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

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Abstract Chemical wood property traits were analyzed for the presence of quantitative trait loci (QTLs) in a three-generation outbred pedigree of loblolly pine (*Pinus taeda* L.). These traits were assayed using pyrolysis molecular beam mass spectrometry and include mass spectrum peak intensities associated with carbohydrates, α-cellulose and hemicellulose sugars, and lignin. Models for projection to latent structures (PLS) were used to also estimate the chemical composition of cell walls (i.e., α-cellulose, galactan and lignin) from mass spectrum data using multivariate regression. Both earlywood and latewood fractions from the fifth annual ring 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. Eight unique QTLs influencing cell wall chemistry were detected from multiple peak intensities and/or PLS estimates using the one- and two-QTL models. Significant differences in chemical contents were observed among the populations from North Carolina vs Oklahoma, and results from QTL×environment analyses suggest that QTLs interact with environmental location. QTLs should be verified in larger experiments and in different genetic and environmental backgrounds. QTL mapping will help towards eventually identifying genes having a major effect on chemical wood properties.

Keywords *Pinus taeda* L. · QTL mapping · Wood chemistry · α-Cellulose · Hemicellulose · Lignin

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Introduction

The physical and chemical properties of wood affect many characteristics desirable to the timber industry. For example, lumber strength, stiffness and dimensional stability are influenced by the microfibril angle in the cell wall (Megraw 1985). Pulp yield and cohesiveness are positively associated with α-cellulose and hemicellulose content, respectively, and energy consumption during pulping is inversely related to lignin content (Smook and Kocurek 1988). Recent efforts in forestry have focused on identifying genes controlling complex traits, including those for wood quality.

The detection of QTLs (quantitative trait loci) is one approach used to discover genes controlling wood property traits. In loblolly pine (*Pinus taeda* L.), a three-generation pedigree was previously used to detect QTLs associated with physical wood property traits, i.e., (1) wood specific gravity from both whole-core (Groover et al. 1994; Knott et al. 1997) and earlywood and latwood samples; (2) earlywood and latewood microfibril angle; and (3) the volume percentage of latewood (Sewell et al. 2000). In the present study, work with this same pedigree was extended to include chemical wood property traits, e.g., α-cellulose, hemicellulose and lignin.

Wood is essentially a matrix of cell walls and cellular air spaces derived from secondary growth (Megraw 1985). The major chemical components of the cell wall are the polysaccharide fractions (holocellulose) and lignin. Holocellulose is composed of α-cellulose and a complex mixture of polymers formed from simple sugars known collectively as hemicellulose. Alpha-cellulose is a highly stable, unbranched polysaccharide composed of

flat chains of poly-1,4-β–D anhydroglucose (Arioli et al. 2000). Approximately 10000 glucose residues are polymerized to form the α-cellulose macromolecule (Panshin and de Zeeuw 1980). Lignin is derived from the polymerization of three different hydroxycinnamyl alcohols (monolignols): *p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol. These monolignols give rise to the *p*-hydroxyphenyl, guaiacyl, and syringyl units of the lignin polymer, respectively (Baucher et al. 1998).

Wood chemical content and composition varies at all levels from among taxa to different cell types and is influenced by both developmental and environmental conditions (Christensen et al. 2000). For example, in softwoods lignin is predominantly derived from the polymerization of coniferyl alcohol, whereas in hardwoods lignin is generally composed of both coniferyl and sinapyl alcohol (Panshin and de Zeeuw 1980). Wood from different tree species is typically composed of 40–50% α-cellulose, 20–35% hemicellulose and 15–35% lignin. In pine, for example, *Pinus elliottii* (slash pine) and *Pinus strobus* (white pine) are composed of approximately 40% α-cellulose and approximately 30% of both hemicellulose and lignin (Panshin and de Zeeuw 1980).

The polysaccharide fraction of the cell wall is arranged into long microfibril strands composed of a crystalline α-cellulose core and surrounded by short-chained hemicellulose sugars. After the microfibrils are formed, lignin is deposited around them and binds them into a rigid structure (Panshin and de Zeeuw 1980). This physical relationship between holocellulose and lignin affects many fiber characteristics that are important to the pulp and paper industry. For example, in the production of fine papers, chemical pulping is designed to maximize the removal of lignin while minimizing the degradation of α-cellulose and hemicellulose. However, this process comes at considerable expense and with detrimental effects on the environment.

Despite the importance of chemical composition in the pulping process, no studies have analyzed QTLs directly associated with chemical wood properties in forest trees. This deficiency results from the high investment of time and money for traditional wet chemistry (e.g., the Klason method for lignin quantification), and the limited accuracy in estimates for total cell wall composition from these analyses. New technologies promise to decrease time and cost, while increasing the accuracy of chemical assessments. Several high-throughput analytical methods have been developed that use a combination of a rapid spectroscopic technique, such as near infrared spectroscopy, and multivariate regression modeling to estimate the amount of a particular cell wall constituent (Schultz and Burns 1990; Wright et al. 1990; Ona et al. 1997). In this study, pyrolysis molecular beam mass spectrometry (pyMBMS) was used to estimate the chemical content of wood (Davis et al. 1999). The analysis of a wood sample by pyMBMS takes approximately 2 minutes per sample (excluding minimal sample preparation), compared to traditional analytical methods that generally require several days. The pyMBMS technique has the added advantage that a peak in the mass spectrum can be related to a specific cell wall chemical component. The intensities of these peaks therefore provide information regarding the amount of chemical component in the wood sample. Consequently, these specific peak intensities can also be used as traits in a QTL analysis.

