Genetic Mapping of Quantitative Trait Loci Influencing Wood Specific Gravity in Loblolly Pine *(Pinus taeda).*

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Abstract.--We are attempting to map quantitative trait loci affecting wood specific gravity in a three-generation loblolly pine pedigree. Forty-eight progeny were measured for wood specific gravity and genotyped for 178 restriction fragment length polymorphism markers. Each marker was tested for linkage to variable genetic factors which influence wood specific gravity by comparison of progeny marker genotype class means for wood specific gravity. Fourteen markers showed differences in genotypic class means at the 0.01 level. An additional 127 progeny were genotyped and analyzed for one of the 14 significant markers. The marker, Pt1IFG149a, accounted for 5% of the total phenotypic variation in the progeny for wood specific gravity.

Introduction

Phenotypic variation of quantitative traits such as height, insect and disease resistance, diameter, and wood specific gravity can be partitioned into additive and non-additive genetic and environmental components. Such partitioning describes the collective effects of all variable genes which influence the phenotype. The opportunity exists, using molecular markers and genetic maps, to identify and characterize individual genetic factors which influence quantitative traits, hereafter referred to as quantitative trait loci (QTL).

Quantitative trait loci have been identified for numerous traits in several crop species. Edwards *et al.* (1987) identified QTL for each of 82 traits evaluated in two F2 populations of maize. Individual QTL accounted for between 0.3% and 16% of the total phenotypic variation for a given trait, and the cumulative effects of all QTL explained between 8% and 40% of the total phenotypic variation per trait. Dominant and overdominant gene action at individual QTL was prevalent, especially for yield-related traits. QTL were also identified which influenced trait stability. Stuber *et al.* (1992) showed that F2 maize plants which were heterozygous for QTL alleles influencing grain yield had higher yields than individuals homozygous at the same locus. The authors hypothesized

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overdominant gene action at these QTL and concluded that the QTL play a role in explaining heterosis. There was little evidence for interactions of QTL with environments in this study, despite replication across diverse environments.

QTL which influenced fruit size, soluble solids concentration, and pH were identified in tomato for progeny of a cross between *Lycopersicon esculentum* (cultivated tomato) and *L. cheesmanii* (a related wild species) (Paterson *et al.* 1991). The trial, conducted in three environments, detected numerous QTL X environment interactions, as only four of the 29 QTL detected had a significant effect in all three environments. There was evidence for QTL with pleiotropic effects. As with maize (Edwards *et al.* 1987), digenic epistasis was not common between QTL.

Wood specific gravity is an important determinant of wood quality and influences lumber quality and pulp yield. Heritability estimates for wood specific gravity are generally high and there is abundant additive genetic variation segregating in advanced generation pedigrees of several conifers. These factors make wood specific gravity a good trait for QTL mapping (Williams and Neale, 1992).

A linkage map has been constructed for loblolly pine *(Pinus taeda* L.) based on RFLP and isozyme markers using a single, three-generation pedigree (Devey *et al.,* in preparation). We report here the use of similar marker technology to locate and characterize QTL which influence wood specific gravity in loblolly pine.

Methods

Pedigree selection

A three-generation, full-sib loblolly pine pedigree with extreme wood specific gravity (WSG) values within grandparental pairs and a high variation for WSG in the F2 progeny was available from the Weyerhaeuser Company (Figure 1) (Williams and Neale, 1992). A total of 175 progeny trees on six sites, four in North Carolina and two in Oklahoma were available for analysis.

WSG measurement

Radial cores were 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 eight. Wood specific gravity was determined on an ovendry weight, green volume basis.

RFLP and Isozyme mapping

Forty-eight progeny with extreme WSG values (24 lowest and 24 highest) were selected for genotyping with RFLP and isozyme markers. Selecting individuals with extreme quantitative trait value minimizes the number of individuals that must be genotyped for a given power of detection of QTL (Lander

and Botstein, 1989). Loblolly pine complementary DNAs (cDNA) were the main source of probes for producing RFLPs (using methods described by Devey *et al.* (1991)). In addition, several cloned genes from loblolly and Scots pine, random loblolly pine genomic clones, and random radiata pine genomic clones were used as probes. Four isozyme loci (Gdh, Got2, Mdh3, and Sk) were scored. Linkage analysis was performed using GMendel 2.0 (Liu and Knapp, 1990).

