Identification of Quantitative Trait Loci Influencing Wood Specific Gravity in an Outbred Pedigree of Loblolly Pine

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ABSTRACT

We report the identification of quantitative trait loci (QTL) influencing wood specific gravity (WSG) in an outbred pedigree of loblolly pine (*Pinus taeda* L.). QTL mapping in an outcrossing species is complicated by the presence of multiple alleles (>2) at QTL and marker loci. Multiple alleles at QTL allow the examination of interaction among alleles at QTL (deviation from additive gene action). Restriction fragment length polymorphism (RFLP) marker genotypes and wood specific gravity phenotypes were determined for 177 progeny. Two RFLP linkage maps were constructed, representing maternal and paternal parent gamete segregations as inferred from diploid progeny RFLP genotypes. RFLP loci segregating for multiple alleles were vital for aligning the two maps. Each RFLP locus was assayed for cosegregation with WSG QTL using analysis of variance (ANOVA). Five regions of the genome contained one or more RFLP loci showing differences in mean WSG at or below the $P = 0.05$ level for progeny as grouped by RFLP genotype. One region contained a marker locus (S6a) whose QTL-associated effects were highly significant ($P > 0.0002$). Marker S6a segregated for multiple alleles, a prerequisite for determining the number of alleles segregating at the linked QTL and analyzing the interactions among QTL alleles. The QTL associated with marker S6a appeared to be segregating for multiple alleles which interacted with each other and with environments. No evidence for digenic epistasis was found among the five QTL.

THE backcross and F_2 populations routinely used for quantitative trait loci (QTL) mapping in crop species have characteristics which simplify linkage mapping and QTL analysis. For backcross and F_2 pedigrees, segregation in the mapping generation results from meiosis in a single parent. As a result, the number of alleles segregating at a locus is restricted to two (assuming a diploid species), and the phase of marker alleles can be determined *a priori*. QTL mapping in loblolly pine (*Pinus taeda* L.) presents additional difficulties. Loblolly pine is an outcrossing and highly heterozygous forest tree for which inbred or close mating pedigrees are not available. Linkage analysis in a pedigree formed from the cross between two unrelated loblolly pine trees is complex, as segregation in the progeny mapping generation results from separate meioses and crossovers in the two parents. For an outbred pine pedigree, as many as four alleles can segregate at a locus in the progeny generation, and the phase of marker alleles must be determined indirectly using grandparent genotypic information or progeny segregation ratios. A QTL segregating for multiple alleles (>2) presents the opportunity for different interactions (*e.g.*, additive, dominant, overdominant, or underdominant) among alleles. Significant deviations from additive gene action at QTL must be characterized for a quantitative trait to be accurately modeled. Tracking the inheritance of multiple alleles at QTL in an outbred pedigree necessitates the use of codominant, multiallelic markers.

Use of molecular markers and genetic maps has provided new insight into the nature of quantitative traits in some crop species. PATERSON *et al.* (1991) found QTL in an interspecific cross of tomato whose expression was influenced by environment. In maize, dominant and overdominant gene action at individual QTLs seems prevalent, especially for yield related traits (EDWARDS *et al.* 1987), and overdominant gene action at individual QTL is hypothesized to play a role in heterosis (STUBER *et al.* 1992). A pedigree of diploid potato in which the two parents shared a common grandparent was used to identify a QTL affecting tuber shape for which multiple alleles segregated in the progeny mapping population (VAN ECK *et al* 1994).

The experiment described herein utilized restriction fragment length polymorphism (RFLP) markers to construct genetic maps and identify QTL influencing wood density in a single outbred loblolly pine pedigree. Wood density is an important indicator of lumber quality and pulp yield and is of economic importance to forest industry. Wood density is usually expressed in terms of wood specific gravity (WSG), defined as the ratio of oven-dry secondary xylem cell wall constituents to pure

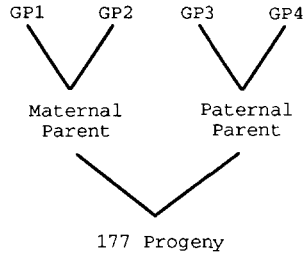

FIGURE 1.—Three-generation loblolly pine mapping pedigree.

water at 4°. WSG is a composite trait influenced by length, diameter and wall thickness of xylem cells, relative proportion of early and late wood, cellulose and lignin content, and extractive content (KOCH 1972; MEGRAW 1985). WSG is an ideal trait for QTL dissection in loblolly pine as reported heritabilities are high and there is abundant genetic variation in advanced generation pedigrees (WILLIAMS and NEALE, 1992). A general strategy for linkage mapping in an outcrossing species for which pedigrees with large sibships are available was developed. In addition to identifying QTL affecting mean WSG in the progeny mapping population, we examined intralocus interaction at QTL, multiple allelism at QTL, QTL interaction with environments and digenic epistasis of QTL.

MATERIALS AND METHODS

The general strategy for identifying WSG QTL was (1) select a single three-generation pedigree with a progeny generation segregating for WSG QTL; (2) for the selected pedigree, genotype the minimum number of progeny necessary to construct a linkage map with a large number of RFLP loci; (3) perform a preliminary analysis of variance (ANOVA) to determine which loci appear to be cosegregating with WSG QTL; (4) genotype all remaining progeny for loci potentially cosegregating with WSG QTL and (5) repeat the ANOVA utilizing the entire progeny data set. This strategy was employed to identify the QTL with the greatest effect using the least amount of experimental effort.

