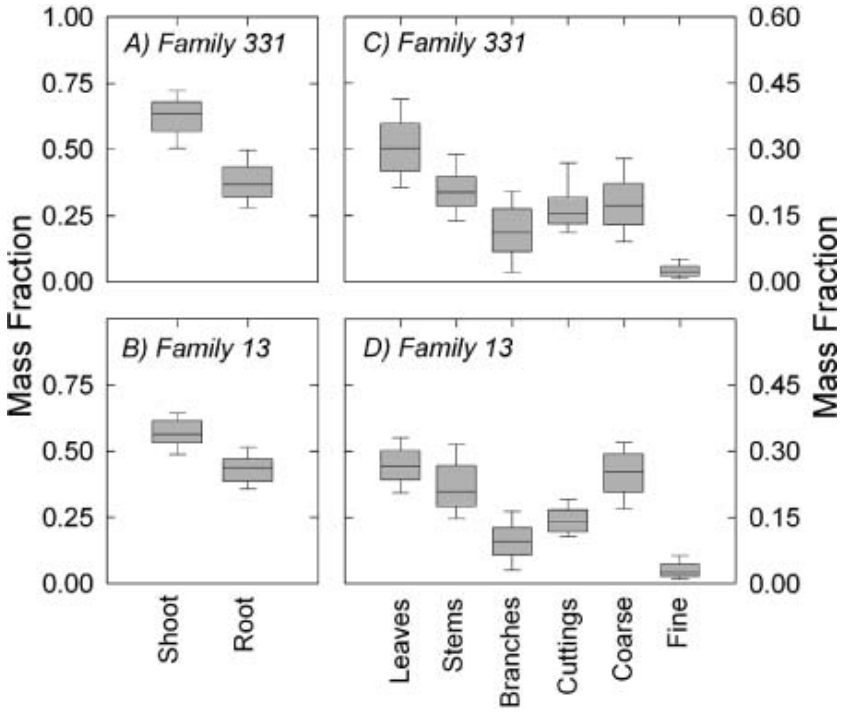


Table 1. Correlation matrices for the distribution of biomass among all plant components for 1-year-old progeny from two pedigrees of hybrid poplar.

	Stem	Branches	Leaves	Cutting	Coarse roots	Fine roots
Family 331						
Stem	1.00					
Branches	0.83	1.00				
Leaves	0.88	0.84	1.00			
Cutting	0.90	0.85	0.86	1.00		
Coarse roots	0.86	0.82	0.80	0.91	1.00	
Fine roots	0.60	0.59	0.62	0.68	0.66	1.00
Family 13						
Stem	1.00					
Branches	0.72	1.00				
Leaves	0.83	0.84	1.00			
Cutting	0.90	0.79	0.86	1.00		
Coarse roots	0.79	0.79	0.79	0.84	1.00	
Fine roots	0.38	0.40	0.44	0.40	0.37	1.00

gression model was between shoot and total biomass, whereas the least significant model was between fine roots and total biomass (Table 2).

