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Multiple-marker mapping of wood density loci in an outbred pedigree of radiata pine

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Abstract The objective of this study was to determine the genetic location and effects of genomic regions controlling wood density at three stages, i.e., rings corresponding to ages 1–5 (WD1_5), rings corresponding to ages 6–10 (WD6_10), and outer wood density (WD14) in a full-sib pedigree (850.055×850.096) of *Pinus radiata*. The number of offspring measured at these three stages were 80, 93 and 93, respectively. Only a single linkage group of the parent 850.55 was considered for mapping quantitative trait loci (QTLs). A multiple-marker least-squares approach was employed for mapping QTLs for each of the three traits, using a single-QTL model. Logistic regression was used for multiple-trait QTL mapping. Critical values for test-statistic were calculated empirically by 'shuffling' the data. A putative QTL with large effect on WD1_5 appears to be segregating at the 73 cM position (experimentwise $P<0.01$). The width of the 95% bootstrap confidence interval for this putative QTL was 40 cM (i.e. 56–96 cM). The effect of this QTL on the expression of wood density at later stages was diminished. From multiple-trait analysis, two marker locations (at 66 cM and 91 cM) were found to be significantly associated (experimentwise $P<0.05$) with the expression of wood density at different ages. These results are encouraging for the application of marker information to early selection in order to increase juvenile wood density, although the putative QTLs detected in this study need to be verified in an independent population.

Key words Quantitative trait loci (QTLs) · *Pinus radiata* · Wood density · Linkage group

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Introduction

Many traits of economic importance in plants and animals are of a quantitative nature. That is, the observed phenotypes are continuously distributed and reflect the action of many quantitative trait loci (QTLs) together with environmental effects. The availability of genetic markers has facilitated experimental studies in a number of species to explore the nature and location of some of these QTLs (de Koning et al. 1998). These studies will provide insight into the control of these economically important traits, and may enhance breeding programmes through opportunities for marker-assisted selection.

QTL mapping studies in crop plants have been performed using segregating populations derived from crosses between inbred lines (e.g. Tanksley et al. 1982; Edwards et al. 1987). Such populations are not available in trees and will be difficult to obtain for many species owing to a high genetic load and long generation times. It is common to use full-sib families as mapping populations in QTL studies in outbred forest trees (e.g. Plomion et al. 1996; Knott et al. 1997; Emebiri et al. 1998). Linkage analysis in a pedigree formed from crosses between two unrelated highly heterozygous trees is complex, as up to four alleles might be segregating at a locus. Tracking the inheritance of multiple alleles at QTLs in an outbred pedigree necessitates the use of co-dominant multi-allelic markers. However, for radiata pine there are presently insufficient multi-allelic markers to construct a dense linkage map.

Grattapaglia and Sederoff (1994) adopted a 'two-way pseudo-testcross' approach with RAPD (random amplified polymorphic DNA) markers to construct linkage maps for each parent of a full-sib family of *Eucalyptus*. RAPD

markers, in a pseudo-testcross mapping configuration, are those which are in a heterozygous state in one parent and homozygous in the other, or vice-versa; therefore, separate sets of linkage data are obtained for each parent. As one of the parents of a full-sib pedigree (in a pseudo-testcross mapping strategy) is non-informative at the RAPD marker loci (which means no information on QTL/marker linkages can come from the non-informative parent), the inheritance of the gametes from the heterozygous parent can be analysed assuming a half-sib design. The advantage with this approach over the conventional half-sib design (where the marker information is usually available only on one parent) is that at each marker locus we know unequivocally which parent allele each offspring has inherited.

It is not uncommon to use the information from one marker at a time to analyse the marker-trait data using analysis of variance (ANOVA) techniques (Weller et al. 1990; Groover et al. 1994). Haley et al. (1994) showed that bias in the estimated position of the QTLs can be reduced by including all markers in a linkage group. Knott et al. (1996), using a simulation study, showed that the multiple-marker technique has a higher power of detecting a putative QTL as compared to the single-marker ANOVA method.