Mapping population: Existing three-generation loblolly pine pedigrees belonging to the Weyerhaeuser Company were evaluated to identify pedigrees segregating for WSG QTL in the progeny generation (WILLIAMS and NEALE 1992). Only pedigrees with a minimum number ($\gg$100) of progeny of at least age 6 (to allow for adequate wood formation for WSG determination) were considered. Specifically, a pedigree with grandparental pairs displaying divergent WSG values and maximum variation for WSG in the progeny generation was desired. This arrangement increases the chances for the parental trees to possess QTL alleles with opposite effect.

One pedigree was selected that best met the above criteria (Figure 1). A total of 177 progeny at age 8–10 years of age were present across four sites in coastal North Carolina, one site in Arkansas and one site in Oklahoma. The sites were originally selected for comparing the performance of coastal North Carolina selections in North Carolina and the two western states, and were not randomly selected from a population of sites. For this reason, the effect of sites is treated as a fixed effect in all analyses.

Wood specific gravity measurement: A radial core of wood was taken for each progeny at the approximate center of the internode below breast height. Each core was cropped at the pith and at the outer edge of the ring boundary corresponding to age 8. Wood specific gravity was determined on an oven dry weight, green volume basis. The distribution of wood specific gravity values in the progeny generation was approximately normal (Figure 2).

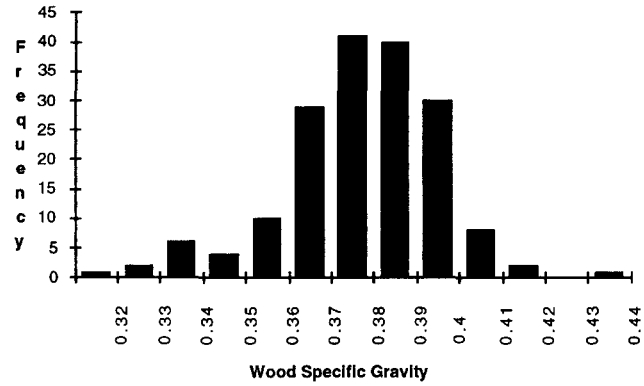

FIGURE 2.—Distribution of progeny wood specific gravity values. Specific gravity is a unitless measure.

RFLP genotyping: A subset of 48 of the 177 progeny was selected as an initial mapping population. The sample size of 48 was empirically determined to be the minimum number of progeny necessary for linkage mapping using segregation data for 95 progeny and 92 loci from an unrelated pedigree (DEVEY *et al.* 1994). Four data sets each of 72, 48 and 24 randomly selected progeny were constructed from the unrelated pedigree. A genetic map was assembled for each data set using GMendel 2.0 (LIU and KNAPP 1990) and compared to the original map constructed from the complete 95 progeny data set. The four maps constructed with 24 progeny were inconsistent whereas the four maps from the 48 progeny data sets, although not as complete as the full 95 progeny map, were nearly identical. Forty-eight progeny with extreme WSG values (24 lowest and 24 highest) were then selected for genotyping for RFLP loci from the QTL mapping pedigree. Genotyping progeny with extreme trait values (selective genotyping) maximizes the ability to detect QTL for a given number of progeny (LANDER and BOTSTEIN 1989). The remaining 129 progeny in the QTL mapping pedigree were genotyped for all loci showing potential linkage to WSG QTL based on analysis of the 48 extreme phenotype progeny, as described in *QTL analysis for WSG* below.

RFLP genotypes were determined as described in DEVEY *et al.* (1991). Loblolly pine cDNAs were the main source of probes, although loblolly pine and Monterey pine (*Pinus radiata* D. Don) genomic DNA probes, and cDNA probes from Scots pine (*Pinus sylvestris* L.) were also used. Each RFLP locus was classified to one of four types, according to the linkage information provided. Loci for which the maternal parent was heterozygous and the paternal parent was homozygous contain linkage information only for the maternal parent, and were termed maternal-informative (MI). Loci for which the paternal parent was heterozygous and the maternal parent was homozygous contain linkage information only for the paternal parent, and were termed paternal-informative (PI). Loci for which both parents were heterozygous for the same two alleles (*e.g.*, *A1A2* × *A1A2*) contain linkage information for both parents, and were termed both-informative (BI). This class has limited linkage information, however, as parental gamete contribution cannot be assigned in the heterozygous progeny class. The fourth class of loci were those for which both parents were heterozygous and three or four alleles were segregating. These loci segregate in a 1:1:1:1 genotypic ratios and both parents' allelic contribution can be assigned in each progeny

genotypic class. In the cross *A1A2* × *A3A4*, for example, the four resulting genotypic classes are *A1A3*, *A1A4*, *A2A3* and *A2A4*. In each class, it is possible to determine which allele came from each parent. There is thus complete linkage information for both parents and we termed these markers as fully informative (FI).

