Theor Appl Genet (2000) 101:1273–1281

M.M. Sewell · D.L. Bassoni · R.A. Megraw
N.C. Wheeler · D.B. Neale

Identification of QTLs influencing wood property traits in loblolly pine (*Pinus taeda* L.). I. Physical wood properties

Received: 15 February 2000 / Accepted: 31 March 2000

Abstract Physical wood property traits for loblolly pine (*Pinus taeda* L.) were analyzed for the presence of quantitative trait loci (QTLs) in a three-generation outbred pedigree. These traits include wood specific gravity (*wsg*), volume percentage of latewood (*vol*%), and microfibril angle (*mfa*). Phenotypic data were collected for rings 2–11 for *wsg* and *vol*%, and rings 3, 5 and 7 for *mfa*. Both earlywood and latewood were analyzed for each trait. An interval mapping approach designed for an outbred pedigree was used to estimate the number of QTLs, the magnitude of QTL effects, and their genomic position. Nine unique QTLs were detected for composite traits (average of all rings) for *wsg*, five for *vol*%, and five for *mfa*. The majority of these QTLs were verified by analyses of individual-ring traits. Additional QTLs for each trait were also detected by these individual-ring analyses. Most QTLs for *wsg* were specific to either earlywood or latewood, whereas each QTL for *mfa* was detected for both earlywood and latewood. Before these QTLs are utilized in a breeding program, they should be verified in larger experiments and in different genetic and environmental backgrounds.

Communicated by P.M.A. Tigerstedt

M.M. Sewell, D.L. Bassoni, D.B. Neale (✉)
Institute of Forest Genetics, USDA Forest Service
Department of Environmental Horticulture,
University of California, One Shields Avenue, Davis,
CA 95667 USA

R.A. Megraw
Weyerhaeuser Company, Weyerhaeuser Technology Center 1 E-19,
Tacoma, WA 98477 USA

N.C. Wheeler
Weyerhaeuser Company, Western Forest Research Center,
Centralia, WA 98531, USA

Present address:
M.M. Sewell, Environmental Sciences Division,
Oak Ridge National Laboratory, PO Box 2008, Oak Ridge,
TN 37831-2008, USA

Keywords *Pinus taeda* L. · QTL mapping · Wood specific gravity · Volume percentage of latewood · Microfibril angle

Introduction

The physical properties of wood affect many characteristics of value to the timber industry. For example, wood density affects both the quality and quantity of pulp production and is highly correlated with the major strength properties of sawn timber (Zobel and Jett 1995). Progress in tree breeding for such traits is hampered by the long generation time of forest trees and the considerable time and expense needed to perform evaluations and selections. Marker-aided breeding offers the potential to reduce the breeding cycle time and increase the overall efficiency of breeding for wood property traits (Williams and Neale 1992). Validation of this concept requires demonstration that quantitative trait loci (QTLs) for wood properties can be detected and verified in an outbred forest-tree pedigree.

Loblolly pine (*Pinus taeda* L.) is the leading timber species in North America and is grown commercially for both solid wood and pulp and paper products. Pines are characterized by their longevity, an outbred mating system, and high genetic variability (Hamrick and Godt 1990). Sufficient genetic variation for wood property traits exists within loblolly pine to consider including these traits in breeding programs (Zobel and Jett 1995; Schultz 1997). Among these wood property traits are wood specific gravity, volume percentage of latewood, and microfibril angle.

Wood is essentially a matrix of cell walls and cellular air spaces from secondary xylem (Megraw 1985). Wood specific gravity is a measure of the total amount of cell-wall substance in secondary xylem and is defined as the ratio of the density of oven-dry wood relative to the density of pure water at 4°C. The specific gravity of a given annual ring is a function of cell size and cell-wall thickness. Both of these properties are heavily dependent upon whether the cells were differentiated during the development of earlywood or latewood. Earlywood is typically composed of large-diameter, thin-walled xylem cells, whereas latewood is typically composed of smaller, thick-walled xylem cells. Therefore, the density of each individual annual ring is a direct combination of its three seasonal determinants: earlywood specific gravity, latewood specific gravity, and the relative percentage of each (Megraw 1985). Wood specific gravity is the most reliable single index of wood quality because it is closely associated with many important wood properties (Zobel and Jett 1995).

Microfibrils are long polysaccharide chains composed of a crystalline cellulose core surrounded by chains of hemicelluloses, which are encased by surrounding lignin and become rigid (Panshin and de Zeeuw 1980). Microfibril angle refers to the mean helical angle that the microfibrils of the S_2 layer of the cell wall make with the longitudinal axis of the cell (Megraw et al. 1998). Lower fibril angles (closer alignment with the axis of the cell) have a positive influence on lumber strength, stiffness, and dimensional stability (Megraw 1985). The thicker cell walls associated with latewood typically have lower fibril angles, although there is no constant relationship within a tree between specific gravity and fibril angle (Megraw 1985).

Average wood specific gravity in a three-generation loblolly pine pedigree was previously analyzed for the presence of QTLs using phenotypic data from whole wood cores (Groover et al. 1994). In the present study, the phenotypic values for this same pedigree were re-estimated for both earlywood and latewood on a ring×ring basis. The objectives of this study were to identify QTLs for earlywood and latewood specific gravity, volume percentage of latewood, and earlywood and latewood microfibril angle; estimate the genetic effects of these QTLs; and analyze for temporal stability of the QTLs for these traits.

Materials and methods

Mapping population

A three-generation outbred loblolly pine pedigree was used to detect marker-trait associations for wood property traits. This pedigree (referred to as the *qtl* pedigree) was used by Groover et al. (1994) and Knott et al. (1997) in earlier QTL mapping studies for wood quality. The *qtl* pedigree was constructed from first-generation selections of the North Carolina State University Industry Cooperative Tree Improvement Program and is maintained by the Weyerhaeuser Company. The grandparental pairs of the *qtl* pedigree displayed divergent values for wood density, and the progeny generation displayed wide variation for wood density. The *qtl* pedigree consists of 172 progeny that are grown at six different sites within southeastern North America. The number of trees per site ranged from 19 to 35.