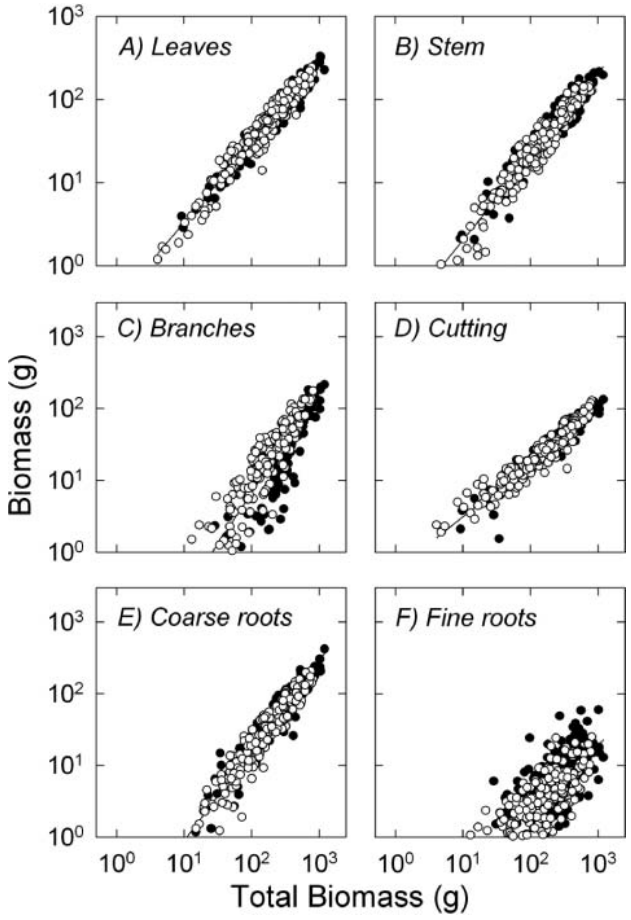
Two-year-old genotypes of family 13 had an average total, shoot, and root biomass of 3455 ± 2115, 2759 ± 1720, and 695 ± 431 g dry mass, respectively (Fig. 5*a*), representing 9.4-, 12.8-, and 4.6-fold increases in total and distributed biomass between first- and second-year growth. Phenotypic variation was large for total and shoot biomass, but remarkably small for roots. Root-to-shoot ratio averaged 0.27 ± 0.07 across genotypes. In contrast to 1-year-old genotypes of this pedigree, the distribution of shoot biomass for 2-year-old trees was stems > leaves > branches (Fig. 5*b*). Fractional distribution of total biomass was 0.79 ± 0.04 to shoots and 0.21 ± 0.04 to roots, again with little variation among genotypes (Fig. 5*c*) Total biomass was distributed among plant components as follows: 0.24 ± 0.03 to leaves, 0.35 ± 0.07 to stems, 0.20 ± 0.05 to branches, 0.09 ± 0.02 to cuttings, and 0.12 ± 0.04 to coarse roots (Fig. 5*d*). Fine roots were not harvested in 2-year-old genotypes.

Correlations among all individual plant components harvested were strong and significant for 2-year-old genotypes (Table 3). The strongest correlations were between leaves and branches, followed by leaves and coarse roots. Allometric analysis also indicated significant relationships when shoot, root, and all plant components when individually plotted against total biomass (Table 4).

QTL detection

All growth and biomass distribution traits in family 13 were associated with at least one QTL, with aboveground and belowground components having similar numbers of QTL (Table 5). There were 14 and 17 QTL detected for 1- and 2-year-old trees, respectively. Generally, these QTL mapped to alternate positions within the genome, with the exception that a single QTL for fractional stem biomass occurred in roughly the same position on LG VI in both the first- and second-year data sets. In addition, six QTL for multiple biomass traits mapped to identical positions within the genome in three cases; for example, total, shoot, root, cutting, coarse root, and branch biomass traits for 2-year-old trees mapped to the same position on LG XIII (Fig. 6). Total and fractional biomass QTL for 1-year-old trees occurred solely on LGs I, II, III, IV, and VII; QTL for 2-year-old trees occurred

Fig. 4. Allometric relationships between individual plant components and total whole-tree biomass. Data are for 1-year-old trees from two hybrid poplar pedigrees. Family 331 data are shown as open circles; family 13 data, as closed circles.



solely on LGs XII, XIII, and XIV; LGs IV and VI shared QTL for both first- and second-year traits. LOD scores for significant QTL ranged from 2.52 to 8.40 and averaged 3.9 (Table 5). The percent phenotypic variance explained (PVE) by any single QTL ranged from 7.5% to 18.3% and averaged 11.2%. Percent PVE did not vary substantially among QTL for either 1- or 2-year-old trees (12.2% and 10.3% average, respectively). The allele with a positive phenotypic effect came from the *P. deltoides* grandparent for 23 of the 31 QTL detected (Table 5). Ten QTL involving biomass distribution mapped to positions indepen- dent of biomass traits (Fig. 6).

Discussion

Field-grown hybrid poplars in this study exhibited patterns of growth and biomass distribution similar to those reported in earlier investigations (Ceulemans et al. 1996; Scarascia-Mugnozza et al. 1997). Total biomass at the end of the establishment year was largely distributed to shoots, consistent with a root/shoot ratio that averaged 0.65 and 0.77 in families 331 and 13, respectively. Previous studies report similar root/shoot ratios for 1-year-old hybrid poplars (Ibrahim et al. 1997; Pregitzer et al. 1990; Dickson et al. 1998), and several document that the distribution of biomass to shoots

Table 2. Allometric coefficients and regression ($\log(Y) = \log(\beta) + \alpha \log(X)$) parameters describing relationships between individual plant components and total tree biomass for 1-year-old plants from two hybrid poplar pedigrees grown near Wallula, Washington.