Map construction: RFLP genotypes in the progeny generation result from separate meioses and crossovers in the maternal and paternal parent. It was thus possible to consider maternal and paternal crossover events separately, and to construct individual maps for each parent. The maternal map included segregation data for: (1) maternally informative loci, (2) fully-informative loci recoded to contain only maternal segregations (*i.e.*, the paternal parent was recoded to be homozygous), and (3) both-informative loci, excluding linkages between pairs of both-informative loci for 129 non-extreme phenotype progeny (accomplished using independent two-point data accessory files described in the JoinMap manual (STAM 1993).The paternal map included segregation data for: (1) paternally informative loci, (2) fully-informative loci recoded to contain only paternal segregations and (3) both-informative loci, excluding linkages between pairs of both-informative loci for the 48 extreme phenotype progeny. Partitioning of data from both-informative loci and recoding of fully-informative loci achieved statistical independence of maternal and paternal data sets. JoinMap (STAM 1993) was used for map construction. A LOD score of 4.0 was used for forming linkage groups, and a "map LOD" (see JoinMap manual) score of 1.0 was used for multipoint linkage analysis.

QTL analysis for WSG: A two-staged approach was used to identify WSG QTL. In the first stage, 48 extreme phenotype progeny were genotyped for 146 RFLP loci. Each locus scored for the 48 extreme phenotype progeny was analyzed for differences in mean WSG for progeny as grouped by marker genotype using the ANOVA model:

$$\mathrm{WSG}_{ijk} = \mu + S_i + G_j + \epsilon_{ijk}$$

where,

μ = mean progeny WSG

S_i = effect of site i, i = 1–6.

G_j = effect of marker genotype j, (j = 1, 2 for MI and PI markers, 1–3 for BI markers, and 1–4 for FI markers)

ϵ_{ijk} = error associated with the kth progeny with marker genotype j in site i.

The effect of the interaction between genotype and site was not included in the model, as the small sample size (48) would result in empty cells.

In the second stage, the remaining 129 progeny were genotyped for all loci showing differences ($P \leq 0.10$) in marker genotypic class means for WSG on 48 extreme phenotype progeny. A second analysis was performed for each locus for which all 177 progeny were genotyped using the ANOVA model:

$$\mathrm{WSG}_{ijk} = \mu + S_i + G_j + S * G_{ij} + \epsilon_{ijk}.$$

The term $S * G_{ij}$ represents the effect of the interaction between marker genotypes and sites. Site and genotype were both considered fixed effects. For all loci showing differences ($P \leq 0.10$) among marker genotypic class means for WSG, the residuals from the ANOVA were plotted and visually inspected for deviation from normality.

RESULTS

Linkage maps: Statistically independent linkage maps were constructed for the maternal and paternal parents (Figure 3). Although all 177 progeny were scored for 64 of the 146 loci presented, the remaining 82 loci were scored only for the 48 extreme phenotyped progeny (see *QTL analysis*, below). Only relatively close linkages could be inferred for loci based on data from 48 progeny, especially the both-informative class loci. Bias in the estimates of linkage of markers near QTLs due to the selective genotyping strategy should have been minimized due to the genotyping of all progeny for markers associated with QTLs. The maternal map contained 87 loci and 17 linkage groups, while the paternal map contained 75 loci and 23 linkage groups. There were 55 (40%) MI markers, 47 (35%) PI markers, 6 (4%) BI markers and 28 (21%) FI markers included on the two maps.

QTL analysis: In the first stage of the QTL analysis, RFLP genotypes were determined at 146 loci for the sample of 48 extreme phenotype progeny. Of 146 loci, nine loci (138b, 726a, 1588b, 2291a, 2615a, 2697a, 2782b, 2958a and 3006a) showed differences in genotypic class mean WSG at the $0.10 \geq P \geq 0.06$ level, 10 loci (66a, 975a, 1623a, 1636a, 1889a, 2442a, 2540b, 2588b, 2718c and Ps3a) at the $0.05 \geq P \geq 0.02$ level, and nine loci (149a, 1918c, 2006b, 2113b, 2541a, 2786a, 2899a, 2986a and S6a) at or below the 0.01 level (Table 1).

In the second stage of the analysis, RFLP genotypes were determined for the remaining 129 progeny for loci that showed significance ($P \leq 0.10$) in the first stage of analysis. Also, several loci that did not show significant results in the first stage of analysis were scored on the additional progeny for an unrelated experiment. Many of the mapping probes revealed multiple loci, thus some additional loci were also scored. The result was that all 177 progeny were genotyped for 64 loci, of which 28 initially showed differences in genotypic class mean WSG at or below the $P = 0.10$ level on the extreme phenotype progeny prescreen.

All 64 loci for which full progeny data was available were re-analyzed for significant differences among marker genotypic classes for mean WSG using data from all 177 progeny. A total of five loci (66a, 975a, 1588b, 2588b, 2986a and Ps3a) showed differences in genotypic class means for WSG at the $0.05 \geq P \geq 0.02$ level, and four loci (1918c, 2006b, 2718c and S6a) at or below the 0.01 level (Table 1). The map position of loci significant at or below the 0.05 level was non-random (Figure 3). Indeed, the significant loci were concentrated in five areas, (1) female group 17, (2) female/male group 3, (3) female/male 5, (4) female/male group 12, and (5) female/male group 11. For each significant locus, the plotted residuals from ANOVA appeared approximately normal. It is possible that some QTL-linked markers which would have been detected had all 177 progeny been genotyped were missed (type II error) by the use of the extreme phenotype prescreen. However, extreme phenotyping allowed more markers to be assayed and resulted in a larger portion of the genome being analyzed. Also, none of the 37 markers scored for all 177

