Theor Appl Genet (2004) 109: 856–864
DOI 10.1007/s00122-004-1699-4

ORIGINAL PAPER

K. Thamarus · K. Groom · A. Bradley · C. A. Raymond · L. R. Schimleck · E. R. Williams · G. F. Moran

Identification of quantitative trait loci for wood and fibre properties in two full-sib pedigrees of *Eucalyptus globulus*

Received: 3 May 2003 / Accepted: 2 April 2004 / Published online: 6 May 2004

Abstract Regions of the genome influencing wood and fibre traits in *Eucalyptus globulus* Labill. have been identified in two full-sib pedigrees that share a common male parent. The first pedigree, cross A, contains 148 progeny, and the second pedigree, cross B, contains 135 progeny. Subsets of progeny of these two controlled crosses were planted at seven sites throughout Australia in 1990. Wood cores were taken at 0.9 m above ground in 1997, and wood and fibre traits were analysed for each individual. Three quantitative trait loci (QTL) affecting wood density, one QTL affecting pulp yield and one QTL affecting microfibril angle have been located in both pedigrees, using single-factor analysis of variance. Other QTLs affecting these traits, as well as fibre length and cellulose content were located in cross A only.

Introduction

Eucalyptus globulus (Labill.) is an important tree species planted in temperate latitudes for the production of kraft pulp for paper. While the natural range of *E. globulus* is restricted to small regions of Tasmania and Victoria in Australia, it is one of the more extensively planted eucalypts in the world with particularly large plantations in Portugal and Spain (Eldridge et al. 1994). The popularity of this species can be attributed to such qualities as its high pulp yield, rapid growth and adaptability to a range of site conditions, all of which enhance the potential of *E. globulus* as a plantation species for pulpwood production (Turnbull and Pryor 1988).

It is economically important to select for wood properties that have a major impact on pulp and paper properties (Raymond and Schimleck 2002). Studies have identified basic density and pulp yield as key variables in the profitability of eucalypt kraft pulp production (Greaves et al. 1997). The traditional method of assessing pulp yield is slow and expensive, restricting the number of samples that may be processed. Alternative methods have been developed, assessing the cellulose content of wood, which have shown a strong correlation with kraft-pulp yield for *E. globulus* (Raymond and Schimleck 2002). Microfibril angle (MFA, the angle between cellulose microfibrils in the cell wall and the cell axis; Donaldson 1993) and fibre length also significantly affect pulp and paper properties. Low MFAs are associated with high tensile strength in paper, while high MFAs are associated with greater stretch and tear indices (Donaldson 1993). Fibre length influences paper strength—especially tear—and paper machine operation (Muneri and Raymond 2001).

These wood properties exhibit continuous variation and as such are viewed as quantitative traits influenced by multiple genetic factors and the environment. Identification of the genetic factors contributing to quantitative trait variation is an important step in many tree breeding programs. Generally in eucalypts these wood quality traits have been found to have moderate to high heritabilities, especially in contrast to growth traits (Raymond 2002). Quantitative trait loci (QTLs) affecting a variety of wood quality, and growth traits have been identified in eucalypts (e.g. Grattapaglia et al. 1996; Byrne et al. 1997; Verhaegen et al. 1997). Because of long generation times, most studies have relied on single pedigrees to determine the number and size of QTLs affecting quantitative trait variation. Because of the highly heterozygous, outbred nature of most tree species, it is important to validate QTLs in additional pedigrees (Kumar et al. 2000)

Communicated by D.B. Neale

K. Thamarus · K. Groom · E. R. Williams · G. F. Moran (✉)
CSIRO Forestry and Forest Products and CRC for Sustainable Production Forestry,
P.O Box E4008 Kingston, ACT, 2604, Australia
e-mail: gavin.moran@csiro.au

A. Bradley · C. A. Raymond
CSIRO Forestry and Forest Products and CRC for Sustainable Production Forestry,
P.O. Box 252-12 Hobart, TAS, 7001, Australia

L. R. Schimleck
CSIRO Forestry and Forest Products,
Private Bag 10 Clayton South, VIC, 3169, Australia

Identification of QTLs affecting wood and fibre properties will lead to greater understanding of these traits and help in selection and manipulation of these traits in breeding programs. In this study we identify regions of the genome affecting wood density, pulp yield, cellulose content, fibre length and MFA in inter-population crosses of *E. globulus*.

Materials and methods

Plant material

Molecular and quantitative analyses were conducted on two full-sib pedigrees, crosses A and B, which share a common male parent. Cross A is an inter-population cross between a King Island female parent (KI-1) and a Taranna male parent (G139) and has 148 progeny. Cross B is an intra-population cross between a Taranna female parent (T-5) and the same male parent as cross A (G139) and has 135 progeny. These controlled crosses were planted in 1990 as part of the larger North Forest Products/CSIRO Division of Forestry hybrid trials (Volker 1995). Subsets of progeny of each cross were planted at seven sites throughout Australia, spanning the natural and planted range of *E. globulus*, with four sites in Tasmania and one site each in Victoria, New South Wales and Western Australia. The crosses were planted in three-, five- or ten-tree row plots in incomplete block designs, with 20–30 trees per site per cross.