Loblolly pine is the leading timber species in North America and is grown commercially for both solid wood and pulp and paper products. The objectives of the present study were to: (1) identify QTLs associated with α-cellulose, hemicellulose and lignin content for both earlywood and latewood; (2) estimate the genetic effects of these QTLs; and (3) analyze for potential QTL×environment (QTL×E) interactions of these QTLs.

Materials and methods

Mapping population, genotypic data and map construction

A three-generation outbred pedigree was used to detect marker-trait associations for wood property traits in loblolly pine. This pedigree (referred to as the *qtl* pedigree) was used by Groover et al. (1994), Knott et al. (1997) and Sewell et al. (2000) in earlier QTL mapping studies for wood quality, and consists of 172 progeny grown at six different sites in North Carolina (four) and Oklahoma (two). The number of trees per site ranged from 19 to 35. Genotypic segregation data from restriction fragment length polymorphism (RFLP) markers were used to construct independent parental framework maps using MapMaker (Lander et al. 1987), and then merged into a single sex-average map using JoinMap (Stam 1993), following Sewell et al. (1999). The genotypic data set and resulting genetic map were exactly as described in Sewell et al. (2000), except several additional markers were added to linkage group eight (LG8).

Wood chemistry phenotypic data

Pyrolysis molecular beam mass spectrometry

A 12-mm radial wood core was taken from each progeny at breast height. Tissue samples from 165 progeny representing the fifth growth ring were collected for both earlywood and latewood and ground in a Wiley mill. Three subsamples (approximately 15 mg) from each ground fraction were passed through the pyrolysis reactor at 550±5°C under a helium flow rate of 5.0 l/min at 21°C, 0.1 MPa. Vacuum expansion created a molecular beam that was introduced into the Extrel Model TQMS C50 mass spectrometer, and mass spectra between 15 and 400 Da were measured using a Teknivent Vector 2TM data acquisition system (Davis et al. 1999). The mass spectrum of the pyrolysis vapor provides a rapid, semi-quantitative picture of the molecular fragments. The mass spectra were normalized to total ion current, and peak intensitites were collected for mass peaks associated with carbohydrates (*crb*; peaks 60 and 73), a five-carbon hemicellulose sugar (*hmc*; peak 114), a six-carbon α-cellulose sugar (*cel*; peak 144) and lignin (*lgn*; peaks 124, 135 and 150) (Evans and Milne 1987). Peak intensity values from each triplicated subsample were averaged and used as traits in the QTL analysis (Table 1). Each trait is referred to by its ‘‘mass spectrum peak/associated trait” (e.g., 60/*crb*).

Multivariate data analysis

Models for projection to latent structures (PLS) were used to predict the chemical composition of cell walls from mass spectrum data using multivariate statistics (Davis et al. 1999). The PLS-2

Table 1 Phenotypic traits for chemical wood properties; all traits were assayed for both earlywood and latewood

Trait type	Phenotypic trait	Chemical association	Trait name
Mass spectrum peaks[a]	60, 73	Various carbohydrates	60/*crb*, 73/*crb*
	114	Hemicellulose sugar	114/*hmc*
	144	Cellulose sugar	144/*cel*
	124, 135, 150	Lignin	124/*lgn*, 135/*lgn*, 150/*lgn*
PLS modeling[b]	α-Cellulose	α-Cellulose	pls/*cel*
	Galactan	Hemicellulose	pls/*gal*
	Lignin	Lignin	pls/*lgn*

[a] Peak intensities from py-MBMS analysis
[b] Multivariate analysis of mass spectrum peaks

Table 2 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

algorithm (Martens and Naes 1989) was used to construct models from 22 standard samples. The chemical composition of these reference standards was determined by Weyerhaeuser Co. These models were used to estimate the concentrations of α-cellulose (pls/*cel*), galactan (pls/*gal*), and lignin (pls/*lgn*) from loblolly pine samples. Data analyses were performed using the Unscrambler software program (version 6.11; CAMO A/S, Trondheim, Norway). The PLS estimates from each triplicated subsample were averaged and used as traits in the QTL analysis (Table 1). Also see Tuskan et al. (1999) for more details.

QTL analysis

An interval mapping method was used to detect associations between the segregation of genetic markers and phenotypic variability for wood chemisty traits. This method uses a least-squares approach (Haley and Knott 1992) to simultaneously analyze multiple markers of an outbred pedigree (Knott et al. 1997). 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 scanning only 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., difference in effect of the alleles inherited from each parent) and an interaction effect (i.e., deviation from additivity, where a value of zero indicates complete additivity) (Table 2; Knott et al. 1997). QTL×E interactions were analyzed at the level of state location (i.e., sites from North Carolina vs Oklahoma) using only the one-QTL model.

QTLs were reported at two thresholds, a significant level ($p \leq 0.005$) and a ''suggestive" level ($0.01 \geq p > 0.005$), for linkage group-wise tests. These *p*-values approximately correspond to a genome-wide level of 0.05 and 0.01, respectively (Knott et al. 1997). Separate thresholds were used in an attempt to avoid Type I and II errors associated with pointwise versus genome-wide analyses (Lander and Kruglyak 1995). Although a few QTLs detected at the suggestive level may subsequently prove to be false positives, the legitimacy of accepting these QTLs increases when a given QTL is detected by multiple peak intensities and/or PLS estimates.

