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ORIGINAL PAPER

# Identification of quantitative trait loci for wood quality and growth across eight full-sib coastal Douglas-fir families

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**Abstract** Typical linkage and quantitative trait locus (QTL) analyses in forest trees have been conducted in single pedigrees with sex-averaged linkage maps. The results of a QTL analysis for wood quality and growth traits of coastal Douglas-fir using eight full-sib families, each consisting of 40 progeny, replicated on four sites are presented. The resulting map of segregating genetic markers consisted of 120 amplified fragment length polymorphism (AFLP) loci distributed across 19 linkage groups. The wood quality traits represent the widest suite of traits yet examined for QTL analysis in a tree species in a single study. Wood fiber traits showed the lowest number of QTLs (3) with relatively small effect (ca. 4%); wood density traits also showed just three QTLs but with slightly larger effect; wood chemistry traits showed more QTLs (7), while ring density traits showed many QTLs with large numbers of QTLs (78) and interesting patterns of temporal variation. Growth traits gave just five QTLs but of major effect (10–16%). Trees, with their long generation times, provide a rich resource for studies of temporal variation of QTL expression.

**Keywords** QTL mapping · Wood quality traits · Douglas-fir

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## Introduction

Quantitative trait locus (QTL) mapping is a potentially powerful approach for dissecting the genetic architecture of quantitative traits, as it can reveal such features as the nature of gene action and the number of genes involved and their interactions. In conifers, recent studies have identified several QTLs of major effect and, in some cases, their temporal and spatial interactions (Byrne et al. 1997; Sewell et al. 2000, 2002; Jermstad et al. 2001a, b; Arcade et al. 2002).

The attributes of "wood quality" are one important class of traits for both the solid wood and pulp and paper industries and, hence, for tree breeders and geneticists. Wood density is arguably the most important wood quality attribute, as it contributes significantly to the overall wood strength and is often negatively correlated with growth traits (Vargas-Hernandez and Adams 1991; St. Clair 1994). Fiber properties, such as fiber length and cell-wall thickness, are also important, but more so for paper quality (Chantre et al. 2002); fiber length and coarseness interact to provide bonding between fibers and improve the ultimate paper strength (Seth and Kingsland 1990). Furthermore, wood strength and stiffness are highly associated with microfibril angle (Cramer et al. 2005; Evans and Ilic 2001), while the chemical constituents of wood (lignin, cellulose, and hemicellulose) influence the overall characteristics of the woody material, and significantly impact pulp and paper processing.

Clearly, the identification of QTLs underlying wood quality traits is therefore highly desirable. QTLs for microfibril angle, wood specific gravity, and volume percentage of latewood have been found in loblolly pine (*Pinus taeda* L.; Sewell et al. 1999, 2000, 2002; Neale et al. 2002; Brown et al. 2003). Similarly, QTLs for lignin and cellulose content provided evidence of environmental

interactions, suggesting a complex pattern of QTL activity (Sewell et al. 2002). Furthermore, QTLs identified for wood quality traits were shown to be stable through time (Sewell et al. 2000; Brown et al. 2003).

Traditional linkage and QTL analyses in forest trees have been conducted in single pedigrees with sex-averaged linkage maps (Jermstad et al. 1998; Sewell et al. 1999; Wu et al. 2000; Chagne et al. 2002) or maps generated for specific individuals using megagametophyte tissue (Travis et al. 1998; Remington et al. 1999). One of the major concerns about the use and implementation of such QTL data is the lack of representation of multiple genetic backgrounds. It has been clearly shown that QTL effects are different in unrelated families (Neale et al. 2002; Brown et al. 2003), and considering that conifers are highly outbred and genetically variable organisms, it would not be unexpected that a QTL be heterozygous and, hence, segregating in only a subset of families. Therefore, surveys across multiple families should provide a mechanism to detect more QTLs.

Coastal Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco var *menziesii*] is the most intensively managed tree species on the west coast of North America (Aubry et al. 1998), as its wood is extremely valuable as a construction material due to its superior strength properties (USDA 2000). Wood density and its components, arguably the most important wood trait, are generally under moderate to strong genetic control, with heritabilities estimated between 0.2–0.7 (Vargas-Hernandez and Adams 1991; St. Clair 1994; Johnson and Gartner 2006). In Douglas-fir, the primary focus of QTL analysis has been on "adaptive traits" such as bud flush and cold hardiness (Jermstad et al. 2001a, b, 1998, 2003; Wheeler et al. 2005). To date, however, no QTL analysis for wood quality traits have been reported in Douglas-fir. In this paper, we present results of a QTL analysis for coastal Douglas-fir for wood quality traits and growth based upon eight full-sib families replicated on four sites.

## Materials and methods

### Sample population

The sample population consisted of eight full-sib Douglas-fir families selected from the British Columbia Ministry of Forests second generation progeny test program. The families were chosen from a total of 15 full-sib families previously characterized for wood properties based on growth and density data and represent completely unrelated genetic material. Families with varying growth and density combinations were selected for linkage map construction and QTL analysis (Table 1). The families were sampled from four sites established in 1977 in southwestern British Columbia (two on Vancouver Island and two on the British Columbia mainland). Forty individuals were selected from each family (10 individuals per family per site; 320 trees in total) for identification of QTLs affecting growth and wood property traits. The trees were sampled in 2004 at the age of 26.

### Growth traits and core sampling

Tree height (HT) and diameter at breast height (DBH) data for each tree at 26 years of age were collected using a Vertex instrument (Vertex III; Haglöf, Sweden) and diameter tape at 1.3 m, respectively. Tree volume was subsequently calculated using Schumacher's equation for Douglas-fir ($0.000047966*[DBH^{1.81382}]*[HT^{1.04242}]$) for each tree. Bark to bark increment core samples (10 mm in diameter) were taken at breast height from each tree in a north to south direction.

### Fiber length and coarseness

Fiber length (FL) and coarseness (CS) were measured specifically on wood material extracted from growth rings corresponding to ages 15–17 from the southern portion of

**Table 1** Average height (HT), diameter (DBH), volume (VOL), earlywood density (EWD), latewood density (LWD), latewood proportion (LWP), and average density (AD) of the full-sib Douglas-fir families employed for QTL analysis

| Trait | Family | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 2 | 7 | 26 | 38 | 62 | 75 | 92 | 151 |
| HT (m) | 18.10 | 16.43 | 17.67 | 18.61 | 18.52 | 18.05 | 15.97 | 15.99 |
| DBH (cm) | 24.60 | 24.08 | 26.93 | 26.50 | 25.76 | 25.85 | 21.89 | 21.40 |
| VOL ($m^3$) | 0.36 | 0.33 | 0.40 | 0.42 | 0.40 | 0.38 | 0.27 | 0.24 |
| EWD ($g/m^3$) | 303.85 | 312.75 | 295.83 | 291.20 | 306.09 | 296.38 | 313.41 | 290.59 |
| LWD ($g/m^3$) | 645.70 | 620.81 | 610.50 | 617.26 | 638.55 | 617.93 | 628.13 | 609.27 |
| LWP | 0.38 | 0.38 | 0.34 | 0.31 | 0.35 | 0.32 | 0.38 | 0.33 |
| AD ($g/m^3$) | 432.06 | 430.94 | 403.22 | 394.42 | 424.10 | 400.20 | 433.70 | 401.85 |

the increment cores. Wood samples were macerated in Franklin solution (1:1, 30% hydrogen peroxide/glacial acetic acid) for 48 h at 70°C. The solution was decanted and the remaining fibrous material was washed under vacuum with de-ionized water until a neutral pH was achieved. Fibers were dried at 50°C overnight, and the moisture content measured to determine fiber mass. Subsamples were then resuspended in 10 ml of de-ionized water and fiber properties determined on a fiber quality analyzer (LDA02, OpTest Equipment, Canada). All samples were run in triplicate. Fiber length was measured in millimeters and fiber coarseness as mass per unit length (mg/m). Both traits are averages for growth rings representing ages 15–17.