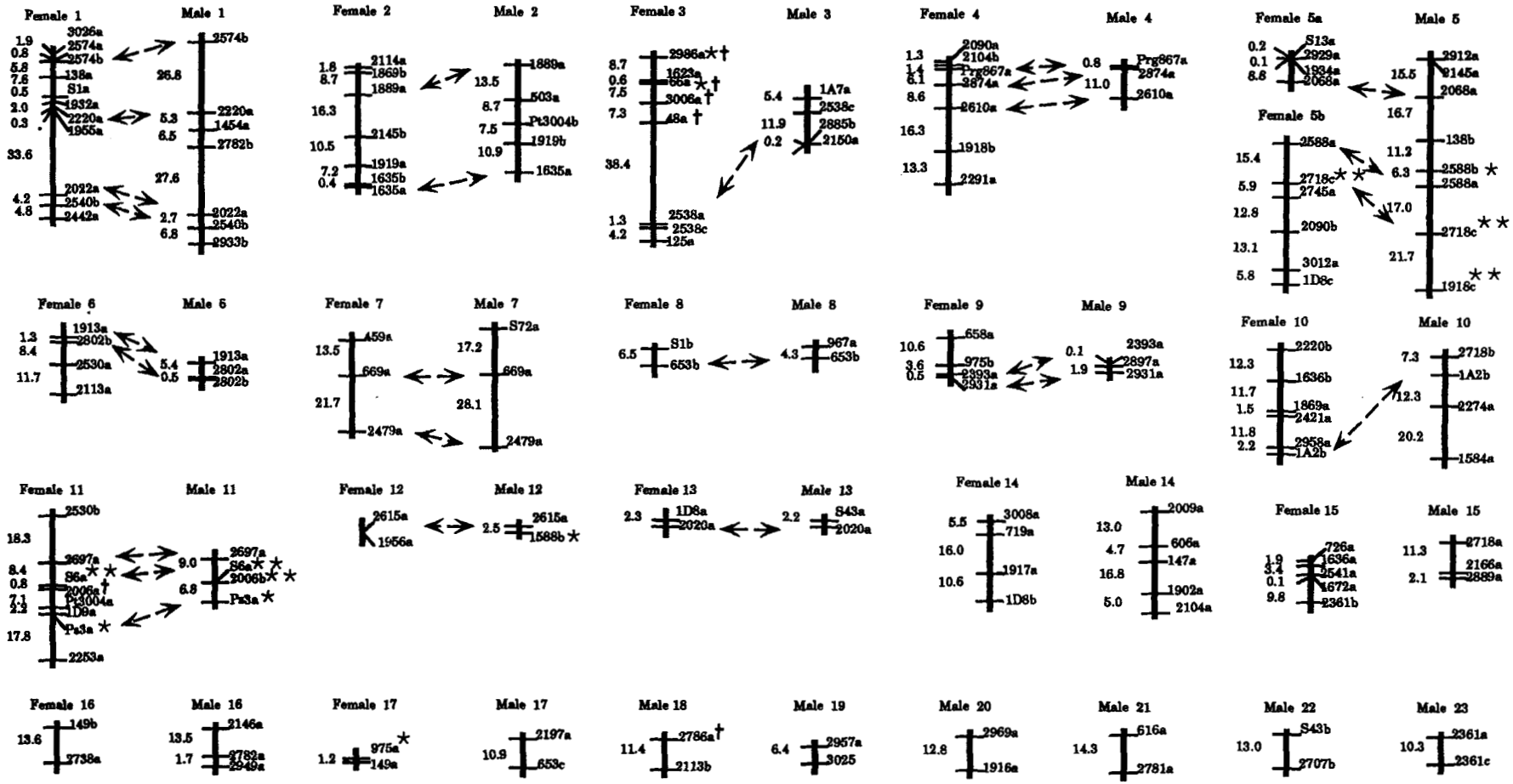

FIGURE 3.—RFLP linkage maps of male and female parents. Loci revealed by probes 1A2, 1A5, 1A7, 1D8, 1D9 1D11, and 4D4 are loblolly pine genomic DNA probes. Probe Prg867 is a *P. radiata* genomic DNA probe. Probes S1, S6, S13, S43 and S72 are *P. sylvestris* Cab cDNAs (JANSSON and GUSTAFSSON, 1990). Probe Ps3 is a *P. sylvestris* SOD cDNA (KARPINSKI *et al.* 1992). Probe Pt3004 is a loblolly pine ADH cDNA (D. HARRY, personal communication). All other loci are revealed by loblolly pine cDNA probes (DEVEY *et al.* 1994). Male and female linkage groups with common marker loci are paired and the common loci indicated with arrows. Marker loci showing significant associations to wood specific gravity based on the full progeny set analysis (Table 1) are indicated by * ($0.05 \geq P \geq 0.02$), and ** ($P \leq 0.01$). Marker loci showing a significant ($P \leq 0.05$) G×E interaction are indicated by †. Unlinked loci in female: 1A5a, 1A7a, 602a, 1940a, 2413a, 2009a, 2889a and 2912a. Unlinked loci in male: 1D11a, 149a, 151a, 602a, 701a, 1576a, 1623a, 1635b, 1672a, 1940a, 2113a, 2253a, 2413a, 2819a and 2899a.