Results and discussion

Number and effect of QTLs associated with chemical wood property traits

Using the one-QTL model and both the significant and suggestive thresholds for declaring the presence of a QTL, a total of 29 QTLs were detected for ten traits associated with wood chemistry (Table 3). Six QTLs were also detected using the two-QTL model (Table 4). Many of these traits were associated with one another (e.g., spectrum peaks 124, 135, 150, and pls/*lgn*, for both earlywood and latewood, were all associated with lignin), and consequently many of these QTLs were expected to be different and/or independent verifications of the same QTL. In addition, some QTLs were detected twice by using both the one- and 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). These are the same criteria as previously used in determining QTLs for physical wood property traits in loblolly pine (Sewell et al. 2000).

In this study, chemical wood property traits were measured based on chemical content per unit weight rather than content per unit volume or per cell. Since wood is composed of approximately 97% lignin and holocellulose, an inverse relationship necessarily exists for lignin vs holocellulose content, while the two components of holocellulose (i.e., α-cellulose and hemicellulose) tend to vary directly (Panshin and de Zeeuw 1980). Therefore an observed increase in lignin content could actually be the result of a decrease in holocellulose, or vice versa. The phenotypic correlations (*r*) among wood chemistry values observed in this study (e.g., *r* is high among pls/*cel*, pls/*gal* and pls/*lgn* for both earlywood and latewood, but negatively so for pls/*lgn*; Table 5), as well as the expression of QTLs related to these different chemical cell wall components (Table 3), also reflected these relationships. For example, on LG8 the QTLs for pls/*lgn*, pls/*cel*, pls/*gal*, 60/*crb* and 73/*crb* all map to the same position and exhibit similar QTL effects, except that pls/*lgn* is in the opposite direction. In light of these relationships, QTLs identified in this study might generally be described as cell wall chemistry (*cwc*) traits, rather

Table 3 Results from QTL analyses of wood cell wall chemistry traits using the one-QTL model

LG	Trait[a]	Wood[b]	cM	*p*	Mat.eff. (SE)[c]	Pat.eff. (SE)[c]	Inter.eff. (SE)[c]	%Var.[d]
1	60/*crb*	ew	45	0.00552*	0.0195 (0.009)	0.0110 (0.009)	0.0231 (0.009)	6.1
	73/*crb*	ew	45	0.00247**	0.0190 (0.008)	0.0126 (0.009)	0.0235 (0.009)	7.1
	144/*cel*	ew	46	0.00471**	0.0042 (0.003)	0.0015 (0.003)	0.0088 (0.003)	6.3
	114/*hmc*	ew	46	0.00189**	0.0142 (0.006)	0.0005 (0.006)	0.0185 (0.006)	7.5
2	pls/*cel*	ew	37	0.00960*	–0.1168 (0.076)	0.0195 (0.076)	0.2540 (0.078)	5.4
	144/*cel*	ew	77	0.00289**	0.0005 (0.003)	–0.0072 (0.003)	–0.0093 (0.003)	6.9
	60/*crb*	ew	77	0.00203**	–0.0072 (0.009)	–0.0218 (0.009)	–0.0315 (0.010)	7.4
	57*crb*	ew	78	0.00263**	–0.0033 (0.005)	–0.0103 (0.005)	–0.0165 (0.005)	7.0
	114/*hmc*	ew	78	0.00003**	0.0003 (0.006)	–0.0194 (0.006)	–0.0287 (0.007)	12.6
5	144/*cel*	ew	104	0.00591*	0.0076 (0.003)	–0.0030 (0.003)	–0.0057 (0.003)	6.0
	144/*cel*	lw	112	0.00378**	0.0097 (0.003)	–0.0007 (0.003)	–0.0015 (0.003)	6.6
	114/*hmc*	ew	119	0.00011**	0.0260 (0.006)	–0.0024 (0.007)	–0.0084 (0.007)	11.0
	124/*lgn*	ew	115	0.00214**	–0.0122 (0.005)	0.0109 (0.006)	0.0133 (0.006)	7.3
	150/*lgn*	ew	115	0.01032*	–0.0040 (0.004)	0.0102 (0.005)	0.0100 (0.005)	5.3
	135/*lgn*	ew	118	0.00003**	–0.0075 (0.002)	0.0049 (0.002)	0.0035 (0.002)	12.7
6	pls/*cel*	ew	87	0.00083**	–0.0963 (0.085)	0.2279 (0.077)	–0.2430 (0.089)	8.6
	144/*cel*	ew	94	0.00509*	–0.0028 (0.003)	0.0073 (0.003)	–0.0074 (0.003)	6.2
	pls/*gal*	ew	94	0.00455**	–0.0013 (0.009)	0.0263 (0.009)	–0.0202 (0.010)	6.4
8	pls/*cel*	ew	39	0.00370**	–0.0549 (0.082)	–0.2968 (0.080)	–0.0306 (0.087)	6.7
	pls/*cel*	lw	22	0.00531*	0.0166 (0.106)	–0.3903 (0.109)	–0.0566 (0.116)	6.2
	60/*crb*	lw	21	0.00272**	–0.0143 (0.012)	–0.0416 (0.012)	0.0169 (0.013)	7.1
	73/*crb*	lw	17	0.00853*	–0.0151 (0.011)	–0.0283 (0.011)	0.0217 (0.012)	5.5
	pls/*gal*	lw	20	0.00486**	–0.0060 (0.013)	–0.0489 (0.013)	0.0000 (0.014)	6.3
	pls/*lgn*	ew	39	0.00760*	0.0090 (0.032)	0.1086 (0.031)	0.0136 (0.034)	5.7
	pls/*lgn*	lw	25	0.00211**	0.0250 (0.039)	0.1523 (0.040)	–0.0342 (0.042)	7.4
9	150/*lgn*	ew	32	0.00685*	–0.0067 (0.004)	0.0124 (0.004)	0.0065 (0.005)	5.8
	124/*lgn*	ew	51	0.00756*	–0.0077 (0.006)	0.0204 (0.007)	0.0100 (0.008)	5.7
11	144/*cel*	lw	1	0.01010*	–0.0014 (0.003)	0.0049 (0.003)	–0.0087 (0.003)	5.3
	135/*lgn*	lw	0	0.00476**	0.0018 (0.002)	–0.0047 (0.002)	0.0069 (0.002)	6.3