The present study demonstrates the use of the multiple-marker techniques for the analysis of data from a pseudo-testcross mapping design, in a two-generation pedigree of radiata pine. A stepwise approach for the QTL analysis is presented here. Initially, we used exploratory analyses (Visscher and Haley 1996; Knott et al. 1997; de Koning et al. 1998), in which trait scores are regressed onto selected marker information, in an attempt to determine whether the inheritance of the trait (for individual linkage group) is compatible with an oligogenic model (several QTLs) or whether a small region of the linkage group (compatible with a single QTL) is important. This was followed by a more-conventional search of the genome using a least-squares analysis (Knott et al. 1996). Churchill and Doerge's (1994) empirical method was used to obtain significant thresholds. Multiple-trait QTL mapping was also undertaken using logistic regression (e.g. Henshall and Goddard 1999).

Materials and methods

Mapping population

Full-sib progeny of parent trees 850.055 and 850.096 of *Pinus radiata* was used to detect putative QTLs. This family was chosen because the parents displayed favourable general combining abilities, for various economic traits, in different mating designs. Tree 850.055 is widely used in seed orchards to produce seed for commercial plantations. The QTL detection population consisted of 80–93 trees (for different traits) from a large block Genetic Gain Trial planted in 1978 in Kaingaroa Forest in New Zealand that had not been subjected to any silvicultural selection.

Framework Linkage Map construction

Different dominant and co-dominant marker systems (RAPD, AFLP and SSR) were used to generate 430 polymorphic loci segregating in this pedigree. The linkage map of 850.055 consisted of 126 framework markers in 21 linkage groups, and covered 1540.2 cM, while the 850.096 map contained 101 markers in 26 linkage groups covering 1223.0 cM (P. L. Wilcox et al., unpublished). Markers were ordered on the map with a 1000:1 support. The genetic distances (cM) were obtained using the Kosambi map function. All of these framework markers were genotyped on 93 randomly selected individuals of this family. Preliminary analysis (P. L. Wilcox et al., unpublished) using single-marker ANOVA found marker-trait association on linkage group 3. Thus, in this study, we considered markers only on linkage group 3 in the framework linkage map of the parent 850.055 for mapping the putative QTLs using the multiple-marker method. The distribution of markers on this linkage group is shown in Table 1.

Table 1 Distribution of markers on linkage group *three*[a]. * Denotes the markers selected for exploratory analysis

No.	Marker	Position on linkage group (cM)
1	A93_b3	0
2	A56_A	15
3	A184_b3	25*
4	RAPD_59	48*
5	RAPD_38	66*
6	A329_c3	83
7	A113_a1	91*
8	A297_b2	99
9	RAPD_270	106
10	A338_a1	115*
11	A219_b2	123
12	A140_c2	130
13	RAPD_192	140*
14	A47_c	153
15	RAPD_209	170*
16	A72_A	187

[a] All markers were in a heterozygous state in parent 850.055 and homozygous null in the other parent (850.096)

Phenotypic data

Cores were extracted at age 14 and, for each individual tree, outer wood density (termed as WD14 in this study) was obtained from two samples of the outer 50 mm of increment cores taken from opposite sides of the tree. To explore the potential for the use of core wood from young trees in QTL detection experiments, one 5-mm bark-to-pith core was taken from each tree in 1997. The wood density from these cores was measured using X-ray densitometry (Cown and Clement 1983). Area-weighted wood densities were obtained for rings corresponding to ages 1–5 and ages 6–10 years. These two area-weighted wood densities were considered as two different traits and the symbols for them in the rest of the text will be WD1_5 and WD6_10, respectively. Thus, three traits (WD1_5, WD6_10 and WD14) were measured in each offspring.

Statistical methods

Exploratory analysis on multiple markers

An exploratory analysis of the linkage group was undertaken to determine whether the chromosomal region under study is associated with variation in the recorded traits (Visscher and Haley 1996). To undertake the analysis, first, the locations of informative and evenly spaced markers were selected from those available in the data set. If too many markers are selected the analysis will take up a significant proportion of the degrees of freedom. Information from closely related markers is highly correlated (de Koning et al.