### Wood density

Wood density traits were measured on the northern portion of tree increment cores by X-ray densitometry. Cores were first Soxhlet-extracted overnight in acetone and cut to 1.68-mm thickness on a precision pneumatic twin blade saw to expose the radial face for analysis. The density samples were then allowed to acclimate to 7% moisture and scanned by X-ray densitometer from pith to bark with a resolution of 0.0254 mm and are reported as relative density on an oven-dry weight basis. Earlywood density (EWD), latewood density (LWD), latewood proportion (LWP), and average ring density (AD) were measured for each ring from pith to bark, for each tree. QTL analyses were performed on composite traits (average across all rings) and individual rings for all traits. Composite traits represent the average density or LWP at age 26.

### Microfibril angle

Microfibril angle estimates were generated by X-ray diffraction and light microscopy (Megraw et al. 1998). The 002 diffraction spectra for individual earlywood growth rings from six sample trees were screened for $T$ value distribution and symmetry on a Bruker D8 discover X-ray diffraction unit equipped with an area array detector (GADDS). Wide-angle diffraction was used in the transmission mode, and the measurements were performed with CuK$\alpha_1$ radiation ($\lambda$= 1.54 Å); the X-ray source fit with a 0.5-mm collimator, and the scattered photon collected by the GADDS detector. Both the X-ray source and detector were set to theta=0°. Thirty-two individual growth rings selected from six sample trees were sectioned using a microtome (0.20–0.30 μm) and processed for compound light microscopy (Wang et al. 2001). Wood sections were placed in 1 ml of 5% (wt/vol) cobalt chloride ($CoCl_2$) and heated to 80°C for 2 h, then floated in a sonicator (47 kHz) for a subsequent 2 h. The sections were mounted on slides and allowed to dry overnight. Differential interference contrast (DIC) microscopy at 400× magnification was used to visualize individual fibers. Images were collected using Qcapture and saved as tagged image file format (TIFF) files. The angles of the microfibrils within individual fibers was measured using ImageJ software. Twenty-nine sections were used to create a standard curve ($R^2$=0.92) using the average $T$ value from the 002 diffraction arc peaks integrated over 2-theta and the known angles (from microscopy). Microfibril angle measurements were then estimated for all samples by collecting the 002 diffraction intensity profiles and measuring the $T$ values for the earlywood portion of the growth ring corresponding to year 17, and comparing them to the best-fit linear relationship generated for known (measured microscopically) microfibril angles and the $T$ values.

### Wood chemistry

Wood chemical composition was measured on increment core material from the northern portion of the tree using a modified Klason analysis (Huntley et al. 2003). The wood material from pith to bark was ground with a Wiley mill to pass a 0.4-mm screen (40 mesh) and acetone extracted. A 0.2-g sample of extracted wood was transferred to a 15 ml reaction vial cooled on an ice bath. A 3-ml aliquot of 72% ($w/w$) $H_2SO_4$ was added to the sample and thoroughly mixed for 1 min. The reaction vial was immediately transferred to a water bath maintained at 20°C and mixed for 1 min every 10 min. After 2 h of hydrolysis, the contents of each test tube were transferred to a 125-ml serum bottle using 112 ml of de-ionized water to rinse all residue and acid from the reaction vial. The serum bottles (containing 115 ml 4% ($w/w$) $H_2SO_4$ plus wood) were sealed and autoclaved at 121°C for 60 min. Samples were allowed to cool, and the hydrolysates vacuum-filtered through pre-weighed medium coarseness sintered-glass crucibles. Each sample was washed with 200 ml of warm (~50°C) de-ionized water to remove residual acid and sugars and dried overnight at 105°C. The dry crucibles were re-weighed to gravimetrically determine Klason lignin (acid-insoluble lignin). The filtrate was then analyzed for acid-soluble lignin by absorbance at 205 nm using a UV/Vis spectrometer (Lambda 45, PerkinElmer Instruments, USA) according to TAPPI useful method UM250 (Tappi Useful Method 1991).

The concentration of the cell wall carbohydrates [arabinose (ARA), galactose (GAL), glucose (GLU), mannose (MAN), and xylose (XYL)] in the reaction hydrolyzates were determined using high-performance anion exchange liquid chromatography (HPLC). The HPLC system (Dionex DX-600, Dionex, USA) was equipped with an ion-exchange PA1 (Dionex) column, a pulsed amperometric detector with a gold electrode, and a Spectra AS50 auto-injector (Spectra-Physics, USA). Before injection, samples were filtered through 0.45-μm HV filters (Millipore, USA). A 20-μl vol-

ume of sample was loaded containing fucose as an internal standard. The column was equilibrated with 250 mM NaOH and eluted with de-ionized water at a flow rate of 1.0 ml/min. Total lignin (TL), ARA, GAL, GLU, MAN, and XYL content were measured as the proportion of the initial mass of wood used in the analysis. Wood chemistry data represent the whole core chemical content at age 26.

## Genotypic data and map construction

Ten amplified fragment length polymorphisms (AFLP) marker combinations were used to develop a comprehensive linkage map for the eight full-sib families used in this study (Ukrainetz et al. 2007). The map was generated by calculating average LOD and recombination rates across families using a joint likelihood function (Hu et al. 2004). The map was generated using Joinmap (Van Ooijen and Voorrips 2001) and contains 120 markers distributed across 19 linkage groups. The total distance covered by the linkage map was 938.6 cM with an average of 9.3 cM between markers.