* $0.01 \geq p > 0.005$, ** $p \leq 0.005$
[a] See Table 1 for explanation of traits
[b] ew=earlywood; lw=latewood
[c] See Table 2; standard error in parentheses
[d] Percentage of phenotypic variance explained by QTL

Table 4 Results[a] from QTL analyses of wood cell wall chemistry traits using the two-QTL model

LG	Trait	Wood	*p*	QTL	cM	Mat. eff. (SE)	Pat. eff. (SE)	Inter. eff. (SE)	%Var.[b]
2	144/*cel*	ew	0.00506*	1st	40	–0.0053 (0.003)	–0.0007 (0.003)	0.0107 (0.003)	11.6
				2nd	72	0.0027 (0.003)	–0.0061 (0.003)	–0.0108 (0.003)	
2	60/*crb*	ew	0.00678*	1st	34	0.0151 (0.009)	–0.0095 (0.009)	0.0186 (0.009)	11.0
				2nd	76	–0.0117 (0.010)	–0.0215 (0.009)	–0.0328 (0.009)	
2	114/*hmc*	ew	0.00044**	1st	72	–0.0089 (0.010)	–0.0163 (0.007)	–0.0161 (0.007)	17.0
				2nd	102	0.0136 (0.014)	–0.0086 (0.007)	–0.0319 (0.009)	

[a] See footnotes for Table 3 [b] Percentage of variance explained by QTL pair

than specifically as QTLs associated with each individual wood chemistry component. Although any given QTL in this experiment is likely to be associated with a specific chemical trait, rather than pleiotrophically associated with each trait simultaneously, these specific associations cannot be made at the current level of QTL detection.

By using these criteria for inferring the number of unique QTLs, eight *cwc* QTLs were identified from multiple peak intensities and/or PLS estimates (Fig. 1). For

Table 5 Correlation coefficients[a] (*r*) among cell wall chemistry traits[b]

Earlywood	60/*crb*	73/*crb*	114/*hmc*	144/*cel*	124/*lgn*	135/*lgn*	150/*lgn*	pls/*lgn*	pls/*gal*	pls/*cel*
60/*crb*	–	0.95	0.77	0.85	–0.84	–0.85	–0.86	–0.92	0.92	0.71
73/*crb*		–	0.72	0.86	–0.85	–0.86	–0.88	–0.91	0.90	0.66
114/*hmc*			–	0.79	–0.58	–0.77	–0.55	–0.62	0.69	0.43
144/*cel*				–	–0.65	–0.87	–0.64	–0.90	0.90	0.80
124/*lgn*					–	0.77	0.91	0.78	–0.71	–0.42
135/*lgn*						–	0.78	0.85	–0.87	–0.66
150/*lgn*							–	0.79	–0.78	–0.46
pls/*lgn*								–	–0.96	–0.88
pls/*gal*									–	0.87
pls/*cel*										–
Latewood	**60/*crb***	**73/*crb***	**114/*hmc***	**144/*cel***	**124/*lgn***	**135/*lgn***	**150/*lgn***	**pls/*lgn***	**pls/*gal***	**pls/*cel***
60/*crb*	–	0.94	0.74	0.82	–0.91	–0.84	–0.88	–0.92	0.85	0.70
73/*crb*		–	0.73	0.84	–0.88	–0.81	–0.82	–0.87	0.77	0.59
114/*hmc*			–	0.73	–0.70	–0.69	–0.62	–0.62	0.64	0.39
144/*cel*				–	–0.73	–0.84	–0.65	–0.84	0.77	0.69
124/*lgn*					–	0.84	0.92	0.85	–0.79	–0.58
135/*lgn*						–	0.83	0.87	–0.85	–0.73
150/*lgn*							–	0.84	–0.85	–0.67
pls/*lgn*								–	–0.95	–0.89
pls/*gal*									–	0.92
pls/*cel*										–