1998) and theoretical studies (Dekkers and Dentine 1991; Visscher 1996) indicate that markers spaced every 25 cM or so should explain most of the variation on a chromosome.

For exploratory analysis, data for each trait are regressed on selected marker positions. For a given position, the conditional probabilities of the offspring inheriting the first gamete of the parent provide an independent variable (as the probabilities of two parental alleles sum to unity) on which the trait values can be regressed (Knott et al. 1996). At the position of an informative marker we know which parental allele each progeny has inherited, so the probability of inheriting one allele will be unity and probability of inheriting the other will be zero. In a pseudo-testcross mapping strategy, one parent is heterozygous at all marker loci but some faint RAPD bands are difficult to classify as present or absent in some individuals. If a marker was not informative in a particular individual, it was replaced by the 'virtual' marker probability calculated for that position based on the nearest markers (e.g. Knott et al. 1996; de Koning et al. 1998). On the assumption that the parental gamete reconstruction is correct, and for each offspring the allele inherited from the parent is known unequivocally, these probabilities are exactly the same as for a backcross situation (Knott et al. 1996).

The model for the exploratory analysis is:

$$Y_j = \mu + \sum_{k=1}^{n} b_k m_{jk} + e_j, \quad (1)$$

where

Y_j is the phenotypic value of offspring j
μ is the overall mean
b_k is effect of parental allele for marker k
m_{jk} is the probability for offspring j of inheriting the parental allele of marker k
e_j is the residual effect for offspring j.

Comparison of alternative genetic models

Oligogenic

Using Eq. (1), the presence of genetic variation associated with a linkage group was tested by fitting the regression of each progeny phenotype on the conditional probabilities at all selected marker locations (n) simultaneously. Under the null hypothesis of no genetic variation for the trait associated with the linkage group under study only a family mean is fitted.

Single region

If an effect of a linkage group or chromosome is significant, further analysis can be used to identify whether there is one or more regions within the linkage group affecting the trait. The regression on all selected marker locations is compared with the regression on every pair of adjacent markers. Where more than one important QTL affects the trait, there will be no single pair of adjacent markers that accounts for as much variance as do all markers jointly (de Koning et al. 1998). If the oligogenic model is not a significant improvement over fitting the best single region, it can be concluded that most of the genetic variance associated with this linkage group is explained by this single region.

Single-trait QTL analysis

The least-squares multiple-marker method described in Knott et al. (1996) was employed in this study. Basically, the model used here is same as that given in Eq. (1) except that the summation is now over the number of QTLs included in the model (one QTL in this study). At each 1-cM position, the value of phenotypes from progeny are regressed on the conditional probabilities. This would provide an estimate of the allele substitution effect (Falconer 1989) for a heterozygous QTL at that position.

Multiple-trait QTL analysis

The three traits measured (WD1_5, WD6_10 and WD14) on each individual can be considered as different states of the same trait. If each trait is analysed separately, it cannot be deduced whether these effects are due to one locus with correlated effects on these traits, or to several loci each affecting a different trait. The genetic correlations (based on an open-pollinated progeny test) among these juvenile and mature wood density traits were more than 0.75 (Bannister and Vine 1981). Multiple-trait QTL mapping could help in understanding a QTL's part in the genetic covariance structure of economically important traits. In this study, the logistic regression method proposed by Henshall and Goddard (1999) was applied for the mapping of multiple-trait QTLs using each marker in turn. The parental allele (coded as 0 and 1) inherited by an individual, at a marker location, becomes the dependent variable and the phenotypes for three traits are the independent variable. Multiple-trait QTL effects, assuming no recombination (r=0), were obtained as (Henshall and Goddard 1999):

$$A=\Sigma\beta/[1+\mathrm{SQRT}(\beta`\Sigma\beta+1)], \quad (2)$$

where A is the vector of half the effect of allele substitution, Σ is phenotypic covariance matrix estimated from the complete experimental data, and β is the vector of parameter estimates. Σ was obtained using a phenotypic coefficient of variation of 0.07 for all three traits (Cown et al. 1992). The estimates of phenotypic correlations among these traits were obtained from Bannister and Vine (1981).