TABLE 1

Significance levels for loci scored for all progeny which displayed QTL linkage ($P \leq 0.1$) for extreme phenotype progeny or QTL linkage ($P \leq 0.05$) for all 177 progeny (main effect of genotype or G×E)

Locus	Type[a]	Linkage group[b]	Extreme phenotype progeny (P)[c]	All progeny (P)	G×E (P)[d]
48a	MI	F 3	0.54	0.35	0.003
66a	MI	F 3	0.03	0.05	0.04
138b	PI	M 5	0.09	0.08	0.45
149a	FI	F 17	0.01	0.09	0.81
726a	MI	F 15	0.08	0.24	0.09
975a	MI	F 17	0.02	0.02	0.14
1588b	PI	M 12	0.06	0.02	0.14
1623a	BI	F 3	0.02	0.09	0.50
1636a	MI	F 15	0.05	0.12	0.23
1889a	FI	F/M 2	0.02	0.48	0.44
1918c	PI	M 5	0.006	0.01	0.53
2006a	MI	F 11	0.54	0.49	0.04
2006b	PI	M 11	0.01	0.005	0.82
2113b	PI	M 18	0.01	0.83	0.13
2291a	MI	F 5	0.09	NA[e]	NA
2442a	MI	F 1	0.03	NA	NA
2540b	FI	F/M1	0.03	0.08	0.44
2541a	MI	F 15	0.009	0.06	0.40
2588b	PI	M 5	0.03	0.02	0.28
2615a	FI	F/M 12	0.10	NA	NA
2697a	FI	F/M 11	0.10	0.38	0.49
2718c	BI	F/M 5	0.02	0.01	0.23
2782b	PI	M 1	0.08	0.63	0.08
2786a	PI	M 18	0.008	0.10	0.008
2899a	BI	Unlinked	0.01	0.28	0.51
2958a	MI	F 11	0.09	NA	NA
2986a	MI	F 3	0.01	0.05	0.02
3006a	MI	F 3	0.06	0.12	0.05
Ps3a	FI	F/M 11	0.05	0.02	0.42
S6a	FI	F/M 11	0.009	0.0002	0.12

[a] Type refers to the type of linkage information contained by the marker: maternal-informative (MI), paternal-informative (PI), both-informative (BI), or fully-informative (FI).

[b] F indicates female map linkage group assignment, M indicates male map linkage group assignment.

[c] Probability that the observed differences in mean WSG for the 48 extreme phenotype progeny as classified by marker genotype are the product of chance alone.

[d] Calculated using data for all 177 progeny.

[e] Marker genotypes were not available (NA) for all progeny for some markers showing significance on the extreme phenotype progeny for technical reasons.

progeny which did not show significance in the extreme phenotype prescreen showed significance at or below the 0.05 level on the full progeny set analysis, suggesting the type II error rate in the prescreen was not unreasonable. Inferences concerning markers for which all 177 progeny were analyzed were not biased by extreme phenotyping.

Analysis of epistasis and GXE: One locus with the highest level of significance was selected from each of the five regions showing significant QTL association, with preference being given to fully-informative markers (149a, 2718c, 66a, S6a and 1588b). Unlinked loci were utilized to avoid correlations due to linkage. The effect of digenic epistasis was analyzed in SAS Proc GLM using an ANOVA model including the main effects of all five loci, the effect of interaction between loci with sites, and the interaction between locus pairs. None of the interaction terms were significant at the 0.10 level, indicating that digenic epistasis is not strong at these loci (data not shown). It is possible that regions without significant main effects could interact with other regions to influence WSG. However, the number of possible interactions to be analyzed is large, and only interactions with very large effects would be discernible from false positives. Thus, all pairwise tests were not computed. Using variance components estimated by SAS Proc VARCOMP for the same model, the variance associated with the main effects of the five loci and the first order interactions with each other explained 23% of the total phenotypic variance.

Six loci (48a, 66a, 2006a, 2786a, 2986a and 3006a) showed a significant ($P \leq 0.05$) genotype × environment interaction (G×E) (Table 1). Four of the six loci mapped to female linkage group 3 (Figure 3). The significant G×E interaction in this region confounds the main effects of the associated QTL on WSG. Locus 48a showed the highest significance for G×E ($P = 0.003$), despite the main effect on WSG at this locus being non-significant ($P = 0.35$).

Gene action of QTL alleles: It is possible to track the inheritance of QTL alleles from both parents using fully-informative marker loci. The number of QTL alleles segregating at a locus and intralocus interaction (deviation from additivity) can thus be estimated. Such an analysis is presented below for locus S6a, representing the region with the largest effect on WSG. None of the other regions identified contained a fully-informative marker whose QTL associated effects were large enough to be amenable to such an analysis.

Locus S6a: Locus S6a was revealed by a light harvesting complex cDNA isolated from Scots pine (JANSSON and GUSTAFSSON 1990) and mapped to female/male group 11. This locus showed the highest level of significance for differences of mean WSG among marker genotypic class of any locus for which all 177 progeny were genotyped ($P = 0.0002$). Locus S6a explained 5.6% of the phenotypic variance. Mean WSG for each of the four S6a genotypic classes is shown in Figure 4. The 48 extreme phenotype progeny analyzed alone showed highly significant ($P = 0.0085$) differences in marker genotypic class means for WSG, as did the 129 non-extreme phenotype progeny (*i.e.*, the progeny in the middle of the distribution for WSG) analyzed alone ($P = 0.003$). This serves as independent confirmation on two progeny sets of the effect of this locus on WSG.