## QTL analysis

QTLs were detected and positioned using the "sib-pair analysis with parents of known genotype" interface of the QTL Express online software package (Seaton et al. 2002). This analysis is based on the sib-pair analysis that was first proposed by Haseman and Elston (1972) and improved upon by Visscher and Hopper (2001). The regression analysis uses the relationship between the identity-by-descent (IBD) probabilities and the squared difference and corrected squared sum of phenotypic values between sibs at each locus. Because site can have a significant effect on trait values, the general linear model (GLM) procedure of SAS (2003) was used to transform phenotypic data to remove site and block effects using the following linear model:

$$Y_{\mathrm{ijlp}} = \mu + F_{\mathrm{i}} + S_{\mathrm{l}} + FS_{\mathrm{il}} + B_{j\,(\mathrm{l})} + FB_{\mathrm{ij(l)}} + E_{\mathrm{p(ijl)}} \qquad (1)$$

where $Y_{\mathrm{ijlp}}$ is the individual phenotypic observation, ì is the overall mean, $F_{\mathrm{i}}$ is the fixed family effect, $S_{\mathrm{l}}$ is the random site effect, $FS_{\mathrm{il}}$ is the random family-by-site interaction, $B_{\mathrm{j(l)}}$ is the random block effect nested within sites, $FB_{\mathrm{ij(l)}}$ is the random family-by-block interaction nested within site, and $E_{\mathrm{p(ijl)}}$ is the random residual effect. The residuals were used as phenotypic input for QTL Express. The linkage map was scanned for each trait using a chromosome-wide bootstrap analysis with 100 iterations to calculate critical $F$ values at the 0.05 and 0.01 alpha levels. If a QTL was detected at either significance level, the linkage group was re-scanned for the trait using 1,000 iterations to calculate the critical $F$ statistics. The final analysis was conducted using a 1-QTL model with a 1-cM step size as described by Visscher and Hopper (2001).

The proportion of variation explained by each QTL was calculated using an analysis of variance (ANOVA) to partition phenotypic trait values into their respective components (Table 2) based on the following linear model:

$$Y_{\mathrm{ijklp}} = \mu + F_{\mathrm{i}} + S_{\mathrm{l}} + FS_{\mathrm{il}} + GF_{\mathrm{k(i)}} + SGF_{\mathrm{lk(i)}} + BS_{\mathrm{j(l)}} + FBS_{\mathrm{ij(l)}} + E_{\mathrm{p(ilkj)}} \qquad (2)$$

where $Y_{\mathrm{ijklp}}$ is the individual phenotypic observation, $\mu$ is the overall mean, $F_{\mathrm{i}}$ is the fixed family effect, $S_{\mathrm{l}}$ is the random site effect, $FS_{\mathrm{il}}$ is the random family-by-site interaction, $GF_{\mathrm{k(i)}}$ is the random genotype nested within family effect (QTL effect), $SGF_{\mathrm{lk(i)}}$ is the random site by genotype nested within family interaction (site by QTL interaction), $BS_{\mathrm{j(l)}}$ is the random block nested within site effect, $FBS_{\mathrm{ij(l)}}$ is the random family-by-block nested within site interaction, and $E_{\mathrm{p(ilkj)}}$ is the random residual effect. The proportion of variation explained by the nearest marker to the QTL position determined by QTL Express was used to estimate QTL effect. QTL effects were estimated as follows:

$$a_{\mathrm{i}} = \frac{SS_{\mathrm{G(F)}}}{SS_{\mathrm{Total}}} \qquad (3)$$

where $a_{\mathrm{i}}$ is the proportion of variation explained by the QTL at the $i$th marker, $\mathrm{SS_{G(F)}}$ is the sum of squares for genotype nested within family (QTL), and $\mathrm{SS_{Total}}$ is the total sum of squares.

**Table 2** Components of variance and degrees of freedom used to calculate the proportion of variation explained by each QTL for the corresponding marker

| | df | Components of variance |
|---|---|---|
| $F$ | $(f-1)$ | $\sigma^2_{\mathrm{E}} + \mathrm{mn}\sigma^2_{\mathrm{FBS}} + \mathrm{bn}\sigma^2_{\mathrm{GFS}} + \mathrm{sbn}\sigma^2_{\mathrm{MF}} + \mathrm{mbn}\sigma^2_{\mathrm{FS}} + \mathrm{smbn}\sigma^2_{\mathrm{F}}$ |
| $S$ | $(s-1)$ | $\sigma^2_{\mathrm{E}} + \mathrm{fmn}\sigma^2_{\mathrm{BS}} + \mathrm{bn}\sigma^2_{\mathrm{GFS}} + \mathrm{fmbn}\sigma^2_{\mathrm{S}}$ |
| $FS$ | $(f-1)(s-1)$ | $\sigma^2_{\mathrm{E}} + \mathrm{mn}\sigma^2_{\mathrm{FBS}} + \mathrm{bn}\sigma^2_{\mathrm{GFS}} + \mathrm{mbn}\sigma^2_{\mathrm{FS}}$ |
| $G(F)$ | $f(m-1)$ | $\sigma^2_{\mathrm{E}} + \mathrm{bn}\sigma^2_{\mathrm{GFS}} + sbn\sigma^2_{\mathrm{GF}}$ |
| $SG(F)$ | $f(s-1)(m-1)$ | $\sigma^2_{\mathrm{E}} + \mathrm{bn}\sigma^2_{\mathrm{GFS}}$ |
| $B(S)$ | $s(b-1)$ | $\sigma^2_{\mathrm{E}} + \mathrm{fmn}\sigma^2_{\mathrm{BS}}$ |
| $FB(S)$ | $s(f-1)(b-1)$ | $\sigma^2_{\mathrm{E}} + \mathrm{mn}\sigma^2_{\mathrm{FBS}}$ |
| Error | $sf\mathrm{mn}(n-1)$ | $\sigma^2_{\mathrm{E}}$ |

## Results

### Numbers and effects of detected QTLs for compound traits

For wood fiber traits, there were three QTLs detected for each fiber trait (length and coarseness) located on four linkage groups, and one QTL for microfibril angle (Table 3, Fig. 1). The three fiber length QTLs were located on linkage groups 14, 17, and 18, and explain 3.6–15.7% of the phenotypic variation. The fiber coarseness QTLs were located on linkage groups 14, 16, and 18, and have effects ranging from 5.2–14.6%, while the microfibril angle QTL was located on linkage group 12 and explains 3.6% of the phenotypic variation. Although the fiber property QTLs on group 14 are located at opposite ends of the linkage group, the QTLs on linkage group 18 are located at the same position. Three of the five unique markers associated with wood fiber property QTLs are not significant in the regression analyses for QTL effects, as is the marker associated with microfibril angle.

Only three QTLs were detected for compound wood density traits, all associated with LWD. The QTLs had effects ranging from 4.0–6.0% of the phenotypic variation and were located on three linkage groups. The QTL on group 14 co-locates with individual ring QTLs, and the QTL on group 15 is located near a QTL associated with several wood chemistry traits. None of the markers associated with compound LWD QTLs were significant for QTL effects (Table 3, Fig. 1).