Significance thresholds

For single-trait QTL analysis

For the genome scans a large number of correlated tests with mixture distributions are being performed and, hence, the standard F distribution cannot be used to obtain the significance thresholds. An empirical distribution is therefore required, in order to test for significance. Test-statistic critical values were calculated empirically from the permutation method described in Churchill and Doerge (1994). The conditional probabilities (m_{jk}s) that the phenotypes are regressed on were not shuffled. The experimentwise critical values, which account for the evaluation of marker-QTL association across the genome and also the three traits being analysed, were calculated from the distribution of test-statistics (see Spelman et al. 1996). Because only one chromosome is being analysed, the experimentwise critical values were calculated using the approximation of standard Bonferroni correction as:

$$\alpha \approx \gamma/n, \quad (3)$$

where α is the nominal threshold level to ensure a γ significance level over the $n(=R*T)$ independent tests. The R (=1540.2/187.0=8.24) denotes the ratio of total map length to the length of the chromosome under study, while T denotes the number of independent traits. The number of independent traits were determined by factor analysis (using SAS 1988) on a genetic correlation matrix for the three traits. It was calculated that two factors account for approximately 98% of the variation. Thus, this suggests that there are only two independent traits (i.e. T=2). The Bonferroni correction factor was applied to all three traits. The 100(1–α) percentile of the distribution of test-statistics provides the experimentwise significance threshold.

Following de Koning et al. (1998), for exploratory analysis, a α level of 0.0006 [=0.01/(8.24*2)] and 0.0030 [=0.05/(8.24*2)] would be required to obtain 0.01 and 0.05 genome-wide levels, respectively, and can be obtained from standard tables. The suggestive level of significance (Lander and Kruglyak 1995), where one significant result is expected by chance in a genome analysis, can be obtained from the binomial distribution as:

$$P_{suggestive}=1/n \quad (4)$$

Many suggestive linkages will subsequently prove to be incorrect, but they are nevertheless reported so that they can be followed up in future studies (Knott et al. 1997).

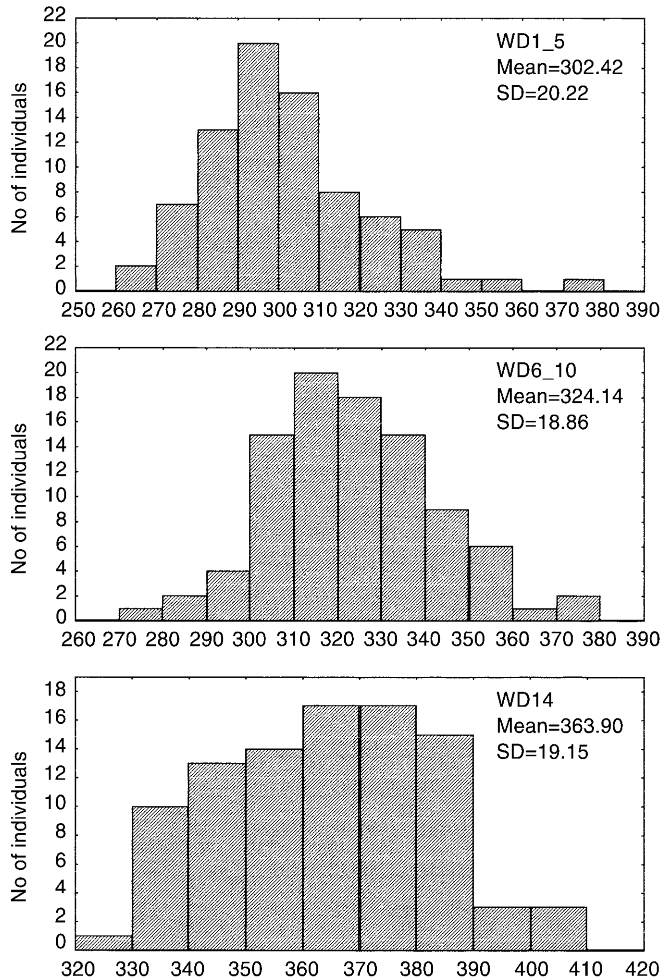

Fig. 1 Phenotypic distributions of WD1_5, WD6_10 and WD14. The mean and standard deviation (*SD*) for each trait are also given

For multiple-trait QTL analysis

The logistic regression was performed at each marker location, in turn. Test-statistic critical values were calculated empirically, at each marker location, from the permutation method that involved repeated shuffling of three quantitative-trait values together. The experimentwise critical value may be obtained by first finding the maximum test statistic over selected marker locations (about 25 cM apart) for each of the shuffled analyses. These values are then ordered and their 100(1–α) percentile will provide the experimentwise significance threshold.