DNA genotyping

DNA genotypes were determined for 286 trees: the 148 progeny of cross A, the 135 progeny of cross B and the three parent trees. DNA extractions from leaf tissue were carried out as described in Byrne et al. (1993). Individuals of cross A were genotyped for 249 co-dominant markers (204 RFLPs, 40 microsatellites, five isozymes), which were mapped as described in Thamarus et al. (2002). Individuals of cross B were genotyped for 100 markers (79 RFLPs and 21 microsatellites), and mapping analyses using JOINMAP, version 2.0 (Stam and van Ooijen 1995), were conducted as described in Thamarus et al. (2002). For fully informative markers, the segregation data from each parent were determined and these data were also included in analyses.

Quantitative trait measurements

For all progeny, three wood core samples were taken at a height of 0.9 m using a 12-mm diameter bark-to-bark core. Basic wood density and fibre length were measured from the first core; cellulose content and predicted pulp yield were estimated from the second core; and MFA was determined from the third core.

Basic wood density was determined by the water displacement method. Core samples were soaked in water until fully saturated, dried with paper towels and green volume was determined by weighting in air and under water. Cores were oven dried at 103°C and dry-weight determined. Basic density was calculated as the ratio of oven dry weight to green volume (kg/m^3). Cores were then delignified in three stages: first with 10% sodium hydroxide, then twice with a 50:50 solution of hydrogen peroxide and glacial acetic acid and then disintegrated in water with an electrical blender. Length-weighted average fibre length was measured using a Kajaani FS200. Length-weighted averages were used as these reduce the influence of cut and broken fibres by placing greater emphasis on the longer, whole fibres (Muneri and Raymond 2001).

The second core was broken into small fragments using an ESSA 200 mm disc pulveriser and then reduced to wood meal in a Wiley Mill. Grinding of all samples was carried out through a 1.0-mm screen for 1 min. Wood-meal samples were used to estimate percentage of pulp yield by near-infrared reflectance analysis (Schimleck et al. 2000) and determine crude cellulose content (percentage) by the diglyme method (Raymond and Schimleck 2002).

Sample strips were prepared from the third core for SilviScan-2 analysis (an automatic wood microstructure analyser, Evans et al. 1999). MFA, the most probable angle of the cellulose microfibrils in the S2 cell wall layer relative to the fibre axis, was estimated by X-ray diffractometry (Evans et al. 2000). For each of the two pith-to-bark portions of a single bark-to-bark core, an average MFA value was determined. These two MFA values were then averaged for a whole core estimate of MFA for each tree.

QTL analysis

For each trait, site was identified as a significant factor affecting the trait mean by analysis of variance (ANOVA, $P \leq 0.001$). Because of small sample sizes at each site ($n \leq 30$), it was necessary to conduct analyses using the full data set with trait data standardized across all sites. For each cross and all traits, row-plot means (n=3, 5 or 10 trees) were estimated, and the deviation from the row-plot mean was determined for each tree sampled, thereby removing additive site effects for each plot. Analyses were conducted using individual deviation from row-plot mean as the sample variable. This shifts the row-plot mean to zero but does not affect the variance. The effect of marker genotype on trait phenotype was determined by single-factor ANOVA of the DNA genotypes on trait deviations. Each marker, trait and cross was analysed separately. For fully informative markers, additional ANOVAs were conducted on female segregating data and male segregating data separately. A simple linear regression approach (Payne et al. 1987) was used to accommodate unbalanced data sets created by missing quantitative or molecular data or segregation distortion. Single-factor ANOVAs were carried out for each marker locus. The following linear model was fitted: $y=\mu+m_i+e_{ij}$, in which y is the individual traits value, μ is the mean, m_i is the ith marker genotype and e_{ij} is the residual associated with the jth individual in the ith genotypic class. F-probabilities were determined for each marker–trait association and percent phenotypic variance accounted for by the most significant marker in a designated QTL region was calculated. The percent phenotypic variance was derived as $100 \times [1-(\text{residual mean square/total mean square})]$. Distribution of the trait data and trait deviations were plotted, and residual plots were created to check the assumptions underlying the ANOVA procedure (Ott 1984).

Initial QTL analyses were conducted using cross A molecular marker genotypes. F-probabilities associated with marker genotypes were sorted by map position and type of segregation (male, female or fully informative). For fully informative markers, F-probabilities associated with male and female segregating data, as well as data segregating from both parents, were included in analyses.

To determine significance thresholds for QTLs, a permutation test was performed according to the procedures of Churchill and Doerge (1994) and Knott et al (1997). The permutation test was performed for each of the linkage groups separately with α=0.05 and n=1,000. The permutation test was done separately for each genotype segregation class corresponding to single classification of analysis of variance with 1, 2 and 3 degrees of freedom, respectively, and for all five traits. So the permutation test was used to identify QTL regions in cross A. Markers in these regions were examined in cross B for purposes of verification.

A QTL region was identified if a marker in that region were significant at the P=0.05% level on the permutation test, if two or more linked markers significantly affected the phenotype ($P \leq 0.05$), and at least one of the markers in the region had an F-probability ≤ 0.01. The segregation of a QTL was determined by noting the segregation of markers with significant F-probabilities. Putative QTL regions were verified in cross B by single-locus ANOVA. QTLs were considered verified if at least one molecular marker in the designated region significantly affected the trait

phenotype ($P \leq 0.05$). In addition, if the QTL were segregating from the same male parent in each cross, common marker alleles linked to the QTL must have a similar effect on the phenotype. This condition did not have to be met for QTL linked marker alleles segregating from the female parent.