A total of seven QTLs were detected for wood chemistry traits (Table 3, Fig. 1). Only one QTL was detected for lignin content and explained 5.0% of the phenotypic variation. This QTL co-locates with QTLs associated with GLU, GAL, and MAN located on group 15, which explains between 4.4 and 6.2% of the phenotypic variation. The markers associated with these QTLs are not significant for QTL effects. A QTL for ARA was also detected on linkage group 17, which explains 4.9% of the variation. A second GAL QTL is located on group 14 with an effect size of 11.9%, and a second GLU QTL was detected on group 13 with an effect size of 6.2%. The marker associated with the

**Table 3** QTLs detected using interval mapping

| Group | Trait | QTL position (cM) | $F$ | $F_{0.05}$ | $F_{0.01}$ | Marker | Marker position (cM) | Effect | $P$ value | $N$ |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | HT | 15 | 8.03 | 6.53 | 13.25 | ACGCCGG_0297 | 17.2 | 0.161 | 0.08 | 70/35 |
| 2 | HT | 31 | 11.02 | 5.86 | 12.09 | ACGCCGG_0325 | 31.6 | 0.177 | 0.00[a] | 106/67 |
| | VOL | 32 | 6.83 | 5.88 | 13.35 | ACGCCGG_0325 | 31.6 | 0.113 | 0.00[a] | 67/73 |
| 4 | DBH | 38 | 24.15[b] | 7.88 | 16.01 | ACGCCCA_0652 | 38.1 | 0.086 | 0.04[a] | 72/75 |
| | VOL | 38 | 24.25[b] | 7.08 | 14.52 | ACGCCCA_0652 | 38.1 | 0.096 | 0.06 | 72/75 |
| 7 | LWD | 37 | 9.16 | 8.2 | 21.09 | ACACCGT_0391 | 37 | 0.040 | 0.34 | 103/39 |
| 12 | MFA | 13 | 9.58 | 7.29 | 18.38 | ACACCGG_0138 | 15.2 | 0.036 | 0.05 | 74/114 |
| 13 | GLU | 0 | 7.59 | 4.18 | 9.43 | ACCCCGC_0327 | 0 | 0.062 | 0.08 | 77/35 |
| 14 | FL | 0 | 16.86 | 6.9 | 19.02 | ACGCCCA_0213 | 0 | 0.074 | 0.02[a] | 75/73 |
| | CS | 50 | 8.05 | 6.35 | 15.31 | ACGCCGT_0595 | 50.1 | 0.052 | 0.16 | 40/73 |
| | GAL | 33 | 9.96 | 5.3 | 12.47 | ACGCCCA_0345 | 32.8 | 0.119 | 0.04[a] | 40/73 |
| | LWD | 41 | 12.98 | 10.67 | 26.81 | ACGCCCA_0345 | 32.8 | 0.060 | 0.10 | 40/73 |
| 15 | LWD | 42 | 24.38[b] | 8.15 | 19.06 | ACGCCTC_0160 | 44.5 | 0.080 | 0.19 | 105/39 |
| | GAL | 44 | 15.64[b] | 5.9 | 11.11 | ACGCCTC_0160 | 44.5 | 0.048 | 0.21 | 105/39 |
| | GLU | 44 | 22.59[b] | 6.39 | 12.65 | ACGCCTC_0160 | 45.5 | 0.044 | 0.24 | 105/39 |
| | MAN | 44 | 27.37[b] | 4.77 | 10.44 | ACGCCTC_0160 | 45.5 | 0.067 | 0.11 | 105/39 |
| | TL | 44 | 24.5[b] | 5.02 | 9.69 | ACGCCTC_0160 | 44.5 | 0.050 | 0.26 | 105/39 |
| 16 | CS | 17 | 8.18 | 6.67 | 13.99 | ACGCCTG_0154 | 16.6 | 0.074 | 0.17 | 74/74 |
| 17 | FL | 10 | 7.66 | 6.36 | 17.52 | ACGCCGG_0311 | 10.1 | 0.036 | 0.32 | 40/40 |
| | ARA | 35 | 13.88 | 7.05 | 19.76 | ACGCCTC_0189 | 34.6 | 0.049 | 0.22 | 74/40 |
| 18 | CS | 0 | 9.6 | 6.53 | 14.3 | ACGCCTC_0144 | 0 | 0.146 | 0.00[a] | 35/35 |
| | FL | 0 | 12.19 | 7.06 | 17.9 | ACGCCTC_0144 | 0 | 0.157 | 0.02[a] | 35/35 |

The $F$ is the $F$ value for the regression analysis, and $F_{0.05}$ and $F_{0.01}$ are critical $F$ values for significance at the corresponding $\alpha$ levels. Marker is the closest AFLP marker to the QTL position and is used to assess the proportion of phenotypic variation explained by the QTL. $P$ values are reported for the significance of genotype nested within family for the single marker QTL analysis.

*N* Number of progeny associated with each marker flanking the QTL (left marker/right marker); *HT* height at age 26; *DBH* diameter at breast height, age 26; *VOL* volume at age 26; *LWD* average core latewood density; *CS* fiber coarseness for rings 15–17; *FL* fiber length for rings 15–17; *GAL* core galactose content; *GLU* core glucose content; *MAN* core mannose content; *ARA* core arabinose content; *TL* core lignin content; *MFA* microfibril angle at age 17