To more completely model the effects of the QTL alleles linked to S6a, an ANOVA was performed which tested the effects of each parent's QTL alleles and the interaction among them (Table 2). The significant interaction of the maternal alleles across sites ($P = 0.018$)

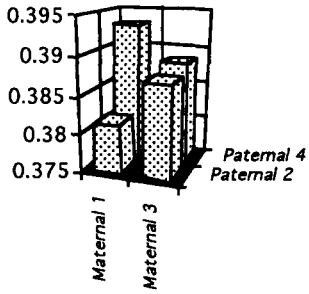

FIGURE 4.—Genotypic class mean WSG (vertical axis) for marker S6a for 177 progeny. *Maternal 1, Maternal 3, Paternal 2* and *Paternal 4* indicate the RFLP alleles donated by the maternal and paternal parent.

TABLE 2

ANOVA for marker S6a testing the effect of maternal and paternal QTL alleles, the effect of the interaction of QTL alleles with each other, and the effect of interaction of QTL alleles with the sites

Source	d.f.	*F*	Pr > *F*
Site	5	4.16	0.0015
Maternal alleles	1	0.91	0.3412
Paternal alleles	1	11.93	0.0007
Maternal alleles × paternal alleles	1	9.00	0.0032
Site × maternal alleles	5	2.84	0.0178
Site × paternal alleles	5	0.56	0.7316
Site × maternal alleles × paternal alleles	5	1.64	0.1526
Error	141		

and highly significant interaction ($P = 0.003$) between maternal and paternal alleles confound the main effects of the two parents' alleles. As a result, the main effect of maternal alleles (*i.e.*, testing the null hypothesis that there is no difference in the effect of the alternative alleles contributed by the maternal parent at this locus on WSG) is nonsignificant ($P = 0.34$). However, the presence of a highly significant interaction between the maternal and paternal alleles is only possible if both parents are segregating for QTL alleles of alternative effect at this locus.

Having established that there is a deviation from purely additive gene action and that both parents are heterozygous for alternative QTL alleles, it is possible to test whether the parents are heterozygous for the same two QTL or if multiple QTL alleles are involved (*i.e.*, testing the null hypothesis that the cross is *Q1Q2* × *Q1Q2*). If only two QTL alleles are segregating, two of the four marker genotypic classes should represent the heterozygous QTL class and have approximately the same mean WSG, regardless of gene action. As the parental trees are unrelated and loblolly pine populations are characterized by a high degree of linkage equilibrium, the phase between marker and QTL alleles cannot be determined *a priori*. A hypothetical modeling of the possible phases between a QTL for which both parent trees are heterozygous for the same two QTL alleles cosegregating with a fully-informative marker is illustrated graphically in Figure 5. Note that, although there are four

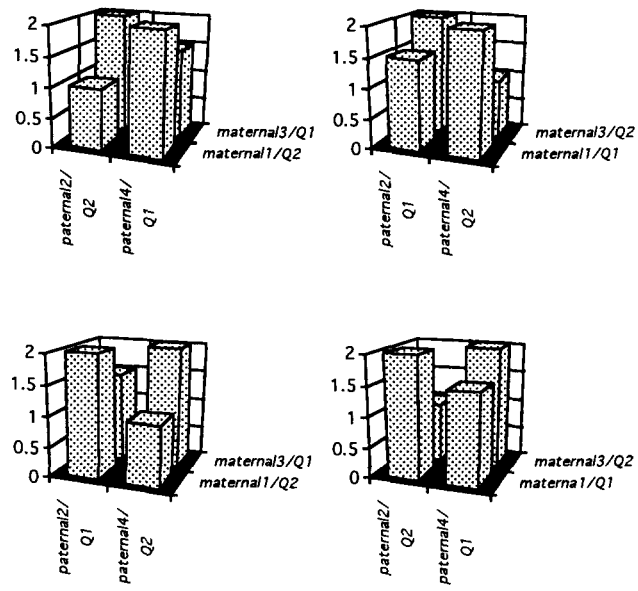

FIGURE 5.—Four possible two-locus phase relationships between a fully informative RFLP marker (*maternal* and *paternal* indicate marker alleles) and a heterozygous QTL where both parents segregate for the same two QTL alleles (*Q1* and *Q2*), assuming complete linkage. *Q1* increases the phenotypic score; *Q2* decreases. The situation depicted displays overdominance, but the model is applicable regardless of QTL gene action. Note that, in every case, the two classes representing the QTL heterozygous class diagonally oppose each other.

possible phase models, there are only two possible configurations of the heterozygous classes in these graphs.

To test the hypothesis that the parents were heterozygous for the same two QTL alleles, the mean WSG of the marker genotypic classes representing the two possible configurations of the QTL heterozygous classes were contrasted (*i.e.*, the marker classes diagonally opposing in Figure 4; *maternal1/paternal2 vs. maternal3/paternal4* and *maternal3/paternal2 vs. maternal1/paternal4*). The mean WSG for the *maternal1/paternal2* and *maternal3/paternal4* genotypic classes differed at the 0.003 level, and the mean WSG for the *maternal3/paternal2* and *maternal1/paternal4* genotypic classes differed at the 0.07 level, indicating (at the 0.07 level) that there are more than two QTL alleles segregating at this locus. The final model for QTL segregation at this locus is that there are more than two alleles segregating and that there is a significant interaction among them. It is not possible to quantify the effect of individual alleles or the nature of the interaction among them, as there are more terms to be solved for than there are equations (Table 3).