[a] Analysis is based on 165 individuals [b] See Table 1 for explanation of traits

example, on LG5 the same QTL (*cwc*_5.12) was detected for various earlywood and/or latewood spectrum peaks (114, 124, 135, 144 and 150) associated with α–cellulose, hemicellulose and lignin. The two QTLs on LG2 (*cwc*_2.4 and *cwc*_2.8) were detected using both the one- and two-QTL models. Six of these eight multi-trait QTLs attained the significant threshold for at least one of the traits in the cluster. The residual variance explained by individual QTLs ranged from 5.3 to 12.7% for these chemical traits. These values are generally small, and are likely to be overestimated because of the parameters of this experiment (Beavis 1995; Frewen et al. 2000). [Note: several additional single-trait QTLs were detectd from only a single peak intensity or PLS estimate (data not shown). These single-trait QTLs are considered less robust than the multi-trait QTLs, and therefore are not discussed in the text.]

Few quantitative genetic studies have been conducted on chemical wood property traits in forest trees (reviewed in Zobel and Jett 1995). In a study of 48 families of loblolly pine, both holo- and α-cellulose yields varied significantly both among families and among individuals within families (Zobel et al. 1966; Jett et al. 1977). These authors also report that the additive variance was essentially zero while the dominance component accounted for about 15% of the phenotypic variance. The genetic control of lignin was indicated in a small study of loblolly pine (van Buijtenen 1967). Einspahr et al. (1964) reported high heritabilities for lignin in slash pine (H^2=0.72 and h^2=0.25), while Dadswell et al. (1961) found no meaningful heritabilities for α-cellulose or lignin in Monterey pine (*Pinus radiata*).

The outbred QTL model (Knott et al. 1997) used in the present study provides a means to estimate gene action for QTLs (Table 2), where an interaction effect of zero implies that the alleles are additive, although this determination is only valid if both parents are heterozygous at that QTL. Five of the eight cell wall chemistry QTLs exhibited a strong non-zero interaction effect, which suggests some degree of non-additive (i.e., dominant or epistatic) expression for alleles at these QTLs. The remaining three QTLs exhibited a weak or zero interaction effect. Of this latter group, the QTLs on LGs 5 and 9 exhibit possible evidence that both parents are heterozygous (evidence for heterozygosity was based on the presence of a non-zero parental effect). This combination provides potential evidence for additive expression for these two QTLs.

Genomic distribution of QTLs associated with chemical wood property traits

A number of studies in forestry have used the same mapping population to identify and map QTLs for multiple traits. In several of these studies, QTLs for different traits have been mapped to the same genomic location (reviewed in Sewell and Neale 2000). For many of these QTL clusters, the traits exhibited a high degree of phenotypic correlation and similar allelic effects. This combined evidence suggests that pleiotrophy of a single QTL, rather than simple linkage among two QTLs, may likely explain these correlations (Bradshaw and Stettler 1995).