Genotypic data and map construction

Genotypic segregation data from restriction fragment length polymorphism (RFLP) markers were used to construct independent parental framework maps from the 172 progeny of the *qtl* pedigree. Methods pertaining to RFLP analysis for loblolly pine followed Devey et al. (1991). Evenly spaced markers were selected for each parental data set from previous (Groover et al. 1994; Devey et al. 1999; Sewell et al. 1999) and current mapping efforts with the outbred *qtl* pedigree. Fully informative markers (i.e., a marker that segregates in both parental populations) were preferentially chosen when available. Each parental data set was organized by ancestry and phase from the three-generation pedigree structure (Sewell et al. 1999). In situations where the ancestry could not be determined, the phase was determined from segregation analyses. The ancestry of alleles was then updated (Sewell et al. 1999), producing a complete three-generation data set that is both ancestry- and phase-known. Parental framework maps were constructed using MapMaker (Lander et al. 1987) and then integrated into a single sex-average map using JoinMap (Stam 1993; Table 1).

Phenotypic data

X-ray densitometry analysis of wood specific gravity and volume percentage of latewood

A radial wood core was taken for each progeny at the approximate center of the internode below breast height. Each core was cropped at the pith and the outer edge of ring 13. Wood specific gravity (*wsg*) and the volume percentage of latewood (*vol*%) was measured by means of continuous X-ray scan from bark to pith. Both earlywood and latewood measurements were taken for each individual ring 2–11.

X-ray diffraction analysis of microfibril angle

The average microfibril angle (*mfa*) of each sample was determined by measuring the relative width of the 002 diffracted X-ray arc using the procedures described by Meylan (1967) and El-Osta et al. (1972). Tangents were drawn at the inflection points for the two sides of the curve, and the relative peak-width measured as the distance between the two tangent intersections with the base line. Relative peak-width values were then converted to actual *mfa*

Table 1 Genomic coverage of marker

Item	Maternal map	Paternal map	Sex-average map
Number of framework markers	118	109	164
Number of fully informative markers	–	–	63
Number of linkage groups[a]	15	15	14
Average spacing of framework markers (cM)	9.0	11.2	–
Genome coverage (cM)[b]	923.9	1053.2	1222.1

[a] Linkage analysis used LOD 5 and the Kosambi mapping function

[b] Recombination in the paternal population is significantly larger than that in the maternal population (Groover et al. 1995; Sewell et al. 1999), producing a larger paternal map

Table 2 Phenotypic traits for physical wood properties

Phenotypic trait	Ring grouping	Method of assay
Wood specific gravity (*wsg*) (both earlywood and latewood)	Individual rings 2–11 Composite rings 2–6 (wt. ave.) Composite rings 7–11 (wt. ave.) Composite rings 2–11 (wt. ave.)	X-ray densitometry
Volume percentage of latewood (*vol*%)	Individual rings 2–11 Composite rings 2–6 (wt. ave.) Composite rings 7–11 (wt. ave.) Composite rings 2–11 (wt. ave.)	X-ray densitometry
Microfibril angle (*mfa*) (both earlywood and latewood)	Individual rings 3, 5 and 7 Composite rings 3, 5, 7 (ave.)	X-ray diffraction

Table 3 Model used to test the effect of QTL alleles (Knott et al. 1997)

Parental cross	$Q_1Q_2 \times Q_3Q_4 \rightarrow Q_1Q_3, Q_1Q_4, Q_2Q_3, Q_2Q_4$
Maternal effect	$= (Q_1Q_3 + Q_1Q_4) - (Q_2Q_3 + Q_2Q_4)$
Paternal effect	$= (Q_1Q_3 + Q_2Q_3) - (Q_1Q_4 + Q_2Q_4)$
Interaction effect	$= (Q_1Q_3 + Q_2Q_4) - (Q_1Q_4 + Q_2Q_3)$; where Q*i* = QTL allele

values using a calibration equation developed from samples measured by light microscopy (Megraw et al. 1998). Both earlywood and latewood measurements were taken for each individual ring 3, 5 and 7.

Each phenotypic trait was analyzed as an individual-ring trait and as a composite trait (i.e., an average of individual-ring traits) (Table 2). Composite traits were considered a more accurate measurement of the phenotypic variation because they represented variation over a longer length of time. Therefore, QTLs were reported primarily for the composite traits. Composite traits (2–6) and (7–11) for *wsg* and *vol*% were considered to generally reflect juvenile and mature wood growth, respectively.

QTL analysis

An interval method designed to simultaneously analyze multiple markers of an outbred pedigree (Knott et al. 1997) was used to detect associations between the segregation of genetic markers and phenotypic variability for wood property traits. This method was modified from Haley et al. (1994) to allow the use of an outbred pedigree constructed from four unrelated, highly heterozygous, grandparents. The analysis first attempts to describe the genotypic marker data in terms of its inheritance in a three-generation pedigree, where a line origin probability was calculated at fixed locations throughout the genome for each of the four possible genotypes in the progeny generation (Haley et al. 1994). The phenotypic trait values were then regressed onto these probabilities using a least-squares analysis (Haley and Knott 1992). Each linkage group was scanned at 1–cM intervals for locations explaining a high proportion of the phenotypic variance (i.e., evidence for a QTL) using a conventional one-QTL model interval analysis. A two-dimensional scan was also performed to fit a two-QTL model for each linkage group (Haley and Knott 1992). The amount of computation time for the two-QTL model was reduced by considering only the composite traits and scanning at 2-cM intervals. Both QTL models included site as a fixed effect. The results are reported in terms of the individual parental effects (i.e., the difference in effect of the alleles inherited from each parent) and an interaction effect (i.e., the deviation from additivity, where a value of zero indicates complete additivity) (Table 3; Knott et al. 1997). QTLs were reported at two thresholds – a significant level ($P \leq 0.005$) and a “suggestive” level ($0.01 \geq P > 0.005$) – in an attempt to avoid Type-I and -II errors associated with pointwise versus genome-wide analyses (Lander and Kruglyak 1995). A few QTLs detected at this suggestive level may subsequently prove to be inaccurate, but they are reported in this study so that they can be followed in future experiments.