[a] Significant at $\alpha$=0.05

[b] Significant at $\alpha$=0.01

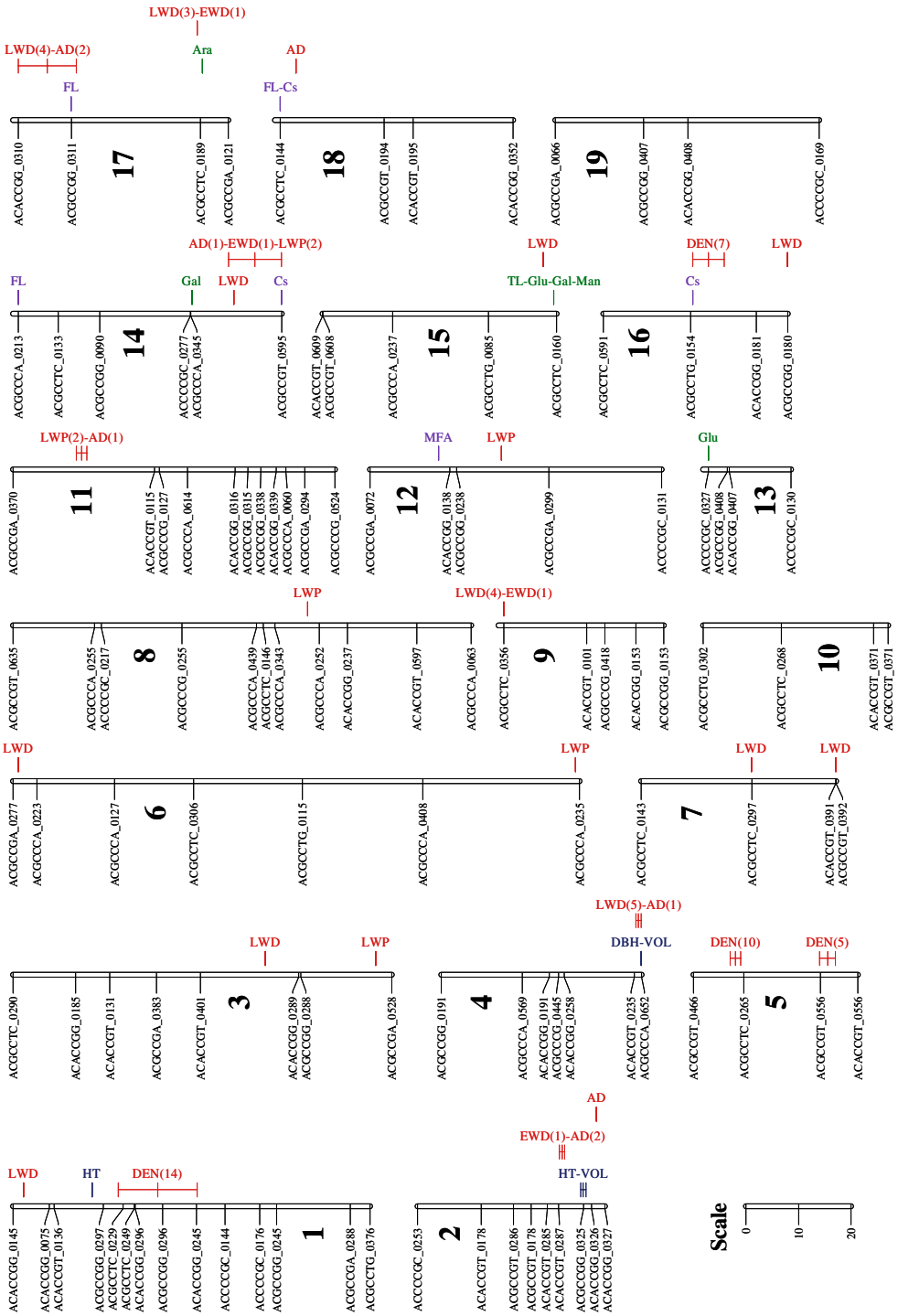


**Fig. 1** QTL map for composite and ring density traits for coastal Douglas-fir. QTLs marked *DEN* contain QTLs detected in multiple years from all four wood density traits. The *numbers in brackets* are the number of QTLs detected within the QTL location. The *interval lines* indicate the range in locations of multiple QTL located within 15 cM of each other on a linkage group. The scale is in cM (Kosambi map function)

second GAL QTL is significant for QTL effects ($p$=0.042); however, the ARA and GLU markers are not ($p$=0.22 and 0.08, respectively).

A total of five QTLs for growth traits in three linkage groups were detected (Table 3, Fig. 1). Two QTLs were detected for height, which explained 16.1–17.7% of the phenotypic variation. Only one QTL was detected for diameter, which had an effect of 8.6%. Two QTLs were detected for volume. Each co-locates with QTLs for height and diameter, as expected given that volume is calculated based on diameter and height. The QTL detected on group 2 co-locates with a QTL for height and explains 11.3% of the variation, while the volume QTL on group 4 co-locates with a QTL for diameter and has an effect of 9.6%. The markers associated with QTL positions for growth traits are significant at the 0.05 level for QTL effects except for ACGCCGG_0297 on linkage group 1 ($p$=0.082) and ACGCCCA_0652 on linkage group 4 ($p$=0.057).

### Numbers and effects of detected QTLs for ring density traits

In total, 78 QTLs were detected for average density, LWD, EWD, and LWP at the 0.05 or 0.01 levels (Table 4). Twenty-two QTLs were detected for average density. These QTLs explained 0.1–14.9% of the phenotypic variation and co-located with other density trait QTLs located on nine different linkage groups. Seventeen QTLs were detected for EWD, with effects ranging from 0.1–6.5%, and they were located on seven linkage groups. Twenty-six LWD QTLs were located on nine linkage groups and explained 0.5–13.1% of the phenotypic variation, while 13 LWP QTLs were detected on nine linkage groups with effect sizes ranging from 0.4–10.2% of the variation. The amount of variation explained by these QTLs is low and many of the markers associated with QTLs are not significant for QTL effects. Several QTLs share similar positions on common linkage groups. When two or more QTLs were located within 15 cM, they were grouped as a single QTL (Fig. 1). Ring density clusters with QTLs from all four wood density traits were labeled "DEN." In total, there were 11 QTL ring density clusters containing 67 QTLs (86% of ring density QTLs) and 11 independent ring density QTLs distributed throughout the linkage map (Fig. 1).

## Discussion

### QTLs and QTL effects

The present study represents a QTL analysis of growth traits and a comprehensive suite of wood quality traits in the commercially important species, Douglas-fir. This is the first QTL investigation evaluating wood quality traits in Douglas-fir, offering a wide array of traits in a single tree species, and is comparable in breadth to the recent work of Thamarus et al. (2004) with *Eucalyptus*.

The resultant QTL map is a synthesis of genotypic and phenotypic information from eight families on four sites. This approach to QTL mapping provides a unique alternative approach (compared to a single pedigree evaluation growing on a single site) elucidating the numbers and effects of QTLs present in the population, but it does present some problems. Although the current investigation involved only 320 offspring (40 per family) to be detected, both a marker and a QTL must be heterozygous in a given parent, and in the best case, a QTL and marker of intermediate frequency (0.5) will occur in this configuration one fourth of the time in one parent, or in (1/4)16=4 families (there are 16 parents among the 8 families). Given that frequencies are normally skewed, more realistically, QTLs will be detectable in only one or two families with the experimental design. Furthermore, if QTLs at slightly different locations occur in multiple families, this confuses and reduces the power of the sib-pair regression method. Therefore, the QTLs presented in this paper are those of largest effect and highest frequency in the population. The number of progeny of the possible 320 individuals, contributing to the estimation of a QTL, ranged from a low of 70 (~22%) for the two fiber traits (CS and FL) on linkage group 18 to a high of a 173 (54%) for a height QTL on linkage group 2. Therefore, these QTLs may be useful and could possibly be used for marker-aided selection, for inferring the genetic architecture of quantitative traits, and for eventual selection of candidate genes (Wheeler et al. 2005).

The number and effects of detected QTLs that were observed concurs with previously reported QTL studies for growth and wood quality traits in several species, particularly those dealing with smaller sample sizes (Bradshaw and Stettler 1995; Grattapaglia et al. 1996; Wu 1998; Yoshimaru et al. 1998; Sewell et al. 2000, 2002; Arcade et al. 2002; Markussen et al. 2003). We note that many of these studies report clustering of QTLs for related and unrelated traits. Yoshimaru et al. (1998) report QTL clusters for height, diameter, and female fertility, while Bradshaw and Stettler (1995) discovered QTL clusters for growth, branch, and leaf area traits. Several QTL clusters were detected in this study for growth, density, fiber properties, and wood chemistry traits. These clusters most likely represent pleiotropic effects, but may be evidence for clusters of linked genes (Grattapaglia et al. 1996). The QTLs for height and volume on group 2 and diameter and volume on group 4 likely represent pleiotropy. Interestingly, the QTL for height on linkage group one was not associated with a volume QTL. Pleiotropic effects are also probably responsible for the co-localization of QTLs affecting fiber length and coarseness on linkage group 18. The large QTL cluster for wood chemistry traits identified on group 15 cannot be attributed to pleiotropy, but rather may represent a cluster of linked genes.