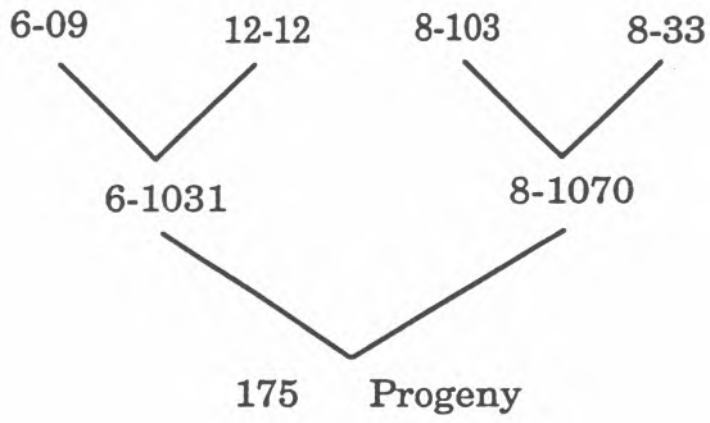

Figure 1. Three-generation loblolly pine pedigree for mapping wood specific gravity QTL.

Marker/QTL association

For a given RFLP marker, progeny were grouped based on their RFLP genotype. Individuals within. a group had in common the region of parental DNA associated with the marker alleles, while regions of DNA unlinked to the marker varied at random among them. The effect of unlinked regions of DNA thus averages to zero within each group, and comparison of mean WSG among the groups tests for the effect of the region of DNA associated with the marker on WSG. Significant differences in mean WSG among the groups is evidence for the presence of a QTL residing in close proximity to the marker. A prerequisite for detecting a WSG QTL in this experiment is for one or both parents to contain alternative alleles for the QTL which differ in their effect on WSG. Incomplete linkage results in the misclassification of individuals with respect to QTL genotype whenever crossing over between the marker and QTL occurs. Consequently, the ability to detect marker/QTL cosegregation decreases with increasing distance between the marker and QTL. Markers with three or four alleles and for which parental trees are both heterozygous are the most informative. Such markers allow the estimation of the influence of both male and female alleles at that locus on WSG.

We are using two approaches to detect linkage between markers and QTL. The first is an analysis of variance approach which, for each marker, compares the mean WSG among progeny RFLP genotype groups. Significant differences in mean WSG among groups indicates linkage between the marker and a QTL. The

data for each marker was analyzed using SAS Proc GLM using the model shown in Table 1. Marker genotype was considered a fixed effect, while site and the site X marker genotype interaction were considered random effects.

Table 1. Effects in ANOVA model for testing differences in marker genotypic classes means.

Source	df	Expected Mean Squares
Site	s-1	$s^2_e + tms^2_s$
Marker	m-1	$s^2_e + ts^2_{s*m} + Q(m)$
Site*Marker	(s-1)(m-1)	$s^2_e + ts^2_{s*m}$
error	n-s-m-1	s^2_e

The second approach under development uses interval mapping as described by Lander and Botstein (1989). This approach uses the information from flanking markers and thus uses more of the information contained in the marker data. The interval mapping software is currently being tested and no results from this method will be presented in this paper. The analysis of variance and interval mapping approaches will eventually be compared for ability to detect QTL.

Results

WSG measurements,

Mean WSG for all progeny was 0.3868 (standard deviation=0.0181) . The distribution was approximately normal (W=0.97).

Genetic Map

To date, 178 RFLP loci have been scored and mapped for the subsample of 48 progeny. Most of the RFLPs were produced using loblolly pine cDNAs. Scots pine Lhc and Sod clones, and loblolly and radiata pine random genomic clones all produced mappable RFLPs. The map, as constructed by GMendel 2.0, is composed of 14 linkage groups of three or more markers and nine linkage groups of two markers; sixteen markers are presently unlinked.