Results and discussion

Number and effect of QTLs associated with wood property traits

A total of 93 QTLs for *wsg*, *vol*% and *mfa* were detected at either the significant or suggestive level using the one- and/or two-QTL model for analysis (Tables 4–7). Many of these traits were highly associated (i.e., either the same trait measured from individual rings or composite traits derived from these individual rings), and therefore many of these QTLs were expected to be independent verifications of the same QTL. In addition, some QTLs were detected twice by using both the one- and the two-QTL models. Therefore, on each linkage group it is necessary to distinquish between the repeated detection of the same QTL versus the detection of multiple unique QTLs. A unique QTL is defined here as the subset of QTLs that map within approximately 15 cM of one another and have the same general profile for their parental and interaction effects (i.e., magnitude and direction of effect). QTLs detected at the significant level using composite traits were considered the most reliable (especially when composite QTLs were verified by individual-ring QTLs). For example, on linkage group 4 (LG4), the same QTL for earlywood *wsg* (*ewsg*) was detected for composite traits *ewsg*-(7–11) and *ewsg*-(2–11) and individual-ring traits *ewsg*-7, *ewsg*-8 and *ewsg*-9 (Table 4).

By using these criteria for inferring the number of unique composite trait QTLs (additional individual-ring trait QTLs are reported in parentheses), nine (14) QTLs were detected for either earlywood and/or latewood *wsg*, five (6) for *vol*%, and five (2) for both earlywood and/or latewood *mfa* (Fig. 1). Each of these composite trait QTLs were also supported by individual-ring QTLs, except for *ewsg*-(2–6) on LG14 and *vol*%-(2–6) on LG2 and LG5 (Tables 4–7). The residual variance explained by each QTL ranged from 5.4 to 15.7% for *wsg*, 5.5 to 12.3% for *vol*% and 5.4 to 11.9% for *mfa*. These values are generally small, and are likely to be overestimated because of the parameters of this experiment (Beavis 1995).

Table 4 Results from QTL analyses of wood density [i.e., wood specific gravity (*wsg*)] using a one-QTL model

LG	Trait[a]	Ring[b]	cM	*P*	Mat. eff. (SE)[c]	Pat. eff. (SE)[c]	Inter. eff. (SE)[c]	%Var.[d]
1	*ewsg*	2	114	0.00294**	–0.0006 (0.002)	0.0030 (0.002)	0.0074 (0.002)	7.2
	ewsg	11	51	0.00098**	0.0079 (0.003)	0.0054 (0.003)	0.0055 (0.003)	8.4
2	*lwsg*	(2–11)	29	0.00975*	0.0026 (0.002)	–0.0006 (0.002)	–0.0067 (0.002)	5.4
	lwsg	11	27	0.00011**	0.0021 (0.004)	–0.0057 (0.004)	–0.0195 (0.004)	11.2
3	*ewsg*	(2–11)	86	0.00390**	0.0007 (0.001)	–0.0051 (0.001)	–0.0020 (0.002)	6.6
	ewsg	2	5	0.00808*	–0.0047 (0.002)	–0.0036 (0.002)	0.0010 (0.002)	5.8
	ewsg	3	84	0.00271**	0.0029 (0.002)	–0.0067 (0.002)	–0.0026 (0.002)	7.0
	ewsg	6	85	0.00201**	0.0003 (0.002)	–0.0083 (0.002)	–0.0019 (0.002)	7.3
	ewsg	10	125	0.00004**	–0.0070 (0.002)	–0.0079 (0.002)	0.0062 (0.003)	12.2
	lwsg	2	13	0.00324**	0.0016 (0.002)	–0.0084 (0.002)	–0.0033 (0.002)	7.8
	lwsg	3	94	0.00059**	0.0004 (0.002)	–0.0054 (0.002)	–0.0099 (0.003)	8.9
	lwsg	7	0	0.00855*	–0.0035 (0.003)	–0.0094 (0.003)	0.0029 (0.003)	5.5
4	*ewsg*	(7–11)	93	0.00026*	0.0057 (0.001)	0.0012 (0.002)	0.0010 (0.002)	10.1
	ewsg	(2–11)	92	0.00174**	0.0043 (0.001)	0.0016 (0.001)	–0.0005 (0.001)	7.7
	ewsg	7	93	0.00204**	0.0054 (0.002)	0.0046 (0.002)	0.0017 (0.002)	7.3
	ewsg	8	92	0.00575*	0.0067 (0.002)	–0.0005 (0.002)	0.0029 (0.002)	6.0
	ewsg	9	95	0.00520*	0.0067 (0.002)	0.0000 (0.002)	0.0015 (0.002)	6.1
	lwsg	2	49	0.00745*	0.0017 (0.002)	0.0087 (0.003)	–0.0024 (0.003)	6.5
	lwsg	7	2	0.00909*	0.0005 (0.003)	0.0085 (0.005)	–0.0161 (0.005)	5.4
5	*lwsg*	(2–6)	67	0.00000**	–0.0025 (0.002)	0.0115 (0.002)	–0.0040 (0.003)	15.7
	lwsg	(7–11)	56	0.00059**	–0.0032 (0.002)	0.0084 (0.002)	–0.0046 (0.002)	9.1
	lwsg	(2–11)	55	0.00018**	–0.0030 (0.002)	0.0083 (0.002)	–0.0043 (0.002)	10.6
	ewsg	6	1	0.00765*	0.0024 (0.002)	0.0003 (0.002)	–0.0066 (0.002)	5.6
	ewsg	9	83	0.00574*	–0.0086 (0.003)	0.0044 (0.002)	–0.0039 (0.003)	6.0
	lwsg	2	31	0.00292**	0.0021 (0.003)	0.0094 (0.003)	0.0021 (0.003)	7.9
	lwsg	3	38	0.00535*	0.0008 (0.002)	0.0094 (0.003)	0.0000 (0.003)	6.1
	lwsg	4	64	0.00091**	–0.0023 (0.003)	0.0110 (0.003)	–0.0066 (0.003)	8.5
	lwsg	5	69	0.00004**	–0.0016 (0.003)	0.0161 (0.003)	–0.0005 (0.004)	12.1
	lwsg	6	58	0.00028**	–0.0046 (0.003)	0.0131 (0.003)	–0.0040 (0.003)	9.8
	lwsg	9	55	0.00399**	–0.0031 (0.003)	0.0103 (0.003)	–0.0073 (0.003)	6.5
6	*ewsg*	9	0	0.00366**	0.0036 (0.002)	–0.0074 (0.004)	–0.0119 (0.004)	6.6
7	*lwsg*	10	38	0.00621*	0.0088 (0.004)	0.0065 (0.004)	–0.0097 (0.004)	5.9
	lwsg	11	13	0.00308**	–0.0044 (0.006)	0.0130 (0.005)	0.0146 (0.006)	7.0
7b	*ewsg*	4	0	0.00146**	–0.0021 (0.002)	0.0054 (0.002)	–0.0054 (0.002)	7.8
8	*ewsg*	(7–11)	36	0.00098**	–0.0053 (0.001)	–0.0001 (0.001)	0.0014 (0.001)	8.4
	ewsg	2	108	0.00766*	0.0058 (0.002)	–0.0023 (0.003)	0.0034 (0.003)	5.9
	ewsg	6	43	0.00034**	–0.0068 (0.002)	0.0004 (0.002)	0.0042 (0.002)	9.6
9	*lwsg*	(2–6)	37	0.00510*	0.0002 (0.002)	–0.0027 (0.002)	0.0071 (0.002)	6.3
	lwsg	5	39	0.00813*	–0.0009 (0.003)	–0.0016 (0.003)	0.0112 (0.003)	5.5
11	*ewsg*	(2–6)	65	0.00676*	0.0026 (0.001)	–0.0027 (0.002)	0.0036 (0.002)	5.9
	ewsg	4	73	0.00640*	0.0047 (0.002)	–0.0051 (0.002)	0.0029 (0.002)	5.9
13	*lwsg*	8	31	0.00641*	0.0122 (0.004)	–0.0020 (0.003)	–0.0035 (0.004)	5.9
14	*ewsg*	(2–6)	0	0.01017*	–0.0001 (0.001)	–0.0066 (0.002)	–0.0018 (0.002)	5.4
	lwsg	3	9	0.00273**	–0.0062 (0.002)	–0.0096 (0.004)	–0.0004 (0.004)	7.0
	lwsg	4	27	0.00225**	0.0016 (0.003)	–0.0023 (0.003)	0.0124 (0.003)	7.3
	lwsg	7	40	0.00533*	–0.0051 (0.003)	–0.0073 (0.003)	0.0087 (0.003)	6.1