Plant components	β	α	r^2	F
Shoot vs. total biomass	0.5187	1.0268	0.983	24 241
Root vs. total biomass	0.4468	0.9738	0.958	9 292
Leaves vs. total biomass	0.3573	0.9560	0.948	7 350
Stem vs. total biomass	0.2164	0.9896	0.926	5 059
Branches vs. total biomass	0.0100	1.4126	0.755	1 097
Cutting vs. total biomass	0.5302	0.7620	0.913	4 260
Coarse roots vs. total biomass	0.0504	1.2544	0.910	4 057
Fine roots vs. total biomass	0.0355	0.9162	0.611	631
Shoot vs. root	1.6346	0.9841	0.894	3 406

Note: Genotypes from family 331 and family 13 were combined (n = 406). In all cases $P < 0.001$.

and roots varied among clones (Heilman et al. 1994; Dickson et al. 1998; Scarascia-Mugnozza et al. 1997; Karim and Hawkins 1999). Most studies, however, have evaluated variation in dry mass distribution for a relatively small number of clones or genotypes. As a result, inferences concerning the genetic control of biomass distribution in hybrid poplar have been limited. Variation among several hundred full-sib genotypes for aboveground and belowground biomass distribution, as measured in our study, was considerable. Root/shoot ratios ranged from 0.23 to 1.92 in family 331 and from 0.24 to 1.25 in family 13. Interestingly, in approximately 9% to 14% of the 1-year-old genotypes harvested in each family biomass was distributed equally to roots and shoots (i.e., root/shoot ratio ≥1.0). Most of these genotypes were small, and results could reflect artifacts associated with low rates of biomass production. However, a few genotypes — notably clones 1093, 1732, 1878, and 1706 in family 331 and clones 827, 695, 609, and 659 in family 13 — were large trees when harvested at the end of the establishment year. Thus, while these root/shoot ratios are high, they nonetheless reflect considerable phenotypic plasticity in terms of the fractional distribution of biomass to roots, achieving above-average stem growth while maintaining above-average root growth.

Previous reports for 2-year-old hybrid poplars indicate that total biomass is distributed among plant components in a manner similar to what we observed in this study: stems, 0.30 to 0.50; branches, 0.13 to 0.20; leaves, 0.12 to 0.27; aboveground biomass, 0.62 to 0.81; and belowground biomass, 0.19 to 0.38 (Horwath et al. 1994; Scarascia-Mugnozza et al. 1997; Friend et al. 1991). Comparison of these estimates with values derived for 1-year-old genotypes reveals marked age-related changes in patterns of biomass distribution. King et al. (1999) point out that as plants increase in size and age, the relative distribution of biomass among organs frequently changes. In a study of four poplar clones, Scarascia-Mugnozza et al. (1997) reported that compared with the establishment year, there was a relative decrease in belowground components during the second growing season and an increase in stem, branches, and leaves for all clones. A similar change in biomass distribution was observed in our study for 1- and 2-year-old genotypes, expressed predominantly as an increase in biomass distribution to stems and branches from one year to the next. While total biomass

Fig. 5. Aboveground and belowground biomass (A and B) and the relative distribution of total biomass (C and D) to leaves, stems, branches, and coarse roots for 2-year-old progeny from family 13.