QTL/Marker associations

The effect of genotype was significant ($p=<0.01$) for 14 of the 178 RFLP markers tested using the analysis of variance approach. For these 14 markers, the remaining 126 progeny are currently being genotyped. We report below the results from a single marker (marker Pt1IFG149a, revealed by a loblolly pine cDNA) for which genotyping of all progeny has been completed and for which a basic analysis has been performed.

Marker 149a

Marker 149a is fully informative in the genetic sense as both parents are heterozygous for marker alleles and it is possible to discern the parental contribution to the marker genotype of all progeny. The genotype of the parent trees and the resulting four genotypic classes in the progeny for marker 149a are shown in Figure 2.

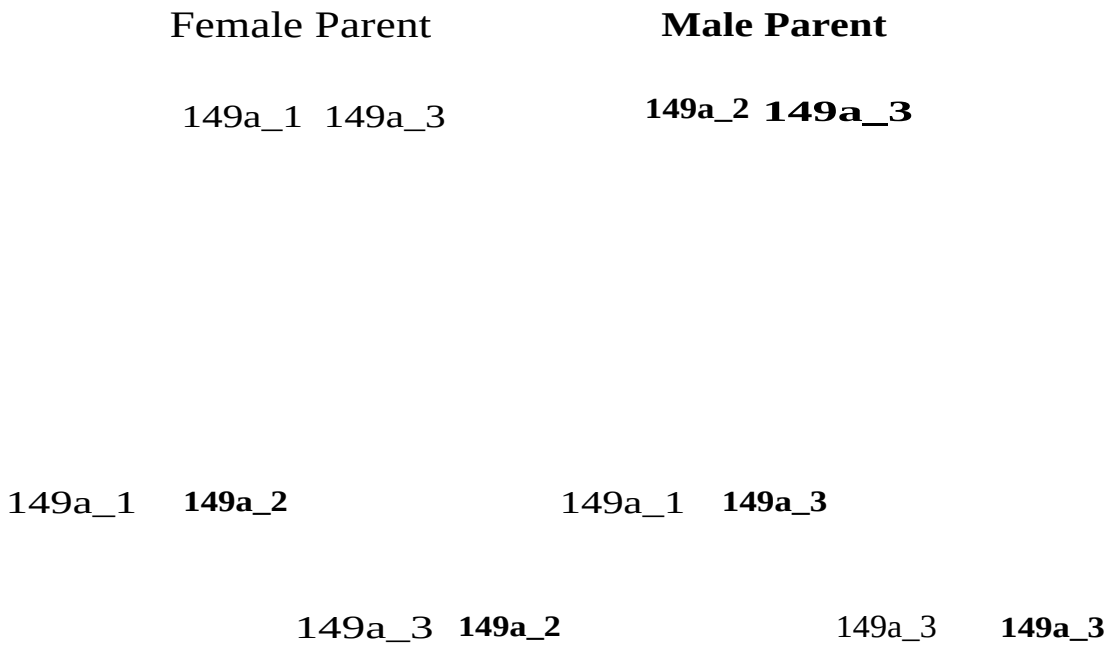

Figure 2. Parental genotypes for marker 149a and resulting four genotypic classes in the F2 progeny. Male marker alleles are indicated in **bold .**

Modeling linkage phase of marker and QTL alleles

The first step of the analysis is to determine whether one or both parents are segregating at the quantitative trait locus. The mean WSG of progeny receiving the 1 marker allele from the female parent (mean WSG=0.3844, SE=0.0023) **is** lower than the mean WSG of the progeny receiving the 3 marker allele from the female parent (mean WSG=0.3892, SE=0.0022). The resulting hypothesis is the female parent is heterozygous for QTL alleles of alternative effect, and that the1 female marker allele is in coupling phase with a QTL allele which decreases WSG, while the 3 female marker allele is in coupling with a QTL allele which increases WSG. Similarly, on the male side of the cross, the mean WSG of the progeny receiving the 3 marker allele from the male parent (mean

WSG=0.3841, SE=0.0016) is lower than the mean WSG of the progeny receiving the 2 marker allele from the male parent (mean WSG=0.3898, SE=0.0015). The resulting hypothesis is the male parent is also heterozygous for QTL alleles of alternative effect, and that the 2 male marker allele is in coupling with a QTL allele which increases WSG and the 3 male marker allele is in coupling with a QTL allele which decreases WSG. Note that the 3 marker allele is apparently associated with QTL alleles of opposite effect in the two parental trees. This is not a surprising result considering the high degree of linkage equilibrium present in coniferous tree populations, and illustrates one of the interesting aspects of QTL mapping in conifers.