Results

Across all sites, all five traits were normally distributed. Descriptive statistics for each trait in both crosses are presented in Table 1. Deviations from row-plot means were also normally distributed (data not shown). The magnitude of the means and range of variation for all five traits are within the expectations for *E. globulus* (Raymond 2002; Raymond and Schimleck 2002). The estimates of traits values are very similar across the two pedigrees.

The loci that were the most significant for each segregation type and linkage group in the permutation test are shown in boldface in Table 2. Initially 12 genomic regions influencing wood and fibre properties were identified in cross A (Table 2); all QTL regions spanned three or more markers, and most regions had at least one marker with an *F*-probability of 0.005 or less. There were three QTLs for basic wood density, two for pulp yield, three for cellulose content, two for fibre length and two for MFA (Fig. 1). The genomic region on linkage group 1 resolved into two wood-density QTLs, bringing the total number of QTLs identified in cross A to 13. Generally the amounts of phenotypic variation explained by significant markers linked to a QTL are quite small. For the most significant loci on the basis of permutation test (loci in boldface in Table 2), the range in proportion of phenotypic variation explained across the five traits was from 3.2–15.75%, with a mean of 6.75%. However these are probably to some extent biased upwards because of sampling sizes (Beavis 1994) and therefore, estimates of variation explained by individual loci are not presented in Table 2. Clearly these traits are quantitative in nature and consist of a number of genes or genome regions of fairly small effect.

Density

Genomic regions influencing wood density were identified on linkage groups 1, 9 and 11 (Fig. 1; Table 2) in cross A. The region on linkage group 1 segregates in the male parent, with significant marker–trait associations detected from 60 to 108 cM. The level of significance of male segregating marker alleles decreases to a probability of 0.003 at marker Mdh-2 at 76 cM, and then increases slightly, then decreases again to a probability of 0.004 at marker c158 at 102 cM. Two groups of markers approximately 40 cM apart on linkage group 1 in cross B also had a significant effect on wood density, validating this region and supporting the premise of two linked QTLs. Analyses of fully informative markers screened in both crosses (En013 and c453) indicate that the common male parental alleles have similar effects on the phenotype in both crosses (Table 4). Progeny with En013 male allele 1 have lower basic wood density than those progeny with male allele 3. Progeny with c453 male allele 1 also have lower basic wood density. This region on linkage group 1 has been considered as two linked QTLs subsequently in the paper.

The wood-density QTLs on linkage groups 9 and 11 segregated in the cross A female parent. Only markers in QTL region on linkage group 9 were significant in cross B. Analysis of the fully informative marker g107 in this region indicated that different QTL-linked female marker alleles, from the different female parents, had a significant effect on wood density (Table 4).

Pulp yield and cellulose

Pulp-yield QTLs were identified in cross A on linkage groups 4 and 10. All pulp-yield QTLs segregated in the female parent (Fig. 1; Table 2). The QTLs on linkage groups 4 and 10 co-located with QTLs for cellulose content in cross A. Not surprisingly the phenotypic correlation coefficient for these traits is positive (0.64, Table 3). The pulp-yield and cellulose QTL region on linkage group 10 includes the lignin candidate gene marker CCR (cinnamoyl coenzyme A reductase). The linkage group 4 pulp-yield QTL was the only one verified in the second cross. The QTL-linked marker, Eg096, at the

Table 1 Descriptive statistics for each trait for all sites combined. *MFA* Microfibril angle

Pedigree	Statistic	Density (kg/m^c)	Pulp yield (%)	Cellulose (%)	Fibre length (mm)	MFA (degree)
Cross A	Mean	526	52.8	41.6	0.68	17.5
	SD	32	1.4	1.7	0.08	2.7
	Maximum	613	55.9	45.8	0.92	28.0
	Minimum	455	49.5	37.2	0.43	12.3
	Sample	139	142	141	137	129
Cross B	Mean	517	52.3	40.9	0.72	17.3
	SD	41	1.1	1.7	0.09	2.4
	Maximum	636	54.4	44.5	0.90	24.6
	Minimum	442	47.2	37.2	0.46	12.6
	Sample	113	115	115	113	105

Table 2 Summary of quantitative trait loci (QTL) analyses in two full-sib pedigrees of *Eucalyptus globulus*. Loci are listed in order of linkage group and map distance for cross A. For fully informative loci (FI), separate analyses of variance were performed on marker genotype data segregating from the female parent (FS), from the male parent (MS) as well as from both parents (BOTH). For each QTL the most significant loci in the permutation test are shown in *boldface*