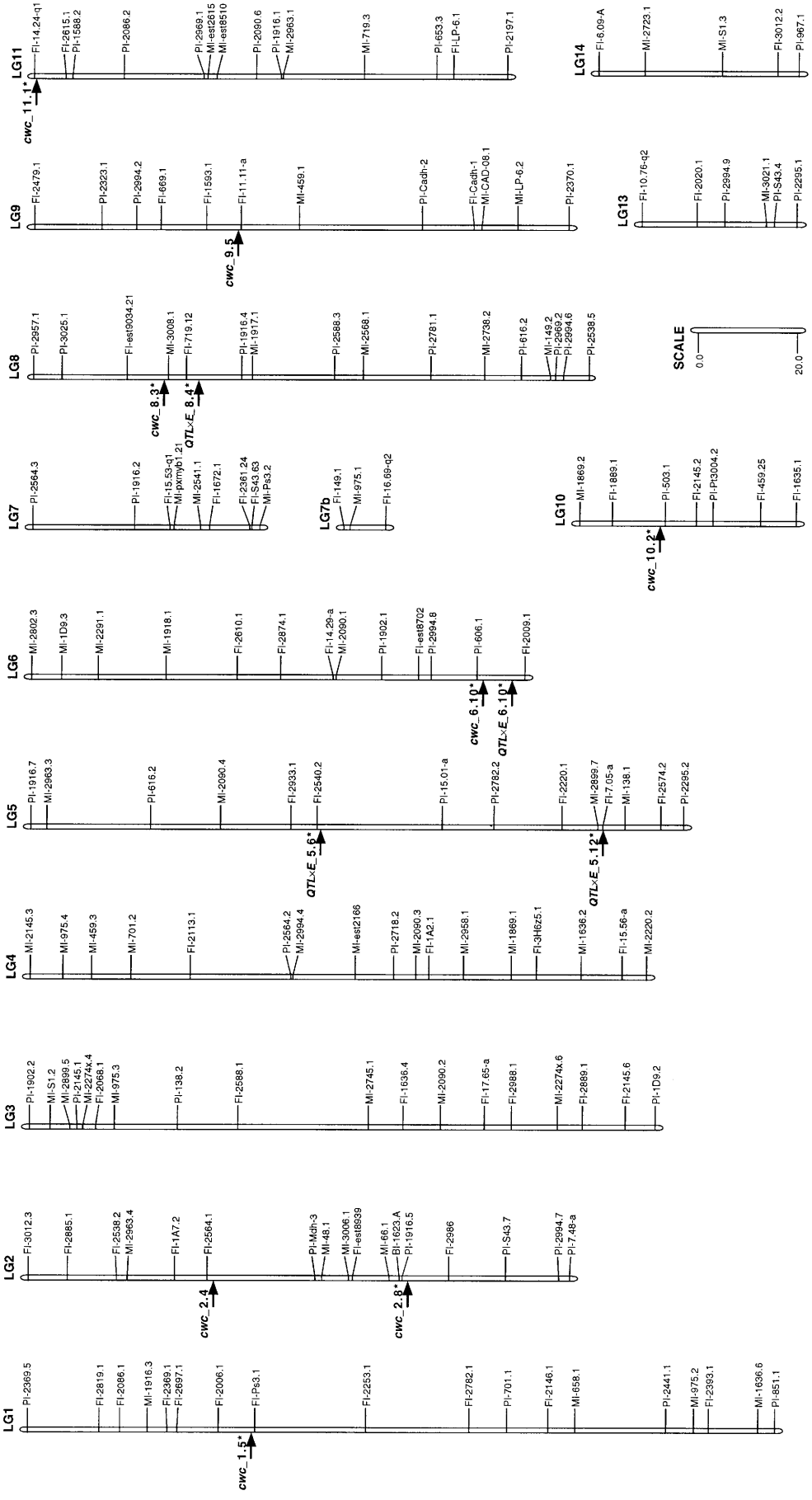

Fig. 1 Map position of unique QTLs for cell wall chemistry traits (*cwc*) and QTL×environment interactions (*QTL*×*E*) for the loblolly pine *qtl* pedigree. The *numerical suffix* indicates the linkage group number and interval for the location of each QTL (e.g., 1.1 represents LG1 and interval 0–10 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 genetic informativeness at that locus; *MI*=maternally informative (*H*×*A*), PI=paternally informative (*A*×*H*), IC=($H_1 \times H_1$) and *FI*=fully informative ($H_1 \times H_2$), where *H*=heterozygote and *A*=homozygote. The map was constructed using the Kosambi mapping function; the scale is in centiMorgans (cM)

Several *cwc* QTLs co-mapped with QTLs for physical wood property traits reported in Sewell et al. (2000). For example, *cwc*_1.5 and *mfa*_1.5 (microfibril angle) both mapped to approximately 45 cM on LG1. Even though both of these traits are associated with microfibrils, there is little phenotypic correlation ($-0.13 \le r \le 0.11$) and little congruence, either positive or negative, among the QTL effects for these traits. Similar observations are found among QTLs for *cwc* and wood specific gravity (*wsg*) and volume latewood percentage, supporting the hypothesis that different QTLs are represented in these QTL clusters.

Factors influencing wood chemical content

The chemical content of wood is influenced by the interaction of developmental, seasonal, and geographic conditions. Variation in wood chemical content is therefore found both vertically and radially within an individual tree trunk, as well as among individuals of the same species. Two aspects of this variation were analyzed in the present study.

Earlywood vs latewood

The components of chemical wood properties are not uniformly distributed throughout the cell wall (Panshin and de Zeeuw 1980). In the tracheids of gymnosperms, lignin concentrations are highest in the middle lamella and lowest in the S2 layer of the secondary cell wall (Baucher et al. 1998), whereas the inverse is true for holocellulose (Panshin and de Zeeuw 1980). However, the S2 layer accounts for a greater percentage of the total cellular lignin and holocellulose due to the larger proportion of the total cell wall volume for that layer (Panshin and de Zeeuw 1980). Variation in chemical concentration of the cell wall layers is also observed throughout the growing season. For example, in loblolly pine the concentration of lignin (g of lignin per g of cell wall substance) for earlywood vs latewood is 0.49 vs 0.51 in the middle lamella and 0.20 vs 0.18 in the S2 layer, respectively (reviewed in Saka and Goring 1985). In addition, the percentage of total cell wall volume for the S2 layer is greater in latewood (80%) than in earlywood (60%; reviewed in Saka and Goring 1985). Therefore, the largest cell wall layer of secondary wood (i.e., the S2 layer of latewood) contains the lowest concentration of lignin. In accordance with these findings, significantly lower lignin and higher holocellulose contents were observed for latewood in this study of loblolly pine (Table 6).

Sewell et al. (2000) previously reported that QTLs for *mfa* were consistently detected for both earlywood and latewood, while the majority of QTLs for *wsg* appeared to be specific to either earlywood or latewood. Phenotypic correlations between earlywood and latewood were in accordance with these results ($0.53 \le r \le 0.70$ for *mfa* and $-0.24 \le r \le 0.13$ for *wsg*). In the present study, only two QTLs were detected from both earlywood and latewood *cwc* traits, while five were detected from only earlywood and one from only latewood (Table 3). However, there were consistently moderate to high phenotypic correlations between earlywood and latewood values for these chemical components ($0.50 \le r \le 0.73$), which supports the notion that many of the same factors (e.g., genes) are involved in the expression of these traits for both earlywood and latewood. Even though the majority of these QTLs were detected for only earlywood, to state that these QTLs are specific to only earlywood is currently premature.