Several QTLs for individual ring density traits map approximately to the same position and, therefore, likely represent the same gene (Neale et al. 2002; Brown et al. 2003). The only compound wood density trait for which QTLs were detected is LWD. The lack of QTL detection for multiple compound wood density traits is likely a result of the large degree of variation in gene expression patterns of these traits in response to temporal climate variation. Also, using average values across multiple rings may reduce the power of detecting QTLs. The detection of QTLs for compound traits is useful, as they incorporate variation across multiple years (Neale et al. 2002); however, the use of individual ring traits is superior for studying temporal variation in gene expression and QTL identification.

**Table 4** Ring density interval mapping QTLs

| LG | Year | Trait | QTL position (cM) | *F* | $F_{0.05}$ | $F_{0.01}$ | Marker | Marker position (cM) | Effect or % variable | *P* | N |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1989 | LWD | 2 | 7.19[a] | 1.18 | 2.18 | ACACCGG_0145 | 0 | 0.005 | 0.082 | 112/74 |
| | 2003 | AD | 20 | 6.57 | 5.59 | 9.4 | ACGCCTC_0229 | 20.9 | 0.010 | 0.802 | 35/67 |
| | 2001 | LWP | 22 | 10.24 | 10.1 | 16.53 | ACGCCTC_0229 | 20.9 | 0.070 | 0.243 | 67/135 |
| | 1998 | EWD | 24 | 4.8 | 4.23 | 7.41 | ACACCGG_0296 | 23.2 | 0.051 | 0.384 | 77/76 |
| | 1995 | AD | 28 | 5.33 | 4.28 | 6.91 | ACGCCGG_0296 | 28.4 | 0.016 | 0.619 | 77/76 |
| | 1994 | AD | 28 | 6.65 | 5.39 | 8.78 | ACGCCGG_0296 | 28.4 | 0.071 | 0.106 | 77/76 |
| | 1995 | EWD | 28 | 5.44 | 3.88 | 5.97 | ACGCCGG_0296 | 28.4 | 0.016 | 0.555 | 77/76 |
| | 1995 | LWD | 28 | 4.04 | 3.37 | 6.09 | ACGCCGG_0296 | 28.4 | 0.073 | 0.003 | 77/76 |
| | 1996 | LWD | 28 | 8.02 | 6.91 | 10.11 | ACGCCGG_0296 | 28.4 | 0.065 | 0.053 | 77/76 |
| | 1996 | AD | 29 | 5.69[a] | 3.97 | 5.63 | ACGCCGG_0296 | 28.4 | 0.013 | 0.469 | 76/143 |
| | 1990 | AD | 29 | 17.49 | 13.22 | 22.98 | ACGCCGG_0296 | 28.4 | 0.110 | 0.053 | 76/143 |
| | 1994 | EWD | 29 | 11.83 | 10.22 | 15.95 | ACGCCGG_0296 | 28.4 | 0.054 | 0.096 | 76/143 |
| | 1989 | AD | 30 | 16.96 | 11.16 | 17.99 | ACGCCGG_0296 | 28.4 | 0.088 | 0.016 | 76/143 |
| | 1989 | EWD | 35 | 9.93 | 8.41 | 13.37 | ACACCGG_0245 | 34.9 | 0.015 | 0.062 | 143/71 |
| | 1990 | EWD | 35 | 22.76 | 14.53 | 26.54 | ACACCGG_0245 | 34.9 | 0.005 | 0.790 | 143/71 |
| 2 | 1993 | AD | 27 | 11.76 | 6.94 | 12.31 | ACACCGT_0287 | 26.8 | 0.052 | 0.006 | 106/67 |
| | 1996 | EWD | 28 | 5.67 | 5.04 | 12.68 | ACACCGT_0287 | 26.8 | 0.035 | 0.260 | 106/67 |
| | 1992 | AD | 34 | 19.53[a] | 8.19 | 17.68 | ACACCGG_0326 | 33.1 | 0.086 | 0.015 | 73/37 |
| 3 | 2000 | LWD | 48 | 8.79 | 8.77 | 21.46 | ACACCGG_0289 | 54.3 | 0.057 | 0.050 | 73/73 |
| | 2002 | LWP | 69 | 11.19 | 7.14 | 14.26 | ACGCCGA_0528 | 72 | 0.004 | 0.762 | 64/34 |
| 4 | 1991 | AD | 37 | 10.2 | 9.51 | 19.17 | ACACCGT_0235 | 36.7 | 0.021 | 0.617 | 72/75 |
| | 1993 | LWD | 37 | 15.08[a] | 6.62 | 15.01 | ACACCGT_0235 | 36.7 | 0.013 | 0.294 | 72/75 |
| | 1985 | LWD | 37 | 22.04[a] | 7.92 | 21.84 | ACACCGT_0235 | 36.7 | 0.022 | 0.602 | 72/75 |
| | 1992 | LWD | 37 | 23.57 | 13.37 | 25.41 | ACACCGT_0235 | 36.7 | 0.012 | 0.511 | 72/75 |
| | 1988 | LWD | 38 | 9.22 | 6.85 | 20.28 | ACGCCCA_0652 | 38.1 | 0.104 | 0.035 | 72/75 |
| | 1991 | LWD | 38 | 17.22 | 13.06 | 24.91 | ACGCCCA_0652 | 38.1 | 0.021 | 0.423 | 72/75 |
| 5 | 1996 | AD | 7 | 6.09[a] | 2.61 | 4.6 | ACGCCTC_0265 | 9.6 | 0.003 | 0.847 | 32/39 |
| | 1999 | AD | 7 | 10.48 | 5.11 | 12.46 | ACGCCTC_0265 | 9.6 | 0.129 | 0.150 | 32/39 |
| | 2002 | AD | 7 | 10.61 | 5.45 | 14.13 | ACGCCTC_0265 | 9.6 | 0.002 | 0.784 | 32/39 |
| | 1998 | AD | 7 | 13.15[a] | 3.88 | 8.08 | ACGCCTC_0265 | 9.6 | 0.013 | 0.707 | 32/39 |
| | 1998 | EWD | 7 | 3.72 | 2.91 | 5.99 | ACGCCTC_0265 | 9.6 | 0.032 | 0.501 | 32/39 |
| | 1995 | EWD | 7 | 4.1 | 2.49 | 6.18 | ACGCCTC_0265 | 9.6 | 0.036 | 0.324 | 32/39 |
| | 1997 | EWD | 7 | 6.65 | 6.33 | 15.29 | ACGCCTC_0265 | 9.6 | 0.025 | 0.552 | 32/39 |
| | 1994 | EWD | 7 | 20.96[a] | 6.1 | 13.21 | ACGCCTC_0265 | 9.6 | 0.001 | 0.772 | 32/39 |
| | 1999 | LWP | 7 | 13.11[a] | 6.02 | 11.68 | ACGCCTC_0265 | 9.6 | 0.100 | 0.202 | 32/39 |
| | 1996 | LWD | 9 | 6.15 | 4.19 | 9.52 | ACGCCTC_0265 | 9.6 | 0.013 | 0.638 | 32/39 |
| | 1990 | LWD | 24 | 9.31 | 7.59 | 15.3 | ACGCCGT_0556 | 24.1 | 0.039 | 0.277 | 39/109 |
| | 1993 | EWD | 26 | 20.24 | 10.39 | 21.28 | ACGCCGT_0556 | 24.1 | 0.026 | 0.356 | 109/105 |
| | 1990 | LWP | 26 | 5.38 | 4.41 | 9.23 | ACGCCGT_0556 | 24.1 | 0.011 | 0.652 | 109/105 |
| | 1990 | AD | 27 | 22.77 | 10.33 | 25.64 | ACGCCGT_0556 | 24.1 | 0.043 | 0.402 | 109/105 |
| | 1991 | EWD | 27 | 8.38 | 5.47 | 10.92 | ACGCCGT_0556 | 24.1 | 0.030 | 0.473 | 109/105 |
| 6 | 1988 | LWD | 1 | 50.1[a] | 8.09 | 15.7 | ACGCCGA_0277 | 0 | 0.061 | 0.316 | 74/37 |
| | 2002 | LWP | 107 | 16 | 11.04 | 20.28 | ACGCCCA_0235 | 107.7 | 0.046 | 0.170 | 71/177 |
| 7 | 2001 | LWD | 21 | 9.68 | 7.91 | 19.04 | ACGCCTC_0297 | 21.1 | 0.035 | 0.433 | 73/103 |
| 8 | 1998 | LWP | 56 | 8.14 | 6.95 | 12.16 | ACGCCCA_0252 | 58.2 | 0.009 | 0.245 | 72/183 |
| 9 | 2001 | EWD | 0 | 11.1[a] | 4.99 | 9.17 | ACGCCTC_0356 | 0 | 0.041% | 0.044 | 70/39 |
| | 1995 | LWD | 0 | 4.08 | 2.74 | 5.82 | ACGCCTC_0356 | 0 | 0.020% | 0.672 | 70/39 |
| | 1994 | LWD | 0 | 5.38 | 2.82 | 6.46 | ACGCCTC_0356 | 0 | 0.021% | 0.431 | 70/39 |
| | 1993 | LWD | 0 | 10.26[a] | 5.31 | 9.7 | ACGCCTC_0356 | 0 | 0.015% | 0.790 | 70/39 |
| | 1985 | LWD | 0 | 27.32[a] | 5.99 | 10.77 | ACGCCTC_0356 | 0 | 0.010% | 0.695 | 70/39 |
| 11 | 2001 | LWP | 12 | 16.64 | 11.51 | 19.49 | ACGCCGA_0370 | 0 | 0.018% | 0.579 | 151/144 |
| | 2003 | AD | 14 | 7.74 | 5.81 | 10.49 | ACACCGT_0115 | 26.9 | 0.021% | 0.394 | 151/144 |
| | 2003 | LWP | 14 | 9.4 | 8.17 | 14.72 | ACACCGT_0115 | 26.9 | 0.041% | 0.068 | 151/144 |
| 12 | 1988 | LWP | 25 | 7.76 | 5.28 | 10.28 | ACGCCGG_0238 | 16.5 | 0.013% | 0.517 | 67/37 |
| 14 | 2003 | AD | 40 | 6.04 | 5.82 | 11.42 | ACGCCCA_0345 | 32.8 | 0.011% | 0.566 | 40/73 |