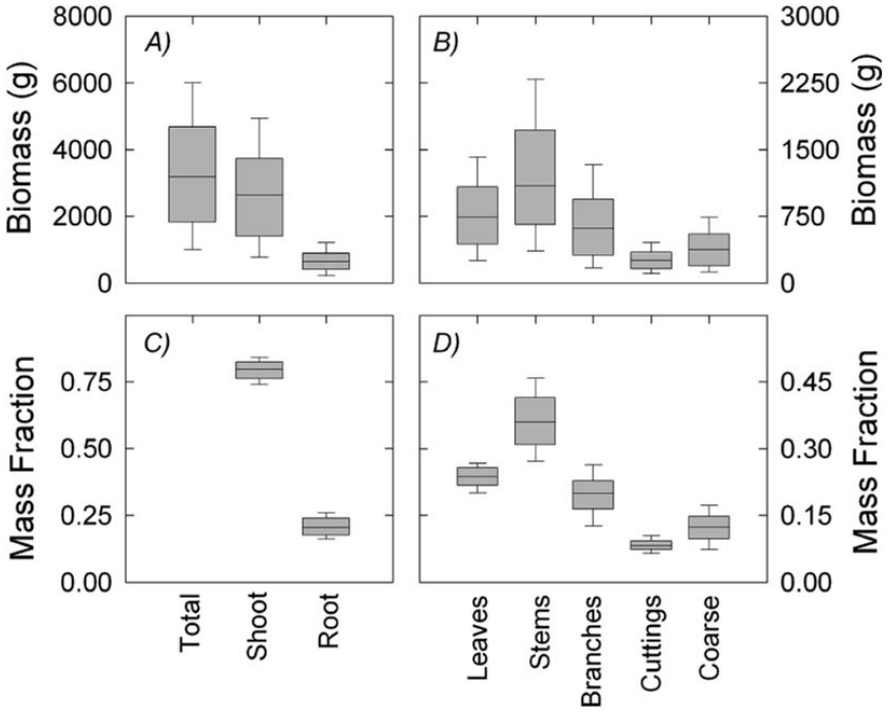


Table 3. Correlation matrices for the distribution of biomass among all plant components for 2-year-old progeny from family 13.

	Stem	Branches	Leaves	Cutting	Coarse roots
Stem	1.00				
Branches	0.78	1.00			
Leaves	0.84	0.96	1.00		
Cutting	0.87	0.79	0.89	1.00	
Coarse roots	0.75	0.75	0.90	0.80	1.00

Table 4. Allometric coefficients and regression ($\log(Y) = \log(\beta) + \alpha \log(X)$) parameters describing relationships between individual plant components and total tree biomass for 2-year-old plants from family 13.

Plant components	β	α	r^2	F
Shoot vs. total biomass	0.6529	1.0241	0.995	25 832
Root vs. total biomass	0.4315	0.9057	0.903	1 327
Leaves vs. total biomass	0.3167	0.9621	0.966	4 095
Stem vs. total biomass	0.2787	1.0298	0.910	1 442
Branches vs. total biomass	0.0852	1.1004	0.890	1 155
Cutting vs. total biomass	0.3330	0.8257	0.903	1 327
Coarse roots vs. total biomass	0.1482	0.9685	0.734	392
Shoot vs. root	3.9326	0.9980	0.856	844

Note: In all cases $P < 0.001$.

for family 13 increased almost 10-fold during the second growing season, there was less than a 5-fold increase in root biomass, but more than a 12-fold increase in shoot biomass. Allometric analysis indicated that as the average genotype increased in age, there was a marked increase in the fractional distribution of biomass to stems and branches and a general decrease in biomass distributed to leaves, cutting, and coarse roots. While multiple studies have examined fractional distribution of biomass in hybrid poplar, few (if any) studies have used allometric analysis to examine variation in biomass distribution among individual plant components. This is despite recommendations that such analyses would help resolve and otherwise avoid size-related complications in interpreting biomass distribution due to environmental and (or) ontogenetic effects (King et al. 1999).

The parental species used in this study, *P. trichocarpa* and *P. deltoides*, and their F_1 hybrids, are known to differ substantially in many morphological, phenological, anatomical, and physiological traits. Such variation is well documented for F_1 progeny and is probably related to the large genetic differences of the two parental species (Stettler et al. 1996). Our interest in using families 331 and 13 was in characterizing the extent of phenotypic variation for advanced-generation (F_2 and BC_1) plant materials. In a study designed to examine nitrogen and phosphorus requirements of a three- generation *Populus* pedigree, Karim and Hawkins (1999) reported that compared with parental species and F_1 clones, the F_2 generation had the poorest growth performance, on average, but exhibited extensive variability in all traits examined. These authors characterized patterns of biomass distribution from which we could calculate the fractional distribution of total biomass to roots (i.e., root mass fraction). For 29 F_2 clones (TD × TD) grown in the greenhouse, root mass fraction averaged 0.24 ± 0.06, with variation among clones ranging from 0.10 to 0.39 (calculated from their table 4). Similar variation in root mass fraction was observed for 1-year-old genotypes from family 331 (0.18 to 0.66) and from family 13 (0.24 to 0.56), and for 2-year-old genotypes from family 13 (0.11 to 0.32) in our field investigation. Moreover, there was no indication in either our study or previous reports that variation in the distribution of biomass to roots was nega-

Table 5. Main characteristics and effects of QTL with a likelihood of odds (LOD) >2.5 for biomass traits measured in family 13.