[a] *e*=earlywood; *l*=latewood

[b] See Table 2

[c] See Table 3; standard error in parentheses

[d] Percentage of phenotypic variance explained by QTL

* $0.01 \geq p > 0.005$, ** $p \leq 0.005$

Table 5 Results[a] from QTL analyses using a two-QTL model

LG	Trait	Ring	cM	*P*	Mat. eff. (SE)	Pat. eff. (SE)	Inter. eff. (SE)	%Var.[b]
1	*emfa*	(3,5,7)	54	0.00064**	0.1653 (0.231)	−0.2855 (0.253)	1.0620 (0.268)	19.8
			134		−0.9298 (0.219)	0.1958 (0.219)	−0.1401 (0.224)	
	lmfa	(3,5,7)	44	0.00574*	0.2293 (0.250)	−0.3027 (0.260)	0.8269 (0.258)	14.7
			134		−0.6626 (0.255)	0.5962 (0.257)	−0.0779 (0.258)	
3	*ewsg*	(2–11)	14	0.00825*	−0.0027 (0.001)	−0.0025 (0.001)	0.0011 (0.001)	12.0
			86		0.0015 (0.001)	−0.0060 (0.001)	−0.0017 (0.001)	
6	*vol*%	(7–11)	68	0.00621*	2.0346 (1.030)	−0.3415 (0.687)	−2.8531 (0.695)	11.0
			82		−1.9189 (1.083)	0.8592 (0.673)	2.0436 (0.704)	

[a] See footnotes for Table 4

[b] Percentage of variance explained by QTL pair

Table 6 Resultsa from QTL analyses of volume percentage of latewood (*vol*%) using a one-QTL model