Testing the proposed model

Based on the apparent effect of parental QTL alleles, progeny were allocated to three groups (Figure 3). The first group contained those trees receiving an RFLP allele coupled to a QTL allele which increases WSG from both parents, and were thus homozygous for QTL alleles increasing WSG (assuming no crossing over between the marker and QTL). A second class was formed by those progeny receiving an RFLP allele from each parent coupled to a QTL allele which decreases WSG, and were thus homozygous for QTL alleles which decrease WSG. The remaining progeny received a QTL allele from one parent which increases WSG and a QTL allele from the other parent which decreases WSG, and thus formed a class heterozygous for QTL alleles of opposite effect.

The means of the three groups were tested for significant differences by ANOVA (Table 2). The effect of genotype was highly significant; the F value is probably of less certainty than the Pr > F of 0.002given by Proc GLM in SAS because of heterogeneity of variances among the classes of genotypes within environments. Closer examination of the data reveals that the trees receiving the 1 female RFLP allele and the 2 male RFLP allele have a distinctly higher variance at four of the six sites when compared to the other 3 RFLP progeny classes. Although this complicates interpreting the results of the ANOVA, it is suggestive of a QTL influencing trait variability, as described by Edwards et al. (1987). We found no evidence for interaction of QTL alleles with environments.

Variance component estimates for the effects in the model are shown in Table 3. The percent of total phenotypic variation explained by the marker genotype was 5%, as estimated by dividing the variance attributed to genotype by the sum of the variance components for genotype, site*genotype, and error. The negative value associated with the site*genotype variance was replaced by zero in this calculation.