Trait	Linkage group	Origin of positive allele	PVE (%)	LOD score	LOD threshold
Year 1					
Aboveground biomass	IV	*P. deltoides*	17.0	4.07	2.02
%belowground	I	*P. trichocarpa*	17.9	8.40	1.96
	IV	*P. trichocarpa*	18.3	4.30	1.99
Coarse root biomass	IV	*P. deltoides*	10.5	3.21	2.00
%coarse root	I	*P. trichocarpa*	9.1	4.86	2.43
Cutting biomass	IV	*P. deltoides*	9.7	3.25	2.10
%fine root	II	*P. trichocarpa*	7.5	2.87	1.86
	III	*P. trichocarpa*	11.9	3.04	1.76
Leaf biomass	IV	*P. deltoides*	17.1	4.72	2.12
%leaves	II	*P. trichocarpa*	9.5	4.11	2.18
Stem biomass	III	*P. deltoides*	10.0	4.27	2.18
	VII	*P. deltoides*	8.2	3.79	1.93
%stem	VI	*P. trichocarpa*	9.8	2.68	2.14
Total biomass	IV	*P. deltoides*	14.2	4.05	1.81
Year 2					
Aboveground biomass	XII	*P. deltoides*	8.9	3.08	1.70
	XIII	*P. deltoides*	11.3	4.51	1.82
Belowground biomass	XII	*P. deltoides*	11.1	4.95	1.99
	XIII	*P. deltoides*	9.4	4.68	1.82
%belowground	VI	*P. deltoides*	11.5	2.89	2.17
Branch biomass	XIII	*P. deltoides*	12.5	4.60	1.65
%branch	XIV	*P. deltoides*	8.3	3.06	2.02
Coarse root biomass	XII	*P. deltoides*	11.8	4.08	1.82
	XIII	*P. deltoides*	8.1	2.66	1.94
%coarse root	VI	*P. deltoides*	9.1	3.19	1.82
Cutting biomass	XII	*P. deltoides*	10.2	2.52	2.01
	XIII	*P. deltoides*	10.1	3.36	2.04
Leaf biomass	XII	*P. deltoides*	9.2	4.18	1.77
%leaves	VI	*P. deltoides*	11.8	3.40	1.85
%stem	VI	*P. trichocarpa*	10.8	3.64	1.80
Total biomass	XII	*P. deltoides*	9.8	3.10	1.57
	XIII	*P. deltoides*	11.2	3.97	1.74

Note: Percent traits (e.g., %belowground) are the relative distribution of total biomass to the individual plant components. PVE, phenotypic variation explained. LOD significance thresholds for each QTL were calculated based on permutations.

tively correlated with whole-tree productivity. Thus, increased distribution of biomass to roots in 1- and 2-year-old trees occurred without apparently compromising aboveground biomass production.

QTL have been identified in forest tree species for a wide variety of traits including growth (Wu et al. 1998), chemical and physical properties of wood (Sewell et al. 2002; Grattapaglia et al. 1996), vegetative propagation capacity (Marques et al. 1999), phenology (Bradshaw and Stettler 1995), and tree architecture (Wu 1998). Our study extends this body of evidence to include total and fractional biomass distribution traits. Because QTL have been identified for various growth-related traits in poplar, we compared linkage group positions of the growth and architecture QTL previously identified in family 331 (Wu 1998; Wu et al. 1998; Bradshaw and Stettler 1995) with those identified in the present study using a consensus genetic map for families 13 and 331. Because of uncertainties in linkage group orientation and marker position, our precision is limited to a whole linkage group level. Still, fewer than 50% of the QTL identified in previous studies were on the same linkage groups as QTL identified in the present study. Correspondence was highest for growth and plant architecture QTL (42% and 48%, respectively) reported by Wu et al. (1998) and lowest for growth QTL (i.e., 20%) reported by Bradshaw and Stettler (1995). Such low correspondence among QTL is possibly because of the small number of genotypes examined in the latter study. It is possible that genetic control of growth and biomass distribution is more consistent than the linkage group level comparisons would suggest because of the coarse resolution of the comparison.

