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QTL influencing growth and wood properties in *Eucalyptus globulus*

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Abstract Regions of the genome affecting physical and chemical wood properties (quantitative trait loci (QTL)), as well as growth, were identified using a clonally replicated, outbred F_2 family (112 genotypes, each with two ramets) of *Eucalyptus globulus*, planted in a field trial in north-west Tasmania. Traits studied were growth (assessed by stem diameter), wood density, cellulose content, pulp yield and lignin content. These traits are important in breeding for pulpwood, and will be important in breeding for carbon sequestration and biofuel production. Between one and four QTL were located for each trait, with each QTL explaining between 4% and 12% of the phenotypic variation. Several QTL for chemical wood properties were co-located, consistent with their high phenotypic correlations, and may reflect pleiotropic effects of the same genes. In contrast, QTL for density and lignin content with overlapping confidence intervals were considered to be due to independent genes, since the QTL effects were inherited from different parents. The inclusion of fully informative microsatellites on the linkage map allowed the determination of homology at the linkage group level between QTL and candidate genes in different pedigrees of *E. globulus* and different eucalypt species. None of the candidate genes mapped in comparable studies co-located with our major QTL for wood chemical properties, arguing that there are important candidate genes yet to be discovered.

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Introduction

Eucalyptus globulus is widely planted in temperate regions of the world for pulpwood production (Doughty 2000; Potts et al. 2004). Favourable characteristics for kraft pulping (Cotterill et al. 1999), in combination with a broad adaptability to different site conditions (Hillis and Brown 1988), have led to the establishment of plantations and many breeding programmes for the species throughout the world (Potts et al. 2004). Breeding programmes for pulp production in Australia currently have the objectives of increasing volume per hectare, basic density and pulp yield (McRae et al. 2004). However, numerous other physical and chemical wood properties affect the efficiency of kraft pulping, by influencing the cost or environmental impact of the process (Poke et al. 2006). For instance, kraft pulping usually involves removing the majority of the extractives and lignin from the cellulose fibres, hence lignin content and quality directly affects the efficiency of pulp production (Smook 1992). Other wood property traits are also important as they can be used to indirectly measure objective traits (Apiolaza et al. 2005). For example, chemical methods for assessing pulp yield are slow and expensive, limiting the number of samples that can be processed. Hence, indirect measures such as the highly correlated cellulose content can be used as an indicator of pulp yield (Raymond and Schimleck 2002). The pulpwood breeding objectives for *E. globulus* are strongly correlated with objectives that maximise profit from increased carbon assimilation for trading of sequestered carbon (Whittock et al. 2007) and are also expected to be highly

correlated with the objectives for biofuel production (Núñez-Regueira et al. 2002).

A sound knowledge of the physiology, biochemistry and genetics underlying wood formation is necessary to support the objectives of breeding programmes (Shepherd et al. 2003). Traditional quantitative genetic analysis has made significant progress toward understanding the genetic control of growth and wood property traits in *E. globulus*, demonstrating substantial variation both within and between provenances and low to moderate heritability (MacDonald et al. 1997; Dutkowski and Potts 1999; Raymond 2002; Miranda and Pereira 2002; Raymond and Apiolaza 2004; Apiolaza et al. 2005; Poke et al. 2006). The vast amount of genetic variation evident in natural populations provides ample scope to improve wood properties for a pulpwood breeding objective.

The analysis of quantitative trait loci (QTL) has the potential to further our understanding of the genetic control of traits relating to wood properties and in the future may lead to more efficient selection by helping to identify the specific genomic regions, and ultimately genes, underlying quantitative traits (Sewell and Neale 2000). Despite initial high expectations, the direct use of QTL for selection has not been realised in forest trees, due to factors including the broad confidence intervals associated with QTL, and large amounts of linkage equilibrium characteristic of forest trees (Strauss et al. 1992; Wu 2002). However, these factors can be overcome by selecting for the specific genes underlying quantitative traits, rather than QTL regions. Hence, several recent molecular genetic studies in *Eucalyptus* have focused on gene discovery, by methods such as monitoring transcription levels and functional analysis (e.g. Kirst et al. 2005; Foucart et al. 2006), as well as association studies (e.g. Thumma et al. 2005). An important issue in association studies is the selection of appropriate candidate genes. The co-location of QTL and candidate genes, particularly across multiple pedigrees, can be useful to reduce the many potential candidate genes for association studies (Myburg et al. 2007; Grattapaglia and Kirst 2008).