Results

Quantitative traits

The phenotypic distributions of three traits examined are shown in Fig. 1. The traits WD6_10 and WD14 were approximately normally distributed as tested by the Kolmogorov-Smirnov distance statistic (*P*-value >0.15). Only WD1_5 showed a significant departure from normality (*P*-value <0.01). The data for this trait was log-transformed to improve normality. However, results from QTL analysis using transformed data did not differ from those with untransformed data. Therefore, similar to some other studies (e.g. Plomion et al. 1996; Emebiri et al. 1998), only untransformed data was used. Phenotypic correlations were estimated among traits within the family studied. Significant ($P\leq 0.001$) correlations were observed between WD1_5 and WD6_10 (r=0.66), WD1_5 and WD14 (r=0.37) and WD6_10 and WD14 (r=0.54).

Table 2 Comparison of alternative genetic models. N denotes the sample size. * A probability level of 0.0006 (=0.01/8.24*2) and 0.0030 (=0.05/8.24*2) would be required to obtain 0.01 and 0.05 genome-wide levels

Traits	WD1_5	WD6_10	WD14
N	80	93	93
# Markers	7	7	7
Oligogenic[a,*]	0.0006	0.0211	0.0021
Oligo vs single interval[b,*]	0.5802	0.3772	0.0476

[a] The probability of the *F* ratio for testing the oligogenic model versus a model with no genetic effect
[b] The probability of the *F* ratio for testing the oligogenic versus the best single-interval mode

Comparison of genetic models

Seven out of sixteen, approximately equally spaced (about 25 cM apart) markers were selected for this exploratory analysis (Table 1). The comparison of alternative genetic models is shown in Table 2. There is significant evidence, at a genome-wide 0.05 level, for a genetic component for the traits WD1_5 and WD14. However, the evidence of a genetic effect for the trait WD6_10 is significant only at a suggestive level (Table 2). The oligogenic model, for the traits WD1_5 and WD6_10, is not a significant improvement over the single-region model but, at a suggestive level, is significantly better in case of the trait WD14.

Single-trait QTL analysis

Permutation test

The distributions of the test-statistic, by shuffling the trait value and fitting its regression on all marker positions in the linkage group, were obtained for each trait (Fig. 2). These distributions are hardly distinguishable, which is also reflected from the critical values given in Table 3. The test-statistic distributions in Fig. 2 account for repeated testing across the linkage group but does not account for repeated tests on the three correlated traits. Experimentwise threshold levels (Table 3) were calculated from the distributions in Fig. 2 using the standard Bonferoni correction (Eq. 3).

QTL mapping

For all three traits, the test-statistics calculated for a single-QTL model at each 1 cM are shown in Fig. 3. The analysis revealed a putative QTL for WD1_5 positioned at 73 cM. The test-statistic was significant at the 0.01 genome-wide threshold level. The other traits, WD6_10 and WD14, showed an indication of a possible QTL at 57 cM and 135 cM, respectively (Fig. 3). However, the test-statistics for WD6_10 and WD14 were only significant at the 0.18 and 0.15 genome-wide significance level, respectively. The estimated effects of allele substitution, at the most likely position of a QTL, were 23.17, 15.19 and 14.46 kg/m^3 for WD1_5, WD6_10 and WD14, respectively (Table 4).