**Table 4** (continued)

| LG | Year | Trait | QTL position (cM) | *F* | $F_{0.05}$ | $F_{0.01}$ | Marker | Marker position (cM) | Effect or % variable | *P* | N |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2001 | EWD | 50 | 7.72 | 6.62 | 17.08 | ACGCCGT_0595 | 50.1 | 0.004% | 0.715 | 40/73 |
| | 2003 | LWP | 50 | 8.97 | 8.29 | 14.41 | ACGCCGT_0595 | 50.1 | 0.102% | 0.110 | 40/73 |
| | 1988 | LWP | 50 | 10.47 | 6.18 | 13.49 | ACGCCGT_0595 | 50.1 | 0.020% | 0.426 | 40/73 |
| 16 | 1998 | LWD | 17 | 21.38 | 12.54 | 26.36 | ACGCCTG_0154 | 16.6 | 0.067% | 0.008 | 74/74 |
| | 2000 | AD | 20 | 8.35 | 7.55 | 15.52 | ACGCCTG_0154 | 16.6 | 0.074% | 0.422 | 74/74 |
| | 2000 | EWD | 20 | 7.8 | 5.73 | 18.42 | ACGCCTG_0154 | 16.6 | 0.065% | 0.456 | 74/74 |
| | 1998 | EWD | 21 | 5.38 | 4.68 | 9.22 | ACGCCTG_0154 | 16.6 | 0.023% | 0.660 | 74/74 |
| | 2001 | LWP | 22 | 15.24 | 7.41 | 16.7 | ACGCCTG_0154 | 16.6 | 0.066% | 0.342 | 74/74 |
| | 2003 | LWD | 23 | 11.55 | 9.42 | 20.62 | ACGCCTG_0154 | 16.6 | 0.012% | 0.561 | 74/74 |
| | 1996 | LWP | 23 | 16.52 | 10.41 | 19.86 | ACGCCTG_0154 | 16.6 | 0.014% | 0.693 | 74/74 |
| | 1986 | LWD | 35 | 17.74 | 9.92 | 22.71 | ACGCCGG_0180 | 35.1 | 0.027% | 0.602 | 74/35 |
| 17 | 1993 | LWD | 0 | 22.25[a] | 3.44 | 10.06 | ACACCGG_0310 | 0 | 0.018% | 0.260 | 40/40 |
| | 1995 | AD | 6 | 5.87 | 2.46 | 7.42 | ACGCCGG_0311 | 10.1 | 0.149% | 0.117 | 40/40 |
| | 1991 | AD | 6 | 10.67 | 4.9 | 13.54 | ACGCCGG_0311 | 10.1 | 0.004% | 0.574 | 40/40 |
| | 1988 | LWD | 6 | 6.11 | 2.59 | 9.56 | ACGCCGG_0311 | 10.1 | 0.129% | 0.072 | 40/40 |
| | 1992 | LWD | 6 | 39.46[a] | 6.84 | 22.72 | ACGCCGG_0311 | 10.1 | 0.087% | 0.163 | 40/40 |
| | 1995 | LWD | 11 | 5.26 | 1.74 | 5.75 | ACGCCGG_0311 | 10.1 | 0.131% | 0.144 | 40/74 |
| | 1993 | AD | 34 | 13.26 | 4.74 | 14.11 | ACGCCTC_0189 | 34.6 | 0.027% | 0.116 | 40/40 |
| | 2001 | EWD | 34 | 10.02[a] | 2.96 | 6.59 | ACGCCTC_0189 | 34.6 | 0.007% | 0.696 | 40/40 |
| | 1994 | LWD | 34 | 8.59[a] | 1.72 | 5.56 | ACGCCTC_0189 | 34.6 | 0.124% | 0.104 | 40/40 |
| | 1985 | LWD | 34 | 43.82[a] | 2.89 | 10.39 | ACGCCTC_0189 | 34.6 | 0.029% | 0.322 | 40/40 |
| 18 | 1988 | AD | 3 | 3.09 | 2.59 | 6.89 | ACGCCTC_0144 | 0 | 0.001% | 0.837 | 35/35 |

*F* is the *F* value for the regression analysis and $F_{0.05}$ and $F_{0.01}$ are the critical *F* values at the corresponding $\alpha$ value. Marker is the closest marker to the QTL position and "*P*" is the *P* value of the single locus analysis testing genotype nested within family. Year is the year for which each ring corresponds.