Our results suggest that regions of the genome associated with genetic control of the biomass traits of 1- and 2-year-old trees are for the most part independent of one another, which is consistent with observations of QTL across years in other studies in *Populus* (Bradshaw and Stettler 1995), *Salix* (Tsarouhas et al. 2002), and *Eucalyptus* (Verhaegen et al. 1997). This may reflect ontogenetic variation in biomass accumulation (Wu et al. 2003), as demonstrated by marked differences in biomass distribution between 1- and 2-year-old

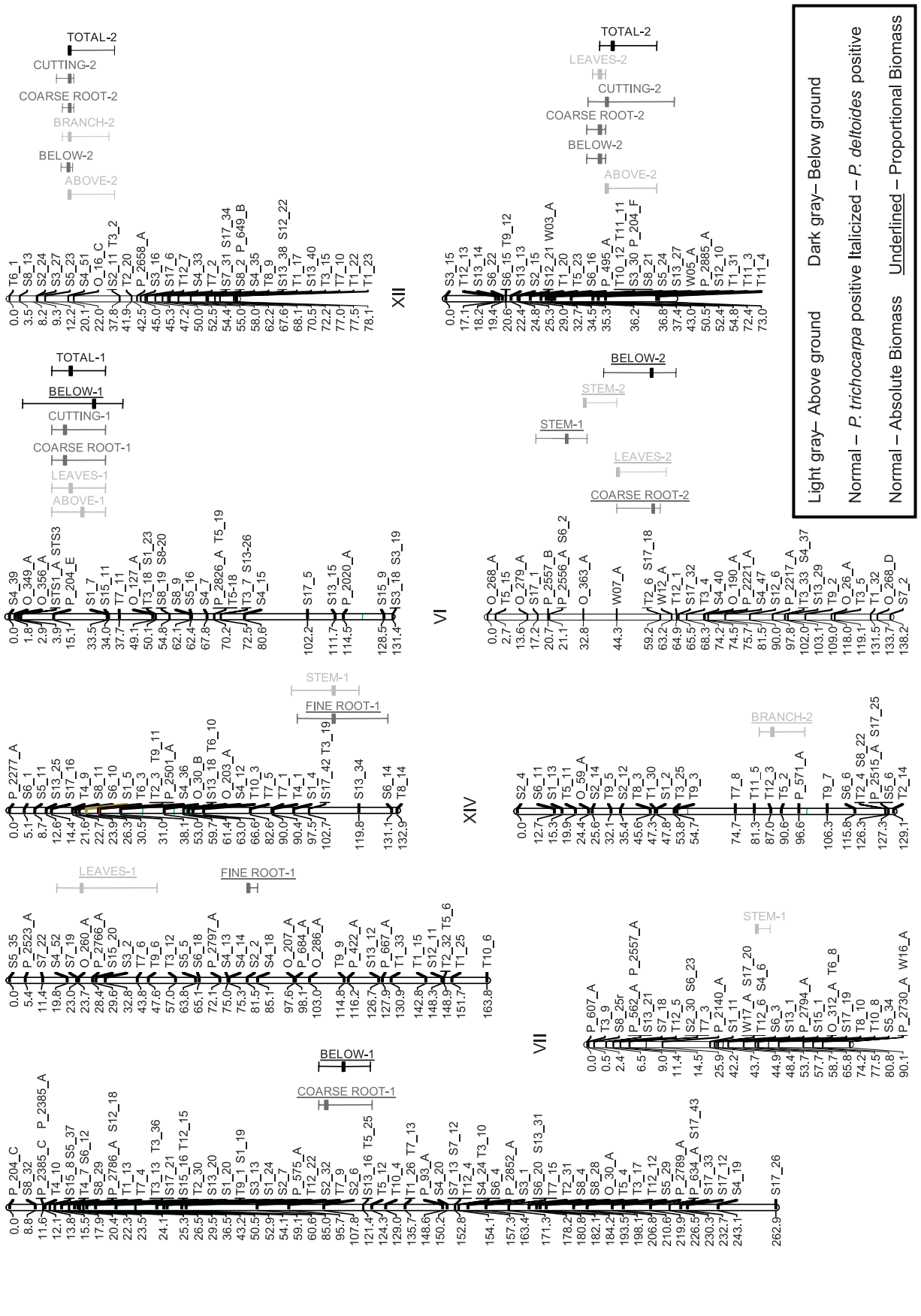


Fig. 6. Graphic representation of quantitative trait loci (QTL) for biomass traits in a (*P. trichocarpa* × *P. deltoides*) × *P. deltoides* hybrid poplar backcross pedigree. Vertical bars indicate the mapping interval for each QTL, delineated by markers with significant trait associations. The peak position of the QTL is indicated by a heavy horizontal line. For details on marker codes, see Yin et al. (2004*a*).

trees (i.e., root/shoot ratios). Alternatively, first-year QTL may reflect establishment effects such as differential rooting, while second-year QTL may be more directly associated with long-term productivity (Wu et al. 1998).