LG	Trait	Ring	cM	*P*	Mat. eff. (SE)	Pat. eff. (SE)	Inter. eff. (SE)	%Var.
1	*vol*%	10	143	0.00281**	0.640 (0.724)	2.802 (0.782)	0.821 (0.827)	7.0
2	*vol*%	(2–6)	1	0.00782*	0.915 (0.385)	0.672 (0.398)	0.799 (0.404)	5.7
3	*vol*%	7	83	0.00055**	0.867 (0.693)	−2.237 (0.842)	−2.923 (0.875)	9.0
5	*vol*%	(2–6)	67	0.00259**	−0.190 (0.464)	1.317 (0.445)	−1.447 (0.543)	7.2
	vol%	2	111	0.00143**	1.441 (0.434)	0.345 (0.441)	1.002 (0.467)	9.0
6	*vol*%	(2–6)	65	0.00268**	0.775 (0.401)	1.181 (0.415)	−0.729 (0.445)	7.1
	vol%	(2–11)	66	0.00379**	0.691 (0.365)	0.781 (0.370)	−0.953 (0.401)	6.7
	vol%	5	62	0.00177**	1.317 (0.743)	2.004 (0.809)	−2.124 (0.830)	7.5
	vol%	8	7	0.00521*	2.501 (0.718)	0.654 (1.399)	1.717 (1.447)	6.1
7	*vol*%	4	35	0.00562*	−4.072 (1.267)	−0.244 (0.530)	2.351 (1.300)	6.1
8	*vol*%	4	72	0.00005**	0.969 (0.573)	1.914 (0.614)	2.695 (0.692)	12.0
	vol%	5	33	0.00646*	1.200 (0.741)	0.892 (0.752)	2.230 (0.742)	5.8
10	*vol*%	6	24	0.00067**	0.866 (0.546)	−1.925 (0.550)	1.065 (0.558)	8.7
11	*vol*%	10	0	0.00132**	2.809 (0.752)	−0.482 (0.755)	0.956 (0.822)	8.0
14	*vol*%	(7–11)	2	0.00121**	0.338 (0.450)	−2.527 (0.638)	−0.043 (0.679)	8.2
	vol%	(2–11)	4	0.00084**	0.580 (0.372)	−2.073 (0.548)	0.099 (0.583)	8.6
	vol%	3	17	0.01012*	−0.623 (0.579)	−1.740 (0.804)	2.077 (0.886)	5.3
	vol%	5	40	0.00406**	2.501 (0.819)	0.210 (0.810)	1.391 (0.896)	6.5
	vol%	6	4	0.00833*	0.443 (0.612)	−2.768 (0.901)	0.870 (0.958)	5.5
	vol%	7	0	0.00813*	−0.118 (0.795)	−3.768 (1.084)	−0.274 (1.128)	5.6

[a] See footnotes for Table 4

In previous studies of forest trees, a remarkably small number of QTLs have been identified for each trait (Sewell and Neale 2000). The number of QTLs identified ranged from 0 to 7 per trait (mean=2.7) and each QTL explained 3.4 to 62.6% of the phenotypic variance. Although most target traits for QTL detection are thought to be of polygenic inheritance (Zobel and Talbert 1984), many of these results could be explained under an oligogenic model (e.g., Bradshaw and Stettler 1995; Grattapaglia et al. 1996; Verhaegen et al. 1997; Kaya et al. 1999). For several reasons, these results are most likely biased so that the number of QTLs per trait is underestimated and the phenotypic effect associated with each trait is overestimated. For example, if the present study was considered on a yearly basis, only 0 to 5 (mean=2.5) earlywood and/or latewood QTLs were identified per trait, which is in the same range as that for previous studies. However, over the multi-year sampling period, 23 unique earlywood and/or latewood QTLs were identified for *wsg*, 16 for *vol%*, and seven for *mfa* (mean=15.3). These differences between the single year and multi-year analyses suggest that possible geno-

Table 7 Results[a] from QTL analyses of microfibril angle (*mfa*) using a one-QTL model

LG	Trait	Ring	cM	*P*	Mat. eff. (SE)	Pat. eff. (SE)	Inter. eff. (SE)	%Var.
1	*emfa*	(3,5,7)	134	0.00005**	−1.046 (0.222)	0.204 (0.229)	−0.067 (0.233)	11.9
	lmfa	(3,5,7)	42	0.00102**	0.363 (0.250)	−0.672 (0.254)	0.719 (0.262)	8.3
	emfa	5	134	0.00180**	−1.045 (0.273)	0.176 (0.282)	−0.107 (0.287)	7.6
	emfa	7	134	0.00038**	−1.262 (0.287)	−0.108 (0.296)	0.036 (0.301)	9.5
	lmfa	5	55	0.00060**	0.494 (0.297)	−0.721 (0.316)	1.073 (0.348)	9.1
	lmfa	7	40	0.00164**	0.578 (0.336)	−0.824 (0.342)	0.928 (0.348)	8.0
5	*lmfa*	3	86	0.00088**	−0.617 (0.321)	−0.864 (0.258)	−0.150 (0.363)	9.1
7	*emfa*	(3,5,7)	44	0.00484**	0.750 (0.230)	−0.074 (0.225)	−0.345 (0.226)	6.3
	lmfa	(3,5,7)	43	0.00290**	0.926 (0.252)	−0.076 (0.246)	−0.221 (0.252)	6.9
	emfa	5	42	0.00158**	1.096 (0.286)	−0.104 (0.277)	−0.300 (0.292)	7.7
	lmfa	5	43	0.00182**	1.096 (0.282)	−0.090 (0.274)	−0.141 (0.282)	7.7
	lmfa	7	44	0.00715*	1.040 (0.337)	−0.143 (0.329)	−0.533 (0.331)	6.0
8	*lmfa*	7	55	0.00816*	−0.962 (0.377)	−0.931 (0.387)	0.324 (0.441)	5.8
9	*emfa*	(3,5,7)	12	0.00095**	−0.605 (0.258)	0.162 (0.235)	0.908 (0.273)	8.4
	lmfa	(3,5,7)	13	0.00695*	−0.482 (0.282)	−0.058 (0.251)	0.872 (0.291)	5.8
	emfa	5	8	0.00700*	−0.661 (0.309)	0.142 (0.300)	0.949 (0.343)	5.8
	lmfa	5	14	0.01020*	−0.215 (0.315)	0.015 (0.281)	1.077 (0.325)	5.4
14	*lmfa*	(3,5,7)	25	0.00047**	−0.522 (0.246)	0.742 (0.317)	−0.767 (0.322)	9.3
	emfa	7	21	0.00261**	−0.477 (0.312)	0.697 (0.413)	−1.212 (0.444)	7.1
	lmfa	5	25	0.00038**	−0.584 (0.273)	0.650 (0.353)	−1.021 (0.357)	9.7
	lmfa	7	24	0.00074**	−0.849 (0.339)	1.095 (0.440)	−0.748 (0.454)	9.0

[a] See footnotes for Table 4

type×environment (G×E) interactions influence the temporal expression (and therefore the detection) of different QTLs from year to year. Alternatively, inconsistent QTL detection and overestimation of the phenotypic effect could also be attributed to the relatively small progeny size of the study (Beavis 1995). In addition, multiple QTLs residing on individual linkage groups may mask or distort the effect of individual QTLs.