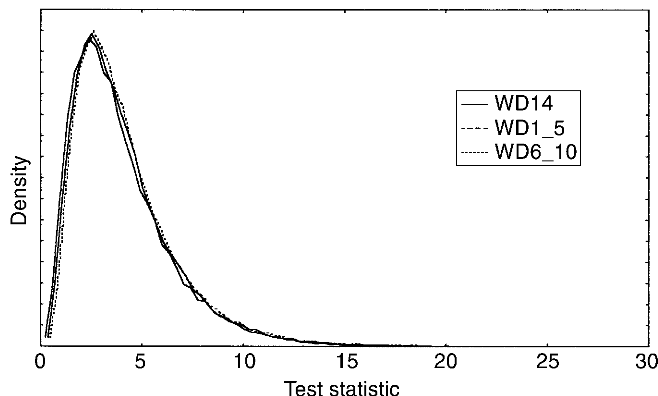

Fig. 2 Approximate density function of test statistics for WD1_5, WD6_10 and WD14 derived from the permutation test (50000 shuffles)

Multiple-trait QTL mapping

The approximate density function of the test-statistic, based on 5000 shuffles, is shown in Fig. 4. Experiment-

Table 3 Experimentwise threshold levels for the three traits (50000 shuffles)

Threshold level	WD1_5	WD6_10	WD14
1%	18.69	18.53	18.99
5%	15.03	15.27	15.61
10%	13.45	13.61	14.02
15%	12.50	12.74	12.98

Fig. 3 Test statistics for different positions (at every 1 cM) on chromosome 3 for different traits. Experimetwise threshold levels (1% and 15%) are also given

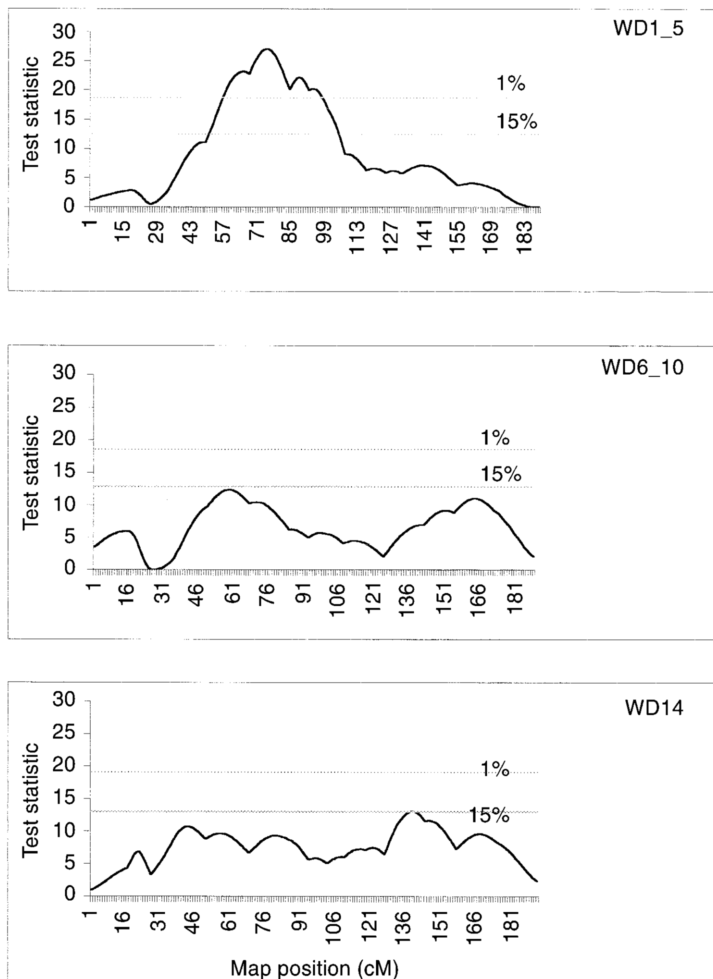

Table 4 Estimated allele substitution effects and standard errors (SEs), at the putative QTL, for different traits

Trait	Position of the QTL	Effect (kg/m^3)	SE
WD1_5	73 cM	23.17	4.45
WD6_10	57 cM	15.19	4.33
WD14	135 cM	14.46	4.00