DISCUSSION

Five regions of the loblolly pine genome were identified as harboring QTL influencing WSG. These results indicate that, despite the continuous variation displayed for WSG, variable genes with measurable phenotypic effects exist for this trait and that they can be identified in

TABLE 3

Equations describing WSG values as predicted by maternal and paternal QTL alleles and their interactions

WSG_{12} = $maternal_1$ +	$paternal_2$ +	$maternal_1 \times paternal_2$
WSG_{14} = $maternal_1$ +	$paternal_4$ +	$maternal_1 \times paternal_4$
WSG_{23} = $maternal_2$ +	$paternal_3$ +	$maternal_2 \times paternal_3$
WSG_{34} = $maternal_3$ +	$paternal_4$ +	$maternal_3 \times paternal_4$

WSG_{nm} refers to the mean WSG of progeny receiving QTL allele n from the maternal parent and QTL allele m from the paternal parent. $Maternal_n$ and $paternal_m$ refer to effects associated with the QTL allele n from the maternal parent and the QTL allele m from the paternal parent. $Maternal_n \times paternal_m$ refers to the effect of the interaction between QTL allele n from the maternal parent and the QTL allele m from the paternal parent. Note that there are more terms to be solved for than there are equations.

an experiment of reasonable size. Together, the effects of the five regions explained 23% of the total phenotypic variance for WSG. These results are consistent with the quantitative genetic model that WSG is controlled by many genes which vary in magnitude of effect. Presumably, we have detected the QTL with the greatest effect on WSG segregating in the mapping pedigree, while QTL of lesser effect have gone undetected. For the QTL with the greatest effect, multiple alleles appeared to be segregating which interacted with each other. Evidence for QTL X environment interaction was found for two QTL. Digenic epistasis was not detected among QTL. Interspecific crosses or crosses between widely divergent individuals of the same species are commonly used for QTL mapping in order to maximize linkage disequilibrium, resulting in frequent marker polymorphisms and segregation at QTL. The loblolly pine pedigree used here was comprised of commercially utilized breeding germplasm, and thus the results are representative of QTL segregation for WSG in a typical pedigree.

A salient feature of a pedigree resulting from the cross of two unrelated outcrossed individuals, as was used here, is that the progeny mapping generation represents two segregating populations. We thus constructed two linkage maps, one for each parent tree, based on segregations measured on a single progeny set. A similar mapping approach has been used successfully in a pedigree of diploid potato in which the two parents shared a common grandparent (VAN ECK *et al.* 1994). Theoretically, a composite map could be made by aligning the two parental maps using loci for which both parents were heterozygous. However, the parental maps were not complete, and the number of connecting loci was insufficient for accurate alignment. Given a larger number of markers, the maps would be expected to coalesce into 12 linkage groups, corresponding to the haploid number of chromosomes in loblolly pine. We have also obtained evidence that the rate of meiotic recombination is greater in male gametes for loblolly pine (GROOVER *et al.* 1994), resulting in slightly different estimates of recombination depending on gamete type. Regardless, the order and distance between markers common to the statistically independent maps was generally in agreement, and no evidence for large rearrangements was found. We are currently adding markers to the maps presented here and a second RFLP map (DEVEY *et al.* 1994) with the goal of merging them into a composite map for loblolly pine. The data from these mapping efforts is being made available through the TreeGenes database (contact dendrome@s27w007.pswfs.gov).

Five regions were identified as harboring putative WSG QTL using an ANOVA based approach which used the data from a single marker at a time. This experiment was, however, limited in its ability to identify WSG QTL because the only detectable QTL were those for which one or both parents were heterozygous for alleles of strong alternative effect which were not masked by dominance. Detection was further limited by marker coverage and by the different marker types with respect to linkage information content. Mapping WSG QTL in related pedigrees will elucidate which regions consistently affect WSG across genetic backgrounds, better characterize effects of alleles at individual QTL, and allow for detection of QTL with smaller effects.

Using molecular markers to test for QTL poses difficult problems for statistical inference. Large numbers of tests are conducted which display partial statistical dependence (LANDER and BOTSTEIN 1989). For this reason, we employed a sequential procedure, with an initial screen of 48 extreme phenotype progeny, followed by detailed analysis of 177 trees. To allow for multiple statistical tests, our initial screen only considered markers with $P < 0.10$. Subsequently, a few detailed analyses employed a threshold F ratio with $P < 0.05$. Although potentially some QTL were overlooked due to type II error in the extreme phenotype prescreen, the prescreen allowed more RFLP loci to be examined and was effective in identifying loci showing linkage to QTL in the full progeny set. Regression of significance levels for loci scored for all 177 progeny onto those for the extreme phenotype progeny subsample indicated that the extreme phenotyped progeny were good predictors of the full progeny set ($R^2 = 0.79$). Regression of significance values for all progeny onto those for the 129 progeny in the middle of the distribution for WSG (*i.e.*, excluding the extreme phenotype progeny) indicated that, despite the fact that there were more observations, their correlation with the full progeny data set was lower ($R^2 = 0.61$) than the 48 extreme phenotype progeny data set.

In the outcrossed pedigree used, up to four alleles could segregate at any given locus. The alleles at a QTL could interact with each other in different degrees of dominance. Determining gene action at a QTL required information for both parents at the linked marker locus. Evidence for a QTL with multiple alleles which interacted with each other was provided by the fully-informative marker S6a. Intralocus interaction at QTL has been reported for maize (EDWARDS *et al.* 1987) and

tomato (WELLER *et al.* 1988), and multiple alleles at a QTL have been reported in diploid potato (VAN ECK *et al.* 1994). The presence of multiple alleles and an intralocus interaction does not allow the estimation of the magnitude of effect of each allele or the nature of the interaction among them in a single cross.