Trait	LG[a]	Marker[a]	Cross A (KI-1 × G-139)					Cross B (T-5 × G-139)					Verification
			CM[a]	Seg[b]	*F*-probability			CM[c]	Seg[b]	*F*-probability			
					FS	BOTH	MS			FS	BOTH	MS	
Density	1	g117	60	FI	0.070	0.030	0.038	53	FI	0.888	0.254	0.090	
		g465	68	FI	0.092	0.027	0.055	63	MS			0.037	
		g334	70	MS			0.063	63	MS			0.023	Yes
		En013	74	FI	0.497	0.036	0.006	67	FI	0.751	0.261	0.055	
		Mdh-2	76	MS			0.003	–		–	–	–	
		e301	83	FI	0.848	0.038	0.005	81	HE	0.542	0.738	0.542	
		e331	94	FI	0.434	0.083	0.022	90	MS			0.173	
		e377	100	MS			0.007	93	MS			0.165	
		c158	102	FI	0.522	0.028	0.004	97	FI	0.392	0.093	0.056	
		c453	107	FI	0.897	0.115	0.028	100	FI	0.640	0.098	0.029	Yes
		e345d	108	FI	0.763	0.161	0.083	101	MS			0.022	
Density	9	g430	74	FI	0.038	0.147	0.332	74	FI	0.163	0.178	0.117	
		AGE2	85	FI	0.034	0.123	0.230	76	FI	0.365	0.368	0.299	
		g080 g	96	FS	0.003			84	FS	0.035			Yes
		g174	102	FI	0.007	0.063	0.836	89	FI	0.080	0.008	0.360	
		g107	110	FI	0.031	0.171	0.817	102	FI	0.048	0.071	0.306	
		Embra5	113	FS	0.057	0.057		111	FS	0.097	0.097		
Density	11	Eg128	17	FI	0.050	0.012	0.039	18	FI	0.837	0.714	0.539	No
		g158	23	FS	0.004			–		–	–	–	
		c401	27	FS	0.003			–		–	–	–	
		c176a	43	FI	0.562	0.008	0.039	44	MS			0.223	
Pulp yield	4	e344b	129	FS	0.001			–		–	–	–	
		g342	139	MS			0.754	148	MS			0.747	
		g198	143	FI	0.000	0.001	0.693	–		–	–	–	
		Eg096	145	FI	0.000	0.000	0.752	159	FI	0.011	0.054	0.395	Yes
Pulp yield	10	c456c	0	MS			0.030	7	MS			0.821	
		CCR	6	FS	0.007			0	FS	0.954			No
		Eg023	8	FS	0.019			–		–	–	–	
Cellulose	4	**e344b**	129	FS	0.001			–		–	–	–	
		g342	139	MS			0.197	148	MS			0.969	
		g198	143	FI	0.000	0.000	0.279	–		–	–	–	
		Eg096	145	FI	0.000	0.000	0.519	159	FI	0.600	0.650	0.818	No
Cellulose	10	CCR	6	FS	0.002			0	FS	0.779			No
		Eg023	8	FS	0.003			–		–	–	–	
		g197	17	FI	0.063	0.119	0.144	27	FI	0.980	0.855	0.551	
		c087b	19	FI	0.005	0.010	0.115	–		–	–	–	
Cellulose	11	Eg030	57	FI	0.313	0.139	0.057	64	FI	0.599	0.777	0.844	
		c456a	60	MS			0.006	65	MS			0.732	
		g338/2	69	MS			0.003	71	MS			0.206	No

Table 2 (continued)

Trait	LG[a]	Marker[a]	Cross A (KI-1 × G-139)					Cross B (T-5 × G-139)					Verification
			CM[a]	Seg[b]	*F*-probability			CM[c]	Seg[b]	*F*-probability			
					FS	BOTH	MS			FS	BOTH	MS	
Fibre length	5	Eg067	2	FI	0.055	0.096	0.423	0	FI	0.978	0.997	0.829	
		g466	2	FS	0.033			–		–	–	–	
		Eg089	6	FI	0.002	0.003	0.884	5	HE	0.951	0.997	0.951	
		g425	16	FI	0.003	0.018	0.788	–		–	–	–	
		c378	19	FS	0.003			–		–	–	–	
		g010	21	FI	0.003	0.026	0.367	22	FI	0.456	0.530	0.186	No
		Eg015a	26	FI	0.004	0.022	0.397	27	MS			0.139	
Fibre length	8	g041	0	FI	0.003	0.010	0.764	0	HE	0.043	0.236	0.043	
		e345a	2	FS	0.001			–		–	–	–	
		ECS1	3	MS			0.424	2	FI	0.336	0.621	0.361	No
		g338/1	5	FI	0.046	0.242	0.657	5	FI	0.069	0.169	0.129	
Micro-fibril angle	7	g069	0	FI	0.017	0.127	0.886	0	FI	0.021	0.110	0.900	
		g133	2	FS	0.019			2	FS	0.023			Yes
		En006	4	FI	0.004	0.034	0.581	12	FI	0.097	0.381	0.705	
		Eg084	6	FI	0.007	0.062	0.823	14	FI	0.196	0.303	0.477	
Micro-fibril angle	8	c116	40	MS			0.026	–		–	–	–	
		g350	58	FI	0.191	0.002	0.001	–		–	–	–	
		g402c	65	MS			0.006	66	MS			0.720	No
		g472	80	MS			0.044	78	FI	0.172	0.543	0.991	

[a]Linkage groups (*LG*), marker names and cross A map distances in Kosambi centiMorgans as described in Thamarus et al. (2002)
[b]Marker segregation type: *FS* two alleles segregating in the female parent only, *MS* two alleles segregating in the male parent only, *FI* three or four alleles segregating in both parents, *HE* two alleles segregating in both parents
[c]Cross B map distance in Kosambi centiMorgans from separate JOINMAP 2.0 analysis (Stam and van Ooijen 1995)

end of linkage group 4, explains 11.8% of the phenotypic variation in pulp yield (Table 2) and of the verified QTL appears to have the largest effect. In cross A, microsatellite Eg096 female allele 3 increases pulp yield (0.34%), while in cross B, the same microsatellite allele 3 decreases pulp yield (−0.16%, Table 4). It is probable that in the two female parents analysed, marker Eg096 is linked to different QTL alleles.