*N* Number of progeny associated with each marker flanking the QTL (left marker/right marker), *EWD* earlywood density, *LWD* latewood density, *LWP* latewood proportion, *AD* average density

[a] Significant at $\alpha$=0.01.

In total, there are 11 QTL clusters for individual ring wood density traits. Many of these clusters are between seasonal traits (earlywood or latewood) and average density (linkage group 2, 4, 11, and 17), and are likely QTL that affect average density via the respective seasonal trait variation. Other QTL clusters for wood density traits include combinations of LWD, EWD, LWP and average density, and reflect genes that have a general effect on wood density throughout the year. There are several other wood density traits that are detected in single years. Neale et al. (2002) suggest that QTLs detected in multiple years are likely verified QTLs, whereas those detected in only a single year have a greater potential to be false positives. QTL clusters, such as on group 1 (Fig. 1), may be evidence of genes that are determinants of wood density, whereas those detected in only a few years may be evidence for gene action in response to biotic or abiotic factors (Brown et al. 2003).

Several QTLs for wood density also co-locate with QTL for other traits. A QTL for height and volume co-locates with a QTL affecting EWD and average density on linkage group two, and a QTL for diameter and volume co-locates with a QTL affecting LWD and average density on linkage group four. The co-location of QTLs for wood density and growth traits is further evidence of pleiotropic effects and may be ideal targets for candidate gene mapping and marker-aided breeding. Other interactions with QTLs for wood density occur with coarseness on groups 14 and 16, fiber length on group 17, fiber length and coarseness on group 18, ARA on group 17, and wood chemistry traits on group 15. These examples may be further evidence of pleiotropy and can help to biologically explain correlations between these traits. However, several traits showed unique QTLs (unlinked to QTLs for other traits), such as microfibril angle on group 12 (Fig. 1); these are likely positions of unique genes with major effect for the respective trait.

## Expected number of QTLs

QTL studies are known to underestimate the total number of loci involved in trait determination (Beavis 1998; Otto and Jones 2000). Strauss et al. (1992) suggest that one of the important applications of QTL analyses is the identification of the number of QTLs controlling quantitative traits.

QTL studies are limited in size and include a restricted number of individuals and markers. As a result, only a small number of the actual QTLs can be detected, and consequently, the detected QTLs are those with large effect (Beavis 1998).

Assuming an exponential distribution of effects, the number of undetected QTLs and their average effect size can be estimated for traits where two or more QTLs have been detected (Otto and Jones 2000). However, depending on the history of each allele (its effect on fitness and the mode of selection acting upon it), an exponential distribution of QTL effects may not be an appropriate assumption, and the results may be biased. In certain circumstances, the more flexible gamma distribution may be appropriate and more accurate when estimating the number of undetected QTLs and their effects (Otto and Jones 2000). Our current study provides estimates for growth and wood traits based on an exponential distribution of QTL effects due to its theoretical support and relative simplicity (Otto and Jones 2000).

We inferred with this analysis that growth traits are affected by a large number of QTL of small effect. Growth occurs at a time of prolific gene activity, which can affect height and volume growth. Volume is affected by fewer QTL than height, suggesting that some height QTLs are negligible when considering their effect on volume. The QTLs detected for height and volume were of rather large effect (>9.5%), whereas the average effect size of undetected QTLs is estimated at 1.6–1.7% (data not shown). This implies a small number of QTL of large effect and a large number of QTL with very small effect.

## Marker-aided selection

One of the potential applications of QTL studies is marker-aided breeding. Knowledge of the number of QTLs affecting traits of interest within breeding populations and individual families is a valuable tool for modeling QTL effects and stability through time in breeding programs (Alvin Yanchuk, personal communication). Therefore, marker-assisted selection (MAS) and marker-assisted early selection (MAES) have been suggested as applications for QTL analyses. Several studies have been conducted to investigate the potential use of QTL mapping in marker-aided selection (Strauss et al. 1992; Johnson et al. 2000; Wu 2002). MAS or MAES may work for selection within families for traits of low heritability, but have limited potential for broader implementation. Strauss et al. (1992) suggest that MAS may be justified for three scenarios: (1) to identify QTLs associated with severe threats to forest health where time is urgent, (2) used in situations where extremely high-value families exist and where a limited number of clones or genotypes will be identified for extensive use, or (3) combine MAS with phenotypic selection in mapped families to improve genetic gains among and within families. Further evaluation of the feasibility of MAS is needed in light of recent reductions in the cost of genotyping and the development of genomic resources for some spruce and pine species.

## Temporal stability of QTLs

Many QTL studies have reported detection of QTLs in multiple years. Yoshimaru et al. (1998) detected two QTLs for height in Japanese cedar (*Cryptomeria japonica* D. Don) from two consecutive years (4 and 5 years of age) at the same location, but a third QTL for year 14 height growth at a separate location. The control of height growth, like any quantitative trait, is complex and varies through time. QTL studies of height growth are snapshots of the accumulation of gene activity through time. This study estimated QTLs for the height at year 26 in Douglas-fir. These height QTLs represent genes of largest effect that control height growth at this particular time and are not likely candidate QTLs for early growth. However, this type of consecutive analysis is warranted and would require the detection of QTL activity within individual years by evaluation of height increments.

Wood density traits also vary through time as climate varies and maturation occurs. Neale et al. (2002) report temporal variation in detected QTLs, suggesting that some QTLs occur over the duration of growth, whereas others occur in only later years. In the current study, the density QTLs on group 5 show interesting patterns of temporal variation. The QTLs detected from 24–27 cM occur in years 1990–1993. The second QTL on the same group (7–9 cM) occurs from 1994–2002. Other QTLs seem to be present throughout the duration of the experiment (20–35 cM on linkage group one), whereas others seem to occur in the early years (0–11 cM on group 17). These patterns of temporal variation may be a result of maturation. Studies have shown that density traits and their heritabilities vary through time for coastal Douglas-fir, and are associated with environmental signals (Vargas-Hernandez and Adams 1992, 1994; Vargas-Hernandez et al. 1994). The pattern of QTL expression through time likely reflects both maturation and responses to biotic and abiotic factors. Trees, with their long generation times, provide a rich resource for future studies of temporal variation of QTL expression.

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