Traditional quantitative genetic studies in forest trees have demonstrated that wood property traits generally exhibit high heritabilities. For loblolly pine, numerous studies have shown that narrow-sense heritabilities (h^2) for *wsg* are typically high ($0.20 \leq h^2 \leq 1.00$; Zobel and Jett 1995). Several researchers have also demonstrated that *wsg* was primarily controlled by additive genetic effects (Talbert et al. 1982; Lowe and Byram 1995). Each of the few studies for *vol*% reported values for h^2 as large or larger than those for wood specific gravity (van Buijtenen 1962; Goggans 1962). Quantitative genetic studies for *mfa* are limited.

Few QTL mapping studies in forest trees have estimated gene action for QTLs. However, the outbred QTL model (Knott et al. 1997) used in the present study provides a means to calculate an interaction effect (Table 3), where an effect of zero implies that the alleles are additive (although this determination is only valid if both parents are heterozygous at that QTL). Nine of the 19 composite-trait QTLs (two for *wsg*, four for *vol*%, and three for *mfa*) exhibited a strong non-zero interaction effect, which suggests some degree of non-additive (i.e., dominance or epistatic) expression for alleles at these QTLs. The remaining ten composite trait QTLs (seven for *wsg*, one for *vol*%, and two for *mfa*) exhibited a weak or zero interaction effect, which provides potential evidence for additive expression. However, of these only the QTL for *ewsg* on LG3 (detected using the two-QTL model) was clearly heterozygous for both parents.

Seasonal expression of QTLs associated with wood property traits

The change from earlywood to the denser latewood is the likely result of a change in auxin balance that promotes a prolongation of the wall-thickening phase (Megraw 1985). Therefore the thicker cell walls that are associated with latewood occur by virtue of a longer accrual period. This change in the auxin balance is possibly associated with the *vol*% trait. The onset of latewood formation appears to coincide with the cessation of height growth and the maturity of new needles, which allows for greater availability of resources for cell-wall thickening (Megraw 1985). Therefore, QTLs for *wsg* may possibly be expressed differently under these changing chemical microenvironments within the cell that are associated with the seasonal development of either earlywood or latewood (e.g., different auxin concentrations).

For the analyses of *wsg* and *mfa*, each trait was examined for both earlywood and latewood components. For *mfa*, each QTL was consistently detected for both ear-