Two female and one male segregating QTL regions for cellulose content were identified in cross A (Fig. 1). As stated above, the two female QTLs were on linkage groups 4 and 10 and co-located with pulp-yield QTL regions. The male segregating QTL region for cellulose content was on linkage group 11. None of the markers for these QTLs was significant in cross B (Table 2). The male segregating marker (g338/2) on linkage group 11 showed similar phenotypic effects with common alleles linked to the QTL in both crosses (data not shown); however, the trend was not significant (P=0.206) in cross B.

Fibre length and MFA

Two female segregating fibre-length QTLs were identified in cross A on linkage groups 5 and 8 (Fig. 1; Table 2). Of the cross A female segregating QTLs, the region on linkage group 5 showed no trend toward validation in cross B. The QTL on linkage group 8, which co-locates with ECS1 (eucalypt cellulose synthase homolog 1), showed a trend towards validation in the second pedigree when marker g338/1 was analysed (P=0.069, Table 2). However, analysis of adjacent marker ECS1 showed no significant effect of female segregating alleles on fibre length in cross B (P=0.336).

Table 3 Phenotypic correlations of wood and fibre traits; all data combined with cross A and cross B data in *parentheses* (A, B)

	Density	Pulp yield	Cellulose	Fibre length
Pulp yield	0.103 (0.139, 0.027)			
Cellulose	−0.270 (−0.171, −0.445)	0.647 (0.614, 0.660)		
Fibre length	−0.306 (−0.145, −0.407)	0.154 (0.162, 0.335)	0.398 (0.421, 0.580)	
Microfibril angle	−0.108 (−0.173, −0.060)	−0.486 (−0.572, −0.402)	−0.292 (−0.349, −0.257)	−0.292 (−0.280, −0.303)