Quantitative genetic analysis of WSG has not usually revealed genotype by environment interaction (TALBERT *et al.* 1983; JETT *et al.* 1991; WILLIAMS and MEGRAW 1994). However, this apparently does not preclude genotype × environment interaction on the QTL level as the action of two QTL appeared to be influenced by environments. QTL × environment interaction was common for tomato (PATERSON *et al.* 1991), but was not prevalent in maize (STUBER *et al.* 1992).

Applying marker-aided selection (MAS) to loblolly pine tree improvement will be complex. Loblolly pine populations are characterized by a high degree of linkage equilibrium and, as a result, linkage phase relationships between markers and QTL will differ among individuals (STRAUSS *et al.* 1992). Thus, phase relationships would have to be established for each parent tree. The pedigree used in the experiment reported here is part of a six-parent half-diallel, which can be used to test for consistent QTL-marker linkages. Also, consistency of QTL must be tested across ages. At the phenotypic level, both juvenile and mature WSG are under similar genetic control (WILLIAMS and MEGRAW 1994).

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LITERATURE CITED

DEVEY, M. E., K. D. JERMSTAD, C. G. TAUER and D. B. NEALE, 1991 Inheritance of RFLP loci in a loblolly pine three-generation pedigree. Theor. Appl. Genet. **83:** 238–242.

DEVEY, M. E., T. A. FIDDLER, B. H. LIU, S. J. KNAPP and D. B. NEALE, 1994 An RFLP linkage map for loblolly pine based on a three-generation outbred pedigree. **88:** 273–278.

EDWARDS, M. D., C. W. STUBER and J. F. WENDEL, 1987 Molecular marker-facilitated investigations of quantitative trait loci in maize. II. Factors influencing yield and its component traits. Crop. Sci. **27:** 639–648.

GROOVER, A. T., C. G. WILLIAMS, M. E. DEVEY, J. M. LEE and D. B. NEALE, 1995 Sex-related differences in meiotic recombination frequency in *Pinus teada*. J. Hered. (in press).

JANSSON, S., and P. GUSTAFSSON, 1990 Type I and type II genes for the chlorophyll *a/b*-binding protein in the gymnosperm *Pinus sylvestris* (Scots pine): cDNA cloning and sequence analysis. Plant Mol. Biol. **14:** 287–296.

JETT, J. B, S. E. MCKEAND and R. J. WEIR, 1991 Stability of juvenile wood specific gravity of loblolly pine in diverse geographic areas. Can. J. For. Res. **21:** 1080–1085.

KARPINSKI, S., G. WINGSLE, O. OLSSON and J. HALLGREN, 1992 Characterization of cDNAs encoding Cu-Zn-superoxide dismutases in Scots pine. Plant Mol. Biol. **18:** 545–555.

KOCH, P., 1972 *Utilization of the Southern Pines*. USDA Agriculture Handbook No. 420. USDA Forest Service, Southeastern Forest Experiment Station, 1663 pp.

LANDER, E. S., and D. BOTSTEIN, 1989 Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics **121:** 185–199.

LIU, B. H., and S. J. KNAPP, 1990 GMENDEL: a program for Mendelian segregation and linkage analysis of individual or multiple progeny populations using log-likelihood ratios. J. Hered. **8:** 407.

MEGRAW, R. A., 1985 Wood quality factors in loblolly pine: the influence of tree age, position in tree, and cultural practice on wood specific gravity, fiber length, and fibril angle. TAPPI Press, Atlanta, 88 pp.

PATERSON, A. H., S. DAMON, J. D. HEWITT, D. ZAMIR, H. D. RABINOWITCH *et al.*, 1991 Mendelian factors underlying quantitative traits in tomato: comparison across species, generations, and environments. Genetics **127:** 181–197.

STAM, P., 1993 Construction of integrated generic linkage maps by means of a new computer package: JoinMap. Plant J. **3:** 739–744.

STRAUSS, S. H., R. LANDE and G. NAMKOONG, 1992 Limitations of molecular-marker-aided selection in forest tree breeding. Can. J. For. Res. **22:** 1050–1061.

STUBER, C. W., S. E. LINCOLN, D. W. WOLFF, T. HELENTJARIS and E. S. LANDER, 1992 Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. Genetics **132:** 823–839.

TALBERT, J. T., J. B. JETT and R. L. BRYANT, 1983 Inheritance of wood specific gravity in an unimproved loblolly pine population: 20 years of results. Silvae Genet. **32:** 33–37.

VAN ECK, H. J., J. M. JACOBS, P. STAM, J. TON, W. J. STIEKEMA *et al.*, 1994 Multiple alleles for tuber shape in diploid potato detected by qualitative and quantitative genetic analysis using RFLPs. Genetics **137:** 303–309.

WELLER, J. I., M. SOLLER and T. BRODY, 1988 Linkage analysis of quantitative traits in an interspecific cross of tomato (*Lycopersicon esculentum* × *Lycopersicon pimpinellifolium*) by means of genetic markers. Genetics **118:** 329–339.

WILLIAMS, C. G., and R. A. MEGRAW, 1994 Juvenile-mature relationships for wood density in *Pinus taeda*. Can. J. For. Res. **24:** 714–722.

WILLIAMS, C. G., and D. B. NEALE, 1992 Conifer wood quality and marker-aided selection: a case study. Can. J. For. Res. **22:** 1009–1017.

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