Despite the economic importance of wood properties, few studies have published QTL for such traits in *Eucalyptus*, limiting the scope for comparison between studies. Most of these studies have considered physical traits, such as density and microfibril angle (e.g. Grattapaglia et al. 1996; Verhaegen et al. 1997; Thamarus et al. 2004; Bundock et al. 2008), while fewer studies have published QTL for chemical wood property traits, such as cellulose and lignin content (e.g. Thamarus et al. 2004; Rocha et al. 2007). Hence, the aims of this study were to locate QTL for chemical and physical wood property traits as well as growth traits in *E. globulus* and to study the co-location of QTL with previously mapped QTL and candidate genes.

Materials and methods

Mapping pedigree

Linkage mapping and QTL analysis were conducted in an outcross F_2 family, with grandparents originating from King Island (KI) in Bass Strait and Taranna (T) in the south-east of Tasmania (T7/KI157//T144/KI5). One hundred and twelve genotypes were used for this study, each of which was replicated clonally (two trees per genotype). The F_2 family was planted in a field trial at Woolnorth in north-west Tasmania in May 1998. The two clones representing each genotype were assigned to separate replicates at random (see Milgate et al. 2005 for full description of the trial design).

Phenotypic measurements

Diameter (cm) at breast height (DBH) of the main stem, was assessed at age 2 (DBH2) and 6 (DBH6) years. For the assessment of wood property traits, two cores 12 mm in diameter were taken from each tree in November 2005 (7 years of age). The two cores were taken from bark-to-bark through the centre of each tree, 10–15 cm apart at a height of ~110 cm and in a north-south direction. One core from each tree was used to determine basic density by the water displacement method (TAPPI 1989). The second core from each tree was used to indirectly assess the content of cellulose, lignin and kraft pulp yield by near infrared spectroscopy (NIR) using a Bruker spectrometer (model Vector 22-N). A sub-set of these cores were also analysed using wet chemistry, in order to validate the NIR models for each trait (excluding kraft pulp yield) using the methodology described in Poke and Raymond 2006 and Downes et al. (2007). Each core was air dried, then ground to wood meal (as described by Poke et al. 2005). Both klason and total lignin were predicted by a global model (220307), developed using *E. globulus* and *Eucalyptus nitens*. Predicted and laboratory values for the validation set (a sub-set of 12 individuals from the mapping population) for both klason and total lignin were highly correlated $R^2=0.74$ and 0.88, respectively. Cellulose was predicted by a global model (1-8derVN.q2), developed using 722 samples from 17 sites, mostly from *E. globulus* but also including *E. nitens* and *Eucalyptus urophylla*. Predicted and laboratory values for the validation set (25 individuals) were again highly correlated $R^2=0.83$. Kraft pulp yield (PPY) was estimated using a large global NIR model (20061214.q2) developed using over 700 samples, including more than 40 eucalypt species from sites around Australia (Downes et al. 2007). The calibration model used to make predictions of PPY had an $R^2=0.92$ and a relative standard error of prediction of 1.22% (Downes et al. 2007). Validation of the model to predict PPY in an independent sample (20 individuals of *E. globulus*) from a nearby trial demonstrated

predicted and laboratory values were highly correlated $R^2 =$ 0.82 (Downes et al. 2007).

Statistical analysis

Estimation of variance components For each trait in this study, the coefficient of phenotypic variation was calculated by dividing the family standard deviation by the family mean. Using PROC MIXED of SAS (version 9.1), the significance of the genetic variation between genotypes was tested, by fitting to the individual tree data a mixed model:

$$y = \text{replicate} + \text{iblk(replicate)} + \text{genotype} + \text{residual}$$

where *y* is the individual tree values for a trait, replicate is the fixed replicate effect, iblk (replicate) is the random incomplete block effect nested within replicate, genotype is the random effect of each genotype and residual is random residual variation. Clonal repeatability was calculated as the ratio of the genotype variance component to the total phenotypic variation, calculated as the sum of genotype, incomplete block and error variance components (Falconer and Mackay 1996) using ASREML, which uses an average information REML procedure (Gilmour et al. 2006). The heritability of clonal means was calculated (using ASREML) following Russell and Libby (1986), fitting just the genotype term.