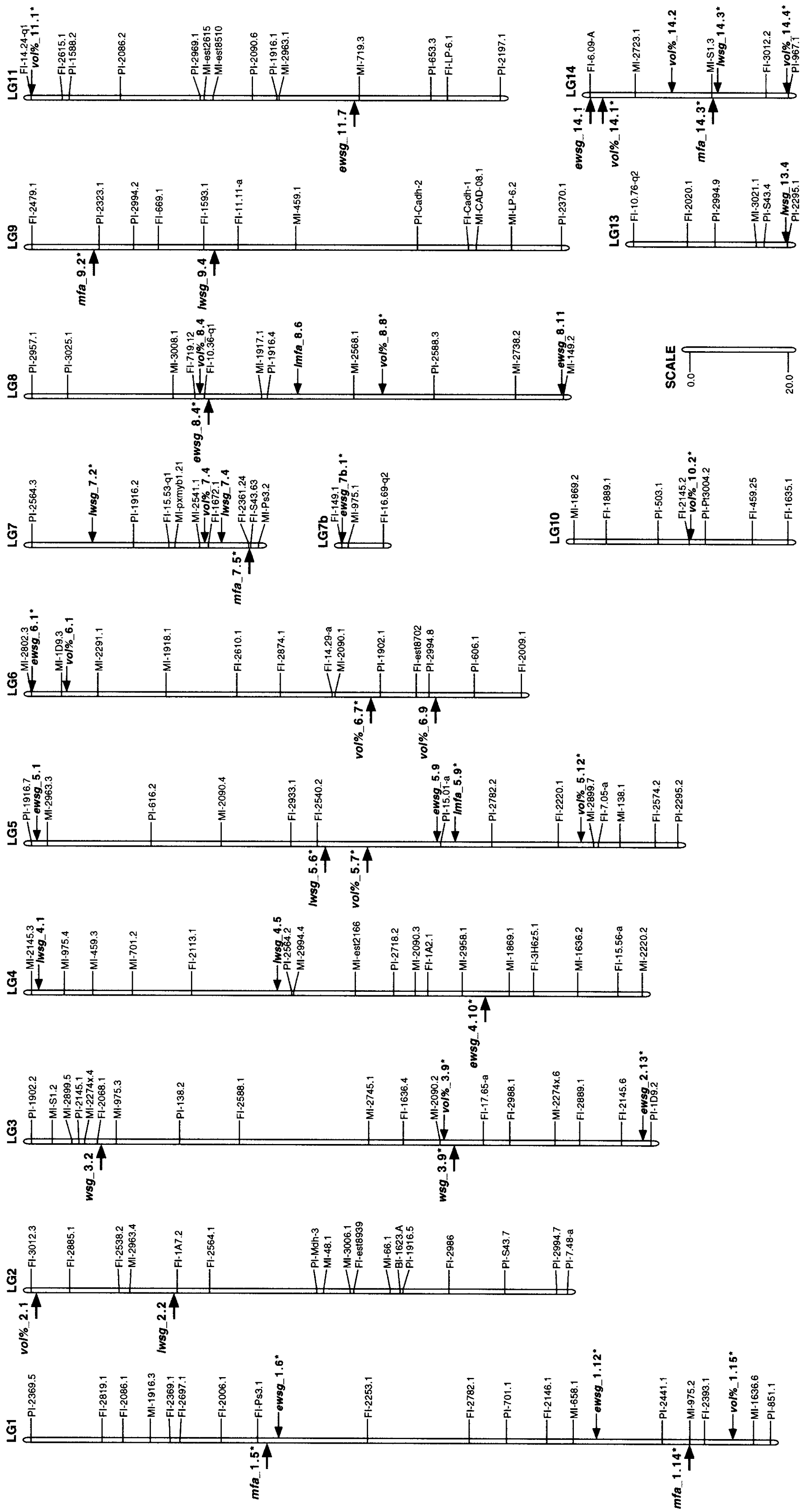

Fig. 1 Map position of unique QTLs for wood specific gravity (*wsg*), volume percentage of latewood (*vol*%) and microfibril angle (*mfa*) for the loblolly pine *qtl* pedigree. Composite trait QTLs are listed to the left and additional individual-ring QTLs are listed to the right of each linkage group (see Discussion). The prefix "*e*" or "*l*" preceding each trait name indicates either earlywood or latewood, respectively; the *numerical suffix* indicates the linkage group number and interval for location of each QTL (e.g., 1.1 represents LG1 and interval 0–10 cM, 2.2 represents LG2 and interval 11–20 cM, etc.). An *asterisk* (*) indicates QTL detection at the significant threshold ($P \leq 0.005$); no asterisk indicates detection at the suggestive threshold ($0.01 \geq P > 0.005$). The prefix preceding each marker name indicates the genetic informativeness at that locus; *MI*=maternally informative (*H*×*A*), *PI*=paternally informative (*A*×*H*) and *FI*=fully informative ($H_1 \times H_2$), where *H*=heterozygote and *A*=homozygote. The scale is in centiMorgans (cM)

lywood and latewood (except for two individual-ring trait QTLs for latewood *mfa* on LG5 and LG8). However, the majority of QTLs for *wsg* appeared to be specific to either earlywood or latewood. Four (8) QTLs for *wsg* were specific to earlywood, three (6) were specific to latewood, and two were detected for both earlywood and latewood (additional individual-ring trait QTLs in parentheses). The phenotypic correlations between earlywood and latewood are in accordance with these results ($0.533 \leq r \leq 0.697$ for *mfa* and $-0.240 \leq r \leq 0.125$ for *wsg*). Gladstone et al. (1970) reported that, although the relative densities of earlywood and latewood within most species are generally strongly correlated, loblolly pine trees can have relatively high earlywood density and low latewood density, thereby potentially lowering the correlation value between earlywood and latewood.

The genetic complexity of a quantitative trait can often complicate the detection of QTLs that are associated with the trait. These results demonstrate the utility of dissecting a complex trait into its different component traits (e.g., earlywood and latewood). QTL detection is enhanced by decreasing the potentially confounding phenotypic variation associated with a trait. This dissection also provides for a better understanding of the genetic architecture of a complex quantitative trait.

Temporal stability of QTLs associated with wood property traits

Forest trees experience a variety of environmental conditions over their lifespans. Long-lived trees also experience different developmental stages of growth (e.g., the change from juvenile to mature wood), which are most likely controlled by different sets of regulatory factors (e.g., tree age is often associated with different patterns of inheritance for wood density; Zobel 1964). Therefore, even within a single site, the temporal expression of QTLs from a single individual will most likely be significantly influenced by G×E interactions and/or the stage of development. Consequently, some QTLs may not be consistently detectable over time. However, those QTLs that are detectable over multiple growing seasons may be most valuable in the widest range of breeding programs.

Several QTL studies in forestry have examined the stability of QTLs over multiple growing seasons. A few of these studies repeatedly detected a subset of QTLs over time (e.g., Newcombe and Bradshaw 1996; Plomion et al. 1996; Verhaegen et al. 1997). Other studies found no evidence for the same QTLs among consecutive growing seasons (e.g., Bradshaw and Stettler 1995; Kaya et al. 1999). In the present study, a subset of QTLs were consistently detected over multiple years and/or at different seasonal stages. Other QTLs were detected only during a single year and seasonal stage. For example, the QTL for earlywood *wsg* on LG4 appears to be consistently expressed over the duration of this study, whereas the QTL for latewood *wsg* on LG5 appears to be expressed only during the later stage of growth and is possibly associated with the onset of the development of mature wood. Therefore, a QTL analysis using averaged phenotypic values may detect QTLs that most consistently influence the trait, yet a temporal dissection of how trees achieve their mature phenotype may give insights both to the developmental responses influencing maturation as well as to potential physiological responses to year to year climatic extremes that occur during the lifespan of a tree (O'Malley and McKeand 1994).

Verification of QTLs

The practical utilization of QTLs in a marker-aided breeding program has in part been hampered by a lack of verification of the results from QTL studies. The instigation of new QTL detection studies designed to verify previously detected QTLs is costly and time consuming. In this study, temporal replication provided an efficient and effective method for the independent verification of QTLs against the same genetic background. However, larger mapping populations are still essential for more precise placement of QTLs, for a more realistic estimation of the magnitude of QTL effects and for detection of QTLs with smaller effect. Experiments are currently being conducted to verify the physical wood property QTLs that were detected in this study. As verification experiments become available, comparative mapping can be used to relate the results from these independent studies at both an intraspecific and interspecific level (Devey et al. 1999; Sewell et al. 1999).

Acknowledgements The authors are grateful to Leon Burris for the collection of germplasm. This research was funded in part by USDA NRI Plant Genome Grant 96-35300-3719 and complies with the current laws of the country in which the experiment was performed.