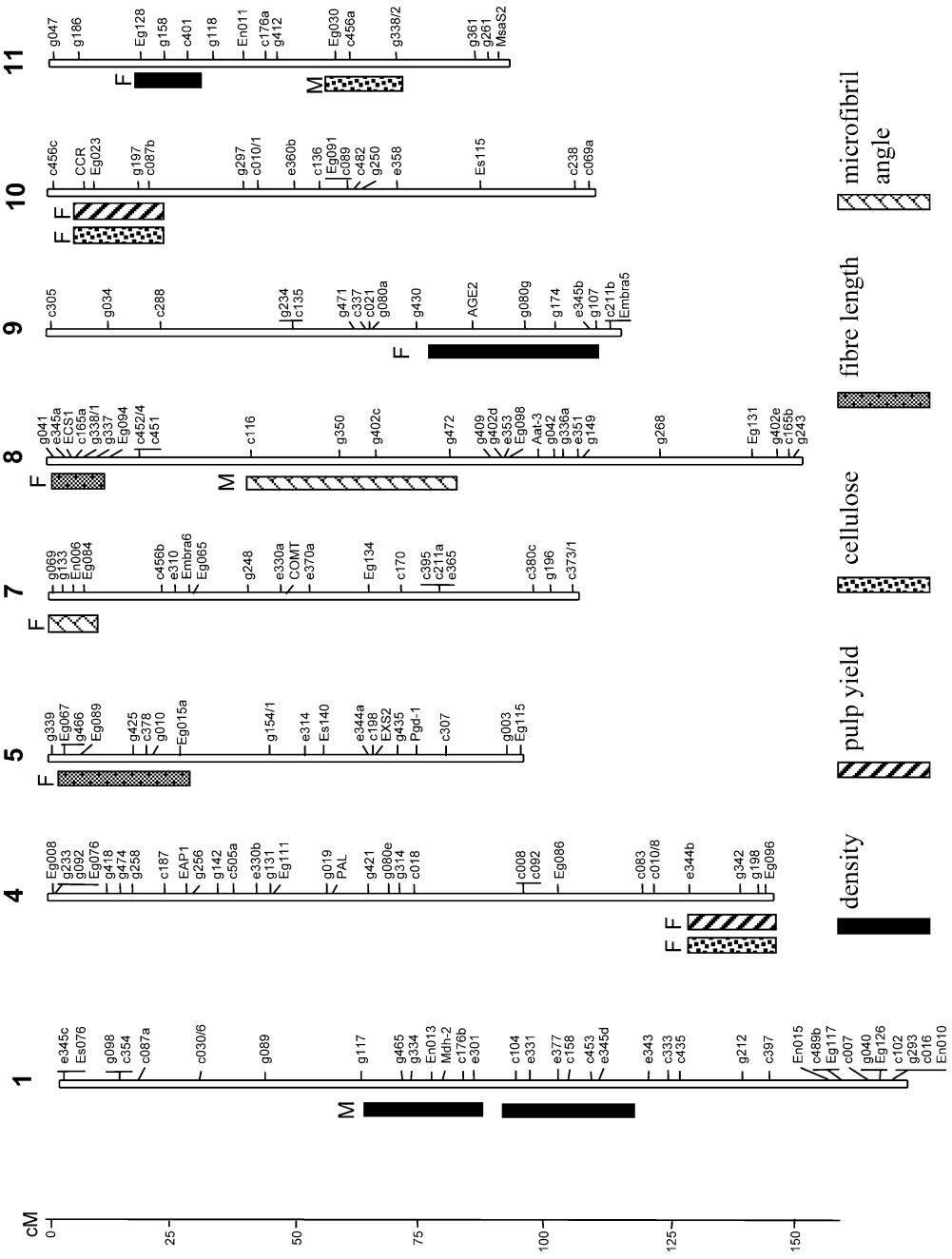

Fig. 1 Location of quantitative trait loci (QTL) for wood and fibre properties identified in *Eucalyptus globulus* cross A (KI-1 × G139). Linkage groups and map loci are as described in Thamarus et al. (2002). QTL are indicated by *bars* adjacent to the linkage group region where a locus had a significant threshold by permutation test. Type of segregation (*M* male, *F* female) for each *QTL* is indicated *adjacent to the bar*

Two MFA QTL regions were identified on linkage groups 7 and 8 in cross A (Fig. 1). The QTL on linkage group 7 segregated on the female side, and the QTL on 8 segregated on the male side. The male segregating QTL region was not verified in cross B (Table 2). Common male alleles of markers screened in both crosses showed opposite effects on the phenotype in the different genetic backgrounds (data not shown). The female segregating MFA QTL was verified in cross B (Table 2). Analysis of the fully informative marker g069 on linkage group 7 showed female allele 4 in cross A and female allele 5 in cross B had a similar effect on MFA (Table 4). Both female marker alleles may be linked to the same QTL allele or to different QTL alleles with similar effect in the different genetic backgrounds.

Discussion

QTLs for wood-fibre traits were identified using two related full-sib families of *E. globulus*. Five QTLs were verified in both pedigrees, with two being verified in the common male parent and three QTL independently segregating in the different female parents from both families. Only the two linked wood-density QTLs on linkage group 1 were verified in cross B. Paternal alleles linked to the MFA region on linkage group 8 and cellulose

Table 4 Crosses A and B allele and genotype means expressed as deviations from row-plot means, for specific markers in each validating QTL region. For each marker, alleles were identified as segregating molecular fragments of different length and assigned a number. If fragments of the same size were identified in each cross, then the number assigned in cross A was retained in cross B. Parental alleles were identified by the letters *f* (female) and *m* (male). Markers were numbered independently

Trait	LG	Marker	Cross	Parental genotypes		Progeny sample size	Progeny genotypes (genotype means)			Alleles (allele means)		
				Female	Male							
Density	1	En013	A	23	13	138	12 (−3.6)	23 (4.8)	13 (−8.7)	33 (4.6)	2f (0.8)	3f (−1.8)	1m (−5.5)	3m (4.7)
			B	45	13	110	14 (−5.2)	34 (4.8)	15 (−1.9)	35 (3.9)	4f (−0.8)	5f (0.5)	1m (−3.7)	3m (4.4)
		c453	A	12	13	139	11 (−2.3)	13 (2.0)	12 (−6.1)	23 (6.0)	1f (−0.2)	2f (0.2)	1m (−4.0)	3m (4.0)
			B	24	13	109	12 (−7.5)	23 (8.1)	14 (−1.1)	34 (4.9)	2f (−1.0)	4f (1.0)	1m (−3.3)	3m (6.2)
	9	g107	A	13	12	139	11 (3.0)	12 (4.5)	13 (−2.7)	23 (−5.6)	1f (3.7)	3f (−4.2)	1m (0.4)	2m (−0.4)
			B	14	12	110	11 (8.5)	12 (−0.8)	14 (−7.1)	24 (−3.4)	1f (3.6)	4f (−4.9)	1m (2.4)	2m (−2)
Pulp yield	4	Eg096	A	23	12	142	12 (−0.40)	22 (−0.26)	13 (0.34)	23 (0.35)	2f (−0.32)	3f (0.34)	1m (−0.03)	2m (0.02)
			B	13	12	115	11 (0.17)	12 (0.10)	13 (−0.08)	23 (−0.26)	1f (0.14)	3f (−0.16)	1m (0.05)	2m (−0.05)
Micro-fibril angle	7	g069	A	24	13	129	12 (0.42)	23 (0.33)	14 (−0.49)	34 (−0.51)	2f (0.37)	4f (−0.50)	1m (0.03)	3m (−0.03)
			B	35	13	96	13 (0.28)	33 (0.59)	15 (−0.29)	35 (−0.60)	3f (0.43)	5f (−0.41)	1m (−0.03)	3m (−0.08)

on linkage group 11 showed no consistent effect on the phenotype in cross B when compared to allelic effect in cross A. Reasons the segregating QTLs in the second family with the same male parent were not verified could include that the hypothesised QTL was not really present in cross A or present but not detectable with available sample sizes or loci segregating in cross B. In addition the effect of different female genetic background (different female parent) could have affected QTL allele expression. Six female segregating QTLs detected in cross A were not detected in the second cross. In the second pedigree, molecular markers may be linked to QTL alleles that simply do not segregate in the different female parent.