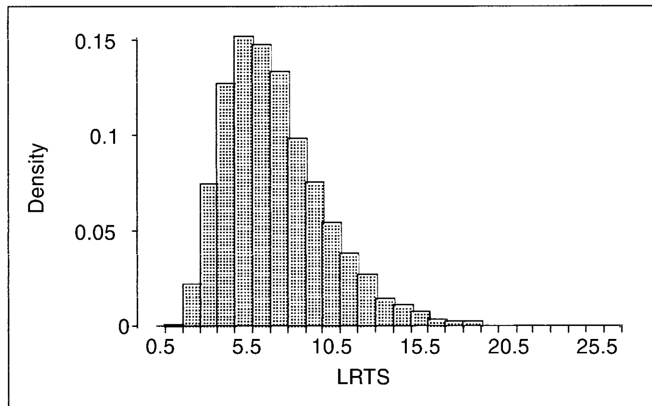

Fig. 4 Approximate density function of the log-likelihood ratio test statistic (LRTS) derived from the permutation test (5000 shuffles)

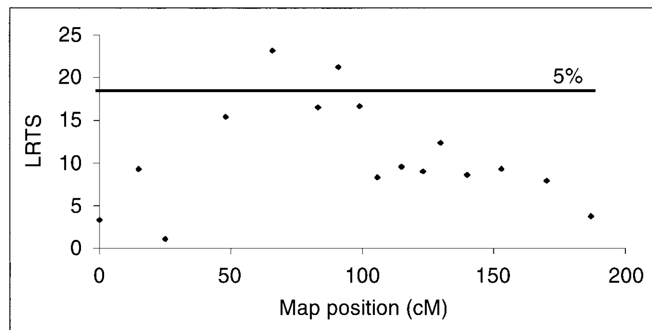

Fig. 5 Log-likelihood ratio test statistic (LRST) at marker positions. The experimentwise threshold level (5%) is also shown

wise threshold levels were calculated from the distribution in Fig. 4 using the standard Bonferroni correction (Eq. 3). The test-statistics calculated at each marker location, using logistic regression, are shown in Fig. 5. Specifically, there were two marker locations (RAPD_38 at 66 cM and A113_a1 at 91 cM) found to have association with putative QTLs at an 0.05 genome-wide significance level. The effect of allele substitution (using Eq. 2) at marker RAPD_38 was 25.58, 23.12 and 17.89 kg/m^3 for the WD1_5, WD6_10 and WD14 traits, respectively. Similarly, the substitution effect was estimated to be 22.56, 17.93 and 18.27 kg/m^3, at marker A113_a1, for the WD1_5, WD6_10 and WD14 traits, respectively.

Discussion

Comparison of alternate genetic models

Since the study by Sax (1923), and more recently with the development of molecular marker technologies, it has been demonstrated in plants and animals that a trait showing a continuous distribution could be under oligogenic control. In *Pinus taeda*, Groover et al. (1994) detected five QTLs for wood specific gravity which together explained 23% of the total variation. Knott et al. (1997) tested different genetic models for each linkage group in loblolly pine. They found different inheritance patterns for different linkage groups. For most of the linkage groups contributing significantly to the genetic variation in a trait, the single-region or oligogenic models were found to best explain the inheritance patterns. The present study revealed that for linkage group 3, the inheritance patterns for different traits under study seem best explained by either a single-region (one QTL) or a small number of QTLs (i.e. oligogenic) (Table 2).

Single-trait QTL mapping

Using a single-QTL model, the highly significant evidence for a putative QTL was found only for the trait WD1_5 (Fig. 3). The test for the presence of genetic variation associated with this linkage group, for trait WD1_5, was also significant at a genome-wide 0.01 significance level and the oligogenic model failed acceptance in favour of a single-region model. There was little indication for the presence of genetic variation, for WD6_10, associated with this linkage group (Table 2) and thus there was no segment of this linkage group having an enormous effect on the trait variation. The putative QTL found for WD6_10 was significant at genome-wide significance level of about 0.18 (Fig. 3). The improvement of the oligogenic model over the single-region model, for WD14, was only significant at a suggestive level and would have been rejected in favour of the single-region model. The QTL analysis revealed a putative QTL at an 0.15 experimentwise threshold for the trait WD14.