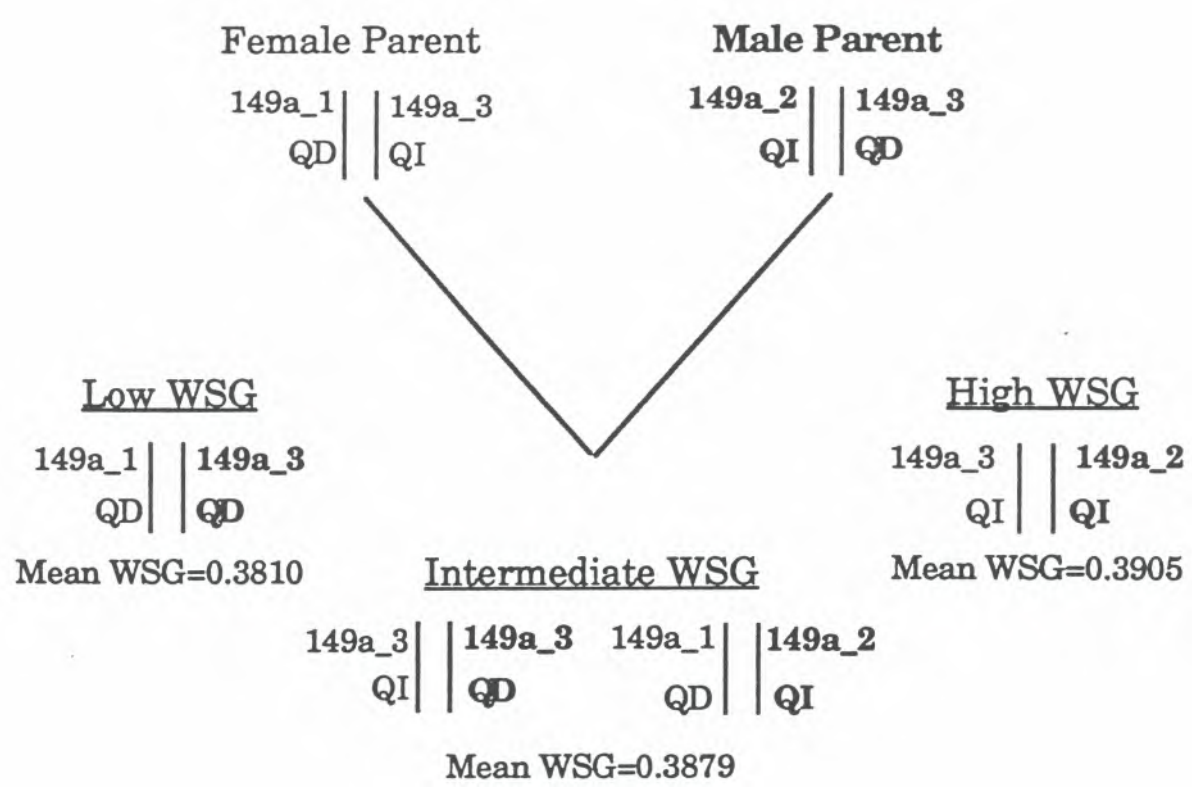

Figure 3. Segregation of marker 149a and linked QTL, *assuming* complete linkage. QTL alleles are described by QI (increasing WSG) or QD (decreasing WSG).

Table 3. ANOVA for marker 149a. The correct test of the effect of genotype in this mixed model (site random, genotype fixed) is MS Genotype divided by MS Site*Genotype.

Source	di	Mean Square	Pr>F
Site	**5**	**0.00072340**	**0.0389**
Genotype	**2**	**0.00101597**	**0.0019**
Site*Genotype	**10**	**0.00008091**	0.9867

Table 3. Variance component estimates for factors in ANOVA model, as produced by Proc Vacomp (SAS). All effects were considered random for this analysis.

Variance component	**estimate**
(Var)Genotype	0.00001499
(Var)Site	0.00002733
(Var)Site*Genotype	-0
(Var)Error	0.00029969

Discussion

We have identified at least one QTL influencing wood specific gravity in a three-generation loblolly pine pedigree, demonstrating that it is possible to identify QTL for a coniferous tree species. Given the pedigree structure and modest sample size of this experiment, the fact that QTL were detected indicates that there exist variable genes with major effect on wood specific gravity. This finding parallels the results of QTL experiments in other plant species, where QTL with differing magnitude of effect have been detected.

Conifer populations are characterized by a high degree of genetic diversity. This is evident at the DNA level from the high frequency of restriction fragment length polymorphism revealed by loblolly pine DNA probes. Often, probes reveal multiple mappable RFLP loci and up to four alleles may be segregating at individual loci in the progeny generation. This differs from backcross or F2 populations where only two alleles can be segregating at any given locus. To simplify the analysis, we presented a reduced genetic model in which QTL alleles were classified as increasing or decreasing WSG. While the simplified model is adequate for detecting the presence of a QTL, it does not describe the interaction among QTL alleles at a locus. A given combination of QTL alleles could interact in an additive, dominant, or overdominant fashion. We plan to expand the analysis to examine the interaction between QTL alleles at a given locus, as well as possible digenic epistatic effects between pairs of QTL.

The full progeny set has been genotyped and analyzed for only one of the 14 markers which gave significant results. However, the results from that analysis illustrates some interesting points. The high degree of linkage equilibrium in coniferous populations was illustrated by marker allele 3 being linked to QTL alleles of different effect in the two parental trees. Also, the data from this marker suggest that QTL which influence trait variability may exist for WSG. This **is** not a surprising result given similar findings for various traits in maize (Edwards *et al.* 1987).

As we identify additional QTL influencing WSG, we hope to further characterize them with respect to gene action and digenic epistasis. Also, we will

be testing other loblolly pine pedigrees for variability in the same QTL detected in this experiment. Because of the high degree of genetic diversity present in conifers, only some of the QTL detected in this experiment would be detectable in a different cross involving unrelated trees. Likely, additional QTL will be detected in these experiments. Eventually, we will also test QTL identified for ontogenetic effects.

Acknowledgments

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