In eucalypts QTL have been characterised for only a few wood-fibre traits such as wood density (Grattapaglia et al. 1996; Verhaegen et al. 1997). In this study, four basic wood-density QTLs were identified on three linkage groups in cross A. The QTL for wood density on linkage group 9 in *E. globulus* could be the same as that reported on linkage group 5 in *E. grandis* and linkage group 8 in RAPD map of *E. urophylla/E. grandis* (Verhaegen et al 1997; Gion et al. 2001) since the linkage groups appear homologous on the basis of a common microsatellite marker Embra5 (Thamarus et al. 2002; Brondani et al. 1998). Determination of whether QTL positions for wood-fibre traits in eucalypts are common across species will require establishment of syntenic relationships between species of genomes and maps from different labs based on genes and microsatellite makers.

Genes in biosynthetic pathways involved in secondary wall formation could influence a number of traits, especially composite ones such as wood density and pulp yield. Collocation of QTLs for different traits might be indicative of such a process. Collocation of pulp-yield and cellulose QTLs occurred on linkage groups 4 and 10 in cross A. These collocations provide a possible genetic explanation for correlation between traits (Patterson et al. 1988). The QTL for pulp yield verified on linkage group 4 while the QTL for cellulose did not. These results suggest that the underlying genes are not acting pleiotropically on these traits. For the co-locating pulp-yield/cellulose region on linkage group 10, neither female QTL was verified in the second pedigree; consequently it remains possible that a single genetic locus is affecting both of these quantitative traits.

The CCR gene locus maps to the region of co-locating pulp yield and cellulose QTLs on linkage group 10. CCR is involved in lignin monomer biosynthesis and was mapped in *E. globulus* as a putative candidate gene for wood quality traits (Thamarus et al. 2002). The ECS1 gene locus maps to the same region as a fibre-length QTL on linkage group 8. ECS1 is a eucalypt homolog of cellulose synthase (Thamarus et al. 2002), a key enzyme in deposition of cellulose microfibrils in plant cell walls. Other gene markers co-locating with QTLs include hydroxymethytransferase and wood-density QTL on linkage group 1 and a *p*-glycoprotein and pulp-yield/cellulose QTLs on linkage group 4. The association of candidate gene loci with QTLs is suggestive, not indicative, of

functional genetic loci contributing to quantitative trait variation. Even tight marker–QTL linkage represents large genomic regions. One centiMorgan in the *E. globulus* map represents approximately 380 kbp of DNA and may contain many functional gene loci (Thamarus et al. 2002). Validation of candidate gene loci as actual genes affecting quantitative trait variation will require assessment of the specific SNP–trait associations in appropriate populations.

QTLs for wood-quality traits have been identified in a number of forest tree species such as eucalypts and pines. On average several QTLs have been demonstrated for each wood-fibre trait such as wood density, MFA and chemical wood properties, and most appear to have a small effect on the trait. In *E. grandis* five wood specific gravity-trait QTLs were identified on four linkage groups, with each QTL explaining between 3.4% and 10.2% of the phenotypic variation (Grattapaglia et al. 1996). Verhaegen et al. (1997) identified seven genomic regions in *E. grandis*/*urophylla* pedigree associated with wood density. Similarly eight and nine QTLs for wood density with small effects have been reported in radiata pine (Devey et al 2004) and loblolly pine, respectively (Sewell et al 2000). Generally wood traits within species are under control of many QTLs/genes of reasonably small effect. With the limited samples sizes available in the *E. globulus* pedigrees, only a small number of significant QTLs were characterised, with most accounting for a small proportion of the variation in the trait. The substantial genetic component for wood-fibre traits in eucalypts (Raymond 2002) has enabled a number of QTLs for various wood-fibre traits to be characterised despite the limited sample sizes available in the full-sib families. However a substantial challenge that remains will be the characterisation of other QTLs of similar effect in the species from additional individuals and pedigrees. Results from this study suggest that QTLs in a number of individuals will need to be characterised. With several important commercial eucalypt species, it is of interest whether the QTLs are generic to the genus. Currently work is in progress to test the commonality of these QTLs in a sister species, *E. nitens*.

This study reports a number of QTLs for wood-fibre traits in *E. globulus*, and a number of these are verified in a second pedigree. Due to a high degree of linkage equilibrium in natural populations of tree species, the probability of detecting similar marker–trait associations in different genetic backgrounds is low (Strauss et al. 1992). This linkage equilibrium coupled with the limited resolution of QTL on tree maps makes it very difficult to use QTL–marker associations broadly in breeding populations. Within-family selection based on marker–trait associations will still be feasible, but deployment in plantations for *E. globulus* will be very inefficient, since large-scale clonal plantations are not currently practicable. Nevertheless the QTL information will form a baseline on the eucalypt genomic map to provide a means of collocating genes to these regions. This collocation, along with other criteria including microarray gene discovery and functional analysis, will determine candidate genes for wood-fibre traits. These candidate genes will provide the starting point to test for associations between variation at the allele level and variation in wood-fibre traits. Ultimately such associations could lead to more efficient and more broadly applicable early selection procedures for key fibre traits in eucalypt breeding populations.