References

Beavis WD (1995) The power and deceit of QTL experiments: lessons from comparative QTL studies. Proc 49th Annu Corn and Sorghum Indus Res Conf 1994, pp 304–312

Bradshaw HD, Stettler RF (1995) Molecular genetics of growth and development in Populus. IV. Mapping QTLs with large effects on growth, form, and phenology traits in a forest tree. Genetics 139:963–973

Buijtenen JP van (1962) Heritability estimates of wood density in loblolly pine. TAPPI 45:602–605

Devey ME, Jermstad KD, Tauer CG, Neale DB (1991) Inheritance of RFLP loci in a loblolly pine three-generation pedigree. Theor Appl Genet 83:238–242

Devey ME, Sewell MM, Uren TL, Neale DB (1999) Comparative mapping in loblolly and radiata pine using RFLP and microsatellite markers. Theor Appl Genet 99:656–662

El-Osta MLM, Wellwood RW, Butters RG (1972) An improved X-ray technique for measuring microfibril angle of coniferous wood. Wood Sci 5:113–117

Goggans JF (1962) The correlation, variation and inheritance of wood properties in loblolly pine. Tech Rep 14, School For Res NC State Univ, Raleigh, North Carolina

Gladstone WT, Barefoot AC, Zobel BJ (1970) Kraft pulping of earlywood and latewood from loblolly pine. For Prod J 20:17–24

Grattapaglia D, Bertolucci F, Penchel R, Sederoff R (1996) Genetic mapping of quantitative trait loci controlling growth and wood quality traits in *Eucalyptus grandis* using a maternal half-sib family and RAPD markers. Genetics 144:1205–1214

Groover A, Devey M, Lee J, Megraw R, Mitchell-Olds T, Sherman B, Vujcic S, Williams C, Neale D (1994) Identification of quantitative trait loci influencing wood specific gravity in an outbred pedigree of loblolly pine. Genetics 138:1293–1300

Groover AT, Williams CG, Devey ME, Lee JM, Neale DB (1995) Sex-related differences in meiotic recombination frequency in *Pinus taeda*. J Hered 86:157–158

Haley CS, Knott SA (1992) A simple regression model for interval mapping in line crosses. Heredity 69:315–324

Haley CS, Knott SA, Elsen J-M (1994) Mapping quantitative trait loci in crosses between outbred lines using least squares. Genetics 136:1195–1207

Hamrick JL, Godt MJ (1990) Allozyme diversity in plant species. In: Brown AHD, Clegg MT, Kahler AL, Weir BS (eds) Plant population genetics, breeding, and genetic resources. Sinauer, Sunderland, Massachusetts, pp 43–93

Kaya Z, Sewell M, Neale D (1999) Identification of quantitative trait loci influencing annual height- and diameter-increment growth in loblolly pine. Theor Appl Genet 98:586–592

Knott SA, Neale DB, Sewell MM, Haley CS (1997) Multiple marker mapping of quantitative trait loci in an outbred pedigree of loblolly pine. Theor Appl Genet 94:810–820

Lander ES, Kruglyak L (1995) Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nature Genet 11:241–247

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newberg L (1987) MapMaker: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Lowe WJ, Byram TD (1995) Heritability estimates for loblolly pine wood specific gravity based on control-pollinated genetic tests. Proc 23rd SFTIC, June 20–22, pp 204–209

Megraw RA (1985) Wood quality factors in loblolly pine. Tappi Press, Atlanta, Georgia

Megraw RA, Leaf G, Bremer D (1998) Longitudinal shrinkage and microfibril angle in loblolly pine. In: Butterfield BA (ed), Microfibril angle in wood. Univ Canterbury Press, Christchurch, New Zealand, pp 27–61

Meylan BA (1967) Measurement of microfibril angle by X-ray diffraction. For Prod J 17:51–58

Newcombe G, Bradshaw HD (1996) Quantitative trait loci conferring resistance in hybrid poplar to *Septoria populicola*, the cause of leaf spot. Can J For Res 26:1943–1950

O'Malley DM, McKeand SE (1994) Marker-assisted selection for breeding value in forest trees. For Genet 1:207–218

Panshin AJ, de Zeeuw CE (1980) Textbook of wood technology: structure, identification, properties, and uses of the commercial woods of the United States and Canada, 4th edn. McGraw-Hill, New York

Plomion C, Durel C-É, O'Malley D (1996) Genetic dissection of height in maritime pine seedlings raised under accelerated growth conditions. Theor Appl Genet 93:849–858

Schultz RP (1997) Loblolly pine: the ecology and culture of loblolly pine (*Pinus taeda* L.). Agricultural Handbook 713, USDA, Forest Service, Washington, D.C.

Sewell MM, Neale DB (2000) Mapping quantitative traits in forest trees. In: Jain SM, Minocha SC (eds) Molecular biology of woody plants, volume 1. Kluwer Academic Publishers, The Netherlands, pp 407–423

Sewell MM, BK Sherman, Neale DB (1999) A consensus map for loblolly pine (*Pinus taeda* L.). I. Construction and integration of individual linkage maps from two outbred three-generation pedigrees. Genetics 151:321–330

Stam P (1993) Construction of integrated genetic linkage maps by means of a new computer package: JoinMap. Plant J 3:739–744

Talbert JT, Bridgwater FE, Jett JB, Jahromi S (1982) Genetic parameters of wood specific gravity in a control-pollinated loblolly pine progeny test. Proc TAPPI Research and Development Division Conf, pp 179–182

Verhaegen D, Plomion C, Gion J-M, Poitel M, Costa P, Kremer A (1997) Quantitative trait dissection analysis in *Eucalyptus* using RAPD markers. 1. Detection of QTLs in interspecific hybrid progeny, stability of QTL expression across different ages. Theor Appl Genet 95:597–608

Williams CG, Neale DB (1992) Conifer wood quality and marker-aided selection: a case study. Can J For Res 22:1009–1017

Zobel BJ (1964) Breeding for wood properties in forest trees. Unasylva 18:89–103

Zobel BJ, Jett JB (1995) Genetics of wood production. Springer-Verlag, Berlin Heidelberg New York

Zobel BJ, Talbert JT (1984) Applied forest tree improvement. John Wiley and Sons, New York