Table 6 Results from a paired Student's *t*-test (two-tailed distribution); analysis compared cell wall chemistry traits for earlywood vs latewood

Trait[a]	Sample[b]	Mean (std)	Range		ew vs. lw *p*
			Min.	Max.	
60/*crb*	ew	1.927 (0.168)	1.597	2.387	*
	lw	2.256 (0.176)	1.781	2.720	
73/*crb*	ew	1.980 (0.160)	1.654	2.399	*
	lw	2.187 (0.153)	1.736	2.584	
114/*hmc*	ew	0.941 (0.090)	0.750	1.207	*
	lw	0.979 (0.082)	0.751	1.217	
144/*cel*	ew	0.524 (0.046)	0.428	0.666	*
	lw	0.564 (0.039)	0.436	0.663	
124/*lgn*	ew	1.748 (0.089)	1.545	1.918	*
	lw	1.577 (0.096)	1.344	1.889	
135/*lgn*	ew	0.731 (0.030)	0.647	0.810	*
	lw	0.676 (0.030)	0.618	0.791	
150/*lgn*	ew	1.252 (0.069)	1.112	1.427	*
	lw	1.167 (0.068)	1.016	1.410	
pls/*lgn*	ew	30.995 (0.522)	29.384	31.880	*
	lw	29.909 (0.594)	28.330	31.702	
pls/*gal*	ew	2.711 (0.147)	2.361	3.156	*
	lw	2.930 (0.183)	2.233	3.352	
pls/*cel*	ew	35.161 (1.167)	32.367	38.535	*
	lw	37.018 (1.383)	32.211	40.276	

* $p \le 0.0001$
[a] See Table 1 for explanation of traits
[b] ew=earlywood; lw = latewood

QTL×environment

Variation in lignin and α-cellulose content has generally been found to occur among individual trees, but not among populations at different sites (Zobel and Jett 1995). However, Schütt (1958) reported significant differences in lignin and α-cellulose contents among provenances of lodgepole pine (*Pinus contorta*) grown in Germany (reported in Zobel and Jett 1995). In the present study of loblolly pine, significant differences in wood chemical contents were also observed among the populations from North Carolina vs Oklahoma (Table 7). Results from QTL×E analyses provide evidence that QTLs also interact with environmental location (i.e., state;

Table 7 Results from a Student's *t*–test (two-tailed distribution with samples of unequal variance); analysis compared cell wall chemistry traits[a] from North Carolina (NC) trees vs Oklahoma (OK) trees

Earlywood Trait	NC Mean (std)	OK Mean (std)	NC vs OK *p*
60/*crb*	1.842 (0.104)	2.095 (0.135)	*
73/*crb*	1.897 (0.102)	2.141 (0.121)	*
114/*hmc*	0.910 (0.079)	1.004 (0.075)	*
144/*cel*	0.503 (0.032)	0.566 (0.043)	*
124/*lgn*	1.786 (0.069)	1.676 (0.079)	*
135/*lgn*	0.744 (0.024)	0.706 (0.026)	*
150/*lgn*	1.282 (0.056)	1.197 (0.058)	*
pls/*lgn*	31.253 (0.301)	30.473 (0.484)	*
pls/*gal*	2.642 (0.093)	2.852 (0.136)	*
pls/*cel*	34.712 (0.854)	36.067 (1.194)	*
Latewood trait	**NC Mean (std)**	**OK Mean (std)**	**NC vs OK *p***
60/*crb*	2.185 (0.132)	2.402 (0.166)	*
73/*crb*	2.125 (0.123)	2.315 (0.133)	*
114/*hmc*	0.947 (0.068)	1.044 (0.070)	*
144/*cel*	0.552 (0.034)	0.589 (0.037)	*
124/*lgn*	1.612 (0.082)	1.499 (0.079)	*
135/*lgn*	0.684 (0.028)	0.657 (0.027)	*
150/*lgn*	1.189 (0.062)	1.118 (0.055)	*
pls/*lgn*	30.136 (0.485)	29.47 (0.538)	*
pls/*gal*	2.866 (0.164)	3.052 (0.154)	*
pls/*cel*	36.661 (1.316)	37.706 (1.251)	*

* p≤0.0001
[a] See Table 1 for explanation of traits

Table 8 Results[a] from QTL analyses of wood cell wall chemistry traits fitting an interaction with a single QTL by environmental location (i.e., state)

LG	Trait	Sample	cM	*p*	%Var[b]
5	73/*crb*	ew	58	0.00106**	10.17
5	pls/*gal*	ew	58	0.00398**	8.36
5	pls/*lgn*	ew	57	0.00139**	9.89
5	144/*cel*	ew	109	0.00305**	8.63
5	114/*hmc*	ew	119	0.00192**	9.32
5	135/*lgn*	ew	118	0.00036**	11.64
6	pls/*cel*	ew	95	0.00001**	16.39
6	144/*cel*	ew	98	0.00172**	9.47
6	pls/*gal*	ew	96	0.00019**	12.63
6	pls/*lgn*	ew	99	0.00075**	10.77
8	144/*cel*	lw	39	0.00872*	7.12
8	60/*crb*	lw	32	0.01014*	6.88
8	73/*crb*	ew	33	0.00473**	8.00
8	pls/*gal*	ew	33	0.00215**	9.26
8	pls/*lgn*	ew	38	0.00139**	9.90

[a] See footnotes for Table 3
[b] Percentage of variance explained by QTL after fitting state

Table 8). Four QTL×E interactions were detected for multiple cell wall chemistry components (*QTL×E*_5.6, _5.12, _6.10 and _8.4; Fig. 1). Two of these QTL×E interactions (25% of total QTLs) co-mapped with previously detected QTLs (*QTL×E*_6.10 and _8.4).