Acknowledgements The authors wish to thank the CRC for Sustainable Production Forestry for financial support of this project. We also thank Rob Evans for Silviscan-2 analyses of wood samples, Kirsty Surridge and Laura Nagy for technical assistance with wood analyses, Mike Devey for comments on the manuscript and Colin Matheson provided statistical advice.

References

Beavis WD (1994) The power and deceit of QTL experiments: lessons from comparative QTl studies. In: Proceedings of 49th Annual Corn and Sorghum Industrial Research Conference. American Seed Trade Association, Washington, pp 304–312

Brondani RPV, Brondani C, Tarchini R, Grattapaglia D (1998) Development, characterization and mapping of microsatellite markers in *Eucalyptus grandis* and *E. urophylla*. Theor Appl Genet 97:816–827

Byrne M, Moran GF, Tibbits WN (1993) Restriction map and maternal inheritance of chloroplast DNA in *Eucalyptus nitens*. J Hered 84:218–220

Byrne M, Murrell JC, Owen JV, Kriedemann P, Williams ER, Moran GF (1997) Identification and mode of action of quantitative trait loci affecting seedling height and leaf area in *Eucalyptus nitens*. Theor Appl Genet 94:674–681

Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative mapping. Genetics 138: 963–971

Devey ME, Carson SD, Nolan MF, Matheson AC, Te Riini C, Hohepu J (2004) QTL associations for density and diameter in *Pinus radiata* and the potential for marker-aided selection. Theor Appl Genet 108:516–524

Donaldson LA (1993) Variation in microfibril angle among three genetic groups of *Pinus radiata* trees. NZ J For Sci 23:90–100

Eldridge K, Davidson J, Harwood C, Van Wyk G (1994) Eucalypt domestication and breeding. Oxford University Press, Oxford

Evans R, Hughes M, Menz D (1999) Microfibril angle variation by scanning X-ray diffractometry. Appita J 52:363

Evans R, Stringer S, Kibblewhite RP (2000) Variation of microfibril angle, density and fibre orientation in twenty-nine *Eucalyptus nitens* trees. Appita J 53:450–457

Gion JM, Boudet C, Grima-Pettenati J, Pichavant FH, Plomion C, Bailères H, Verhaegen D (2001) A candidate gene approach identifies *CCR*, *PAL* and *C4H* as loci for syringyl/guaiacyl ratio in a interspecific hybrid between *E. urophylla* and *E. grandis*. In: Barros S (ed) Developing the eucalypt of the future. Proceedings of IUFRO international symposium, 10–15 September 2001, Valdivia

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

Greaves BL, Borralho NMG, Raymond CA (1997) Breeding objective for plantation eucalypts grown for production of kraft pulp. For Sci 43:465–472

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

Kumar S, Carson SD, Garrick DJ (2000) Detecting linkage between a fully informative marker locus and a trait locus in outbred populations using analysis of variance. For Genet 7:47–56

Muneri A, Raymond CA (2001) Nondestructive sampling of *Eucalyptus globulus* and *E. nitens* for wood properties. II. Fibre length and coarseness. Wood Sci Technol 35:41–56

Ott L (1984) An introduction to statistical methods and data analysis. PWS-Kent, Boston

Patterson AH, Lander ES, Hewitt JD, Peterson S, Lincoln SE, Tanksley SD (1988) Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature 355:721–726

Payne RW, Lane PW, Ainsley AE, Bicknell KE, Digby PGN, Harding SA, Leech PK, Simpson HR, Todd AD, Verrier PJ, White RP (1987) Genstat 5 reference manual. Oxford University Press, Oxford

Raymond CA (2002) Genetics of *Eucalyptus* wood properties. Ann For Sci 59:525–531

Raymond CA, Schimleck LR (2002) Development of near-infrared reflectance analysis calibrations for estimating genetic parameters for cellulose content in *Eucalyptus globulus*. Can J For Res 32:170–176

Schimleck LR, Raymond CA, Beadle CL, Downes GM, Kube PD, French J (2000) Applications of NIR spectroscopy to forestry research. Appita J 53:458–464

Sewell MM, Bassoni DL, Megrew 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

Stam P, van Ooijen JW (1995) JOINMAP, version 2.0: software for the calculation of genetic linkage maps. CPRO-DLO, Wageningen

Strauss SH, Lande R, Namkoong G (1992) Limitations of molecular marker-aided selection in forest tree breeding. Can J For Res 22:1050–1061

Thamarus KA, Groom K, Murrell J, Byrne M, Moran GF (2002) A genetic linkage map for *Eucalyptus globulus* with candidate loci for wood, fibre and floral traits. Theor Appl Genet 104:379–387

Turnbull JW, Pryor LD (1988) Choice of species and seed sources. In: Hillis WE, Brown AG (eds) Eucalypts for wood production. CSIRO, Collingwood, Victoria, pp 6–65

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

Volker PW (1995) Evaluation of *Eucalyptus nitens* × *globulus* for commercial forestry. In: Potts BM, Borralho NMG, Reid JB, Cromer RN, Tibbits WN, Raymond CA (eds) Eucalypt plantations: improving fibre yield and quality. Proceedings of CRC-IUFRO Conference, Hobart, 19–24 February 1995, pp 222–225