The colocation of multiple QTL was not surprising given that many of the individual components of plant biomass are highly intercorrelated and allometric relationships are relatively invariable within species. However, in some cases traits were associated that had no allometric relationship (e.g., QTL for multiple biomass traits were colocated with a QTL for fractional distribution of belowground biomass for year 1 on LG IV, and QTL for percentage of fine roots was colocated with a QTL for stem biomass on LG III; opposite genetic effect). This suggests that there may be a single gene or regulatory element that has broad pleiotrophic effect on multiple biomass traits. Alternatively, multiple genes with different effects may be located closer than the resolution of our QTL mapping, which may span several megabases of sequence and encompass dozens of candidate genes (Yin et al. 2004*b*). Finally, there may be a functional relationship between traits that are colocated (e.g., genotypes with greater distribution of biomass to roots in year 1 may grow better because of superior capture of resources in year 2). We are unable to differentiate these possibilities in the current study, but with availability of the entire *Populus* genome, including the annotated gene models associated with our genetic map, it will be possible to test candidate genes from within these flanked regions to determine the genetic basis for multiple traits mapping to the same position within the genome (DiFazio 2005).

One of the more noteworthy findings of our study was the independent location of biomass trait QTL and fractional biomass trait QTL. Wu et al. (2002) previously demonstrated that allometric variation in family 331 was under genetic control. However, to our knowledge, our study with family 13 is the first time that independent genetic control of aboveground and belowground biomass traits has been demonstrated in a forest tree. Therefore, if proven true, it should be possible to independently modify distribution of biomass to individual plant components. Confirmation of this will require additional independent assessments of QTL effects to validate both QTL position and magnitude, as well as to expand the environmental and genetic backgrounds under which our estimates were determined.

Conclusion

Considerable variation was observed for the distribution of whole-tree biomass to leaves, stems, branches, and roots in two advanced-generation hybrid poplar pedigrees. We have shown that biomass distribution is not fixed, but varies with genotype and age. While similar results have been reported in the past, our study is unique because of the large number of genotypes investigated and the use of these data to identify QTL associated with whole-tree biomass and the relative distribution of total biomass to individual plant components. Our studies have shown that extensive genetic variability does exist in biomass distribution among F_2 hybrids and that there is a genetic basis for this variation. Thus, phenotypic variation as observed in our study, especially as it relates to biomass distribution to roots, is an asset for future tree improvement programs. Interestingly, Heilman et al. (1994) point out that "…for practical reasons, the process of clonal selection does not usually involve collection of data on root systems", with tree plant breeders and geneticists focusing almost exclusively on traits observable above ground. While this statement is understandable, the importance of root distribution to carbon sequestration, phytoremediation, and to an improved understanding of basic tree biology dictates a focused and informed approach to investigating root–shoot relationships. Studies should seek to explore the mechanistic basis for biomass distribution in trees encompassing a range of ages, taking advantage of existing field plantings and the availability of new technologies for quantifying variation in biomass distribution for trees grown under a range of conditions.

Acknowledgments

Special thanks to Peggy Payne, Larry Miller, Tom Agens, Evan Purser, Robin Rayborn, Andrea Silletti, Steve Thomas, Joanne Ledford, Ramie Wilkerson, Kenneth Lowe, Kim Mangrini, and others who provided assistance with plantation establishment, silvicultural management, field harvests, and processing of samples. Our appreciation is extended to Dr. H.D. (Toby) Bradshaw at the University of Washington, Dr. Brian Stanton at GreenWood Resources, and Dr. Jon Johnson at Washington State University for providing us with clonal material for family 331 experiments. Dr. Don Riemenschneider, US Forest Service, kindly provided clonal material for family 13. We acknowledge support from the US Department of Energy, Office of Science, Biological and Environmental Research Program. This project was supported under the Carbon Sequestration Program. Oak Ridge National Laboratory is managed by UT–Battelle, LLC, for the US Department of Energy under contract No. DE-AC05-00OR22725.

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