The results showed that there is no strong statistical evidence of putative QTLs for the traits WD6_10 and WD14. The putative QTL found for WD1_5 had a considerable, but not significant, effect on the expression of wood density at later stages. As the genetic correlations among WD1_5, WD6_10 and WD14 are very high, Bannister and Vine (1981) concluded that in each tree the same genes were acting on wood density, and acting in much the same way, throughout the 15-year period. The results from the present study reflects that the same genes might be acting on wood density but that the effect of gene substitution does not remain similar at all stages. For example, the effect of gene substitution at the putative QTL for WD1_5 (at 73 cM) was 23.17 kg/m^3 and reduced to 13.32 and 12.83 kg/m^3 for the WD6_10 and

WD14 traits, respectively. This might be due to the weak environmental correlations among wood densities at different ages. As observed by Searle (1961), a phenotypic correlation less than its genetic counterpart, together with a small environmental correlation (as is the case among traits in this study), will occur where the genes governing two traits are similar but where the environments pertaining to the expression of these traits have a low correlation. Environmental influence was suspected to be one of the causes for the differential expression of stem growth QTLs in radiata pine seedlings (Emebiri et al. 1998). These authors found that none of the putative QTLs detected at any one stage were strongly expressed at all four stages of measurement. Verhaegen et al. (1997) found that no chromosomal region was consistently expressed across three ages for wood density in two species of *Eucalyptus,* though the analysis demonstrated the existence of a chromosomal segment being involved in the control of the trait across the period studied, independent of age. They also found that some QTLs were specific to a single stage.

Multiple-trait QTL mapping

Multiple-trait QTL mapping was undertaken at each marker location using a logistic regression method. Two marker locations (RAPD_38 at 66 cM and A113_a1 at 91 cM) were found significantly associated (at 0.05 experimentwise threshold level) with the expression of wood density at three stages, i.e. WD1_5, WD6_10 and WD14 (Fig. 5). Incidentally, these two markers are flanking the location of the putative QTL (at 73 cM) found for the trait WD1_5 and which had a considerable, though not significant, effect on other two traits as well. Using the bootstrap technique (see Visscher et al. 1996), we estimated the width of the 95% confidence interval (for the QTL found for WD1_5) to be 40 cM (i.e. 56–96 cM).

Marker-Assisted Selection (MAS)

There is a high level of tree-to-tree genetic variation in *P. radiata* for various morphological traits, wood properties, disease resistance, and many other traits for which actual data have been collected (Burdon 1992). This situation exists even in the progenies generated from intermating parents of high breeding value. Thus, there is an opportunity to use marker-trait associations to increase genetic gain, per unit time, by selecting within the families that are used to establish production populations, and propagating for deployment only those individuals that have favourable marker genotypes. The first important step towards MAS is the detection and verification of QTLs. Forest trees like radiata pine have long generation intervals and undergo various changes at morphological, anatomical and physiological levels during their life span. Instability of QTL expression over age has been reported in poplar for basal area (Bradshaw and Stettler 1995), in maritime pine for juvenile growth (Plomion et al. 1996; Emebiri et al. 1998), and in *Eucalyptus* for wood density (Verhaegen et al. 1997). However, the very high genetic correlations among the expression of wood density at different ages in radiata pine indicate that much the same genes are controlling the trait expression at different stages (Bannister and Vine 1981). The present study suggests that linkage group 3 in 850.055 contains such a locus. However, the effect of gene substitution would change at different stages. The putative QTL found for the trait WD1_5 was not significantly expressed at later stages. The putative QTLs for WD6_10 and WD14 were only significant at a 15–20% genomewide significance level. As the sample sizes used in this study (80–93) are small the power of QTL detection would be low (e.g. Kumar et al. 1999) so that QTLs of low effect can not be detected. Also, with such a small sample size, there is a high probability that the effect of allelic substitution is likely to be overestimated. Therefore, marker-trait associations found in this study need to be verified in independent population. Nonetheless, this study indicates that early selection based on desired marker haplotypes might help increasing juvenile wood density.

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