Breeding for increases in holocellulose yield, or decreases in lignin yield, may prove challenging in loblolly pine. If the genetic control of wood chemistry is primarily non-additive, as the results here suggest, then improved yield can only be obtained through a control-pollination breeding program or by vegetative propagation (Zobel and Jett 1995). This is true even when variation in wood chemistry is large. An alternative approach may be to take advantage of the previously discussed differences in chemical content between earlywood and latewood. Breeding for higher wood density in general, or more specifically for a greater percentage of latewood, might achieve the desired goal of increasing holocellulose content while decreasing that of lignin (Dinus et al. 2001).

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References

Arioli T, Burn JE, Williamson RE (2000) Molecular biology of cellulose biosynthesis. In: Jain SM, Minocha SC (eds) Molecular biology of woody plants, vol. 1. Kluwer Academic Publishers, The Netherlands, pp 205–225

Baucher M, Monties B, van Montagu M, Boerjan W (1998) Biosynthesis and genetic engineering of lignin. Crit Rev Plant Sci 17:125–197

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 van JP (1967) Pulpwood properties and tree breeding: a synthesis. 14th IUFRO Congr Sect 41, Müchen, Germany, pp 243–262

Christensen JH, Baucher M, O'Connell A, van Montagu M, Boerjan W (2000) Control of lignin biosynthesis. In: Jain SM, Minocha SC (eds) Molecular biology of woody plants, vol. 1. Kluwer Academic Publishers, The Netherlands, pp 227–267

Dadswell HE, Fielding JM, Nicholls JW, Brown AG (1961) Tree to tree variations and the gross heritability of wood characteristics of *Pinus radiata*. TAPPI 44:174–179

Davis M, Elam C, Evans R, Wiselogel A, Wheeler N, Megraw R, Jech K, Neale D, Sewell M, Dinus R, West D, Tuskan G (1999) Application of pyrolysis molecular beam mass spectrometry for the determination of loblolly pine and hybrid poplar cell wall composition. TAPPI Pulping Conf, TAPPI Press, Atlanta, Georgia, pp 1077–1082

Dinus RJ, Payne P, Sewell MM, Chiang VL, Tuskan GA (2001) Genetic modification of short rotation poplar wood properties for energy and fiber production. Crit Rev Plant Sci 20:51–69

Evans RJ, Milne TA (1987) Molecular characterization of the pyrolysis of biomass. 1. Fundamentals. Energy and Fuels 1:123–137

Einspahr DW, Goddard RE, Gardner HS (1964) Slash pine wood and fiber property heritability study. Silvae Genet 13:103–109

Frewen BE, Chen THH, Howe GT, Davis J, Rohde A, Boerjan W, Bradshaw HD (2000) Quantitative trait loci and candidate gene mapping of bud set and bud flush in *Populus*. Genetics 154:837–845

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

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

Jett JB, Weir RJ, Barker JA (1977) The inheritance of cellulose in loblolly pine. TAPPI For Biol Wood Chemistry Conf., Madison, Wisconsin, pp 159–162

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

Martens H, Naes T (1989) Multivariate calibration. John Wiley and Son, New York

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

Ona T, Sonoda K, Ito K, Shibata M, Kato T, Ootake Y (1997) Nondestructive determination of wood constituents by fourier transform raman spectroscopy. J. Wood Chem Tech 17:399–417

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

Saka S, Goring DAI (1985) Localization of lignins in wood cell walls. In: Higuchi T (ed) Biosynthesis and biodegradation of wood components. Academic Press, Orlando, pp 51– 62

Schultz TP, Burns DA (1990) Rapid secondary analysis of lignocellulose: comparision of near infrared (NIR) and Fourier transform infrared (FTIR). TAPPI J 73:209–212

Schütt P (1958) Schwankungen in Zellulose- und Ligningehalt bei einigen in Westdeutschland angebauten *Pinus contorta*-Herkünften. Silvae Genet 7:65–69

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

Sewell MM, Sherman BK, 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

Sewell MM, Bassoni DL, Megraw RA, Wheeler NC, Neale DB (2000) Identification of QTLs influencing wood property traits in loblolly pine (*Pinus taeda* L.). I. Physical wood properties. Theor Appl Genet 101:1273–1281

Smook GA, Kocurek MJ (1988) Handbook for pulp and paper technology. TAPPI Press, Atlanta, Georgia

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

Tuskan G, West D, Bradshaw HD, Neale D, Sewell M, Wheeler N, Megraw R, Jech K, Wiselogel A, Evans R, Elam C, Davis M, Dinus R (1999) Two high-throughput techniques for determining wood properties as part of a molecular genetic analysis of loblolly pine and hybrid poplar. Appl Biochem Biotech 77–79: 55–65

Wright JA, Birkett MD, Gambino MJT (1990) Prediction of pulp yield and cellulose content from wood samples using near infrared reflectance spectroscopy. TAPPI J 73:164–166

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

Zobel BJ, Stonecypher R, Browne C, Kellison RC (1966) Variation and inheritance of cellulose in southern pines. TAPPI 49:383–387