QTL analysis Genotype means were used for QTL analysis; hence some of the environmental effects were removed, and the power of QTL detection was enhanced. The distribution of genotype means were examined for each trait (by the Kolmogorov–Smirnov test using PROC UNIVARIATE of SAS), in order to test the assumption of a normal distribution for QTL mapping. QTL analysis was conducted with MAPQTL 4.0 (Van Ooijen et al. 2002), using the consensus linkage map constructed by Freeman et al. (2006), comprised of 165 makers, of which 33 were SSR and 132 were AFLP. Putative QTL were declared at two different levels of significance; the genome-wide level (genome-wide type I error <0.05), and the chromosome-wide level (chromosome-wide type I error rate <0.05). The LOD threshold for genome-wide significance was determined by permutation testing (1,000 replications; Churchill and Doerge 1994). An average LOD threshold (LOD 3) for chromosome-wide significance was determined by empirical simulations (Van Ooijen 1999). Significant QTL at the chromosome-wide level were presented in order to facilitate comparative QTL mapping, as suggested by Van Ooijen (1999), since this significance level will decrease the type II error rate relative to the genome-wide level. Comparative mapping will be particularly useful in a genus such as

Eucalyptus, due to its outbred nature and the fact that QTL mapping is still at a relatively early stage. For each QTL, the parent from which the QTL effect segregated was determined by testing whether adjacent AFLP markers inherited solely from the male or female parent had a significant effect on the trait in question using the Kruskal–Wallis single marker test in MAPQTL 4.0 (Van Ooijen et al. 2002).

Interval mapping was performed initially, using the default parameters of MAPQTL 4.0 (Van Ooijen et al. 2002). Map intervals exceeding the chromosome-wide threshold from interval mapping were subsequently selected as cofactors for the multiple QTL model (MQM) mapping procedure. MQM mapping was performed using an iterative approach and forward selection of cofactors, until a stable set of cofactors was found (Van Ooijen et al. 2002; see Freeman et al. (2008) for more details on the QTL analysis). In order to calculate confidence intervals for each QTL using MQM mapping, the QTL in question was excluded from the list of cofactors (Van Ooijen pers. comm.). The proportion of the total phenotypic variance explained by each QTL was estimated following Bradshaw and Stettler (1995), by multiplying the total genotypic variance explained by the heritability of clonal means for each trait. Where more than one unlinked QTL were discovered for a trait, the closest marker to each was tested for epistasis (i.e. a significant interaction effect) using PROC MIXED of SAS (Freeman et al. 2008).

Results

Analysis of variation in quantitative traits

All wood property traits exhibited highly significant (*P*< 0.001) variation between genotype means, while growth traits exhibited less significant variation, consistent with their lower clonal repeatabilities (Table 1). The chemical wood property traits were highly inter-correlated, as were the two growth measurements (Table 2). The genotype means for cellulose, total and klason lignin and pulp yield exhibited a moderate departure from normality (Kolmogorov–Smirnov ~*P*=0.01); however standard transformations (log, x^2, √) failed to significantly improve normality and therefore the traits were analysed without transformation. The genotype means for all other traits approximated a normal distribution (Kolmogorov–Smirnov *P*>0.05).

QTL for growth and wood properties

Thirteen QTL in total were identified for wood properties and growth traits, at the chromosome-wide significance

Table 1 The range of genotype means, family mean, standard deviation (SD) and coefficient of phenotypic variation (C.V.), the Z value for the between genotype variance component and its significance from zero (P), as well as clonal repeatability (H^2) and its standard error (SE), for each growth and wood property trait analysed in this study

Trait	Min.	Max.	Mean	SD	C.V. (%)	Z	P	H^2	SE
Density (kg/m³)	418.8	551.6	476.7	26.18	5.5	4.55	<0.001	0.50	0.07
Cellulose (%)	41.1	48.0	43.5	0.99	2.3	4.19	<0.001	0.44	0.08
Total lignin (%)	24.5	28.8	27.3	0.68	2.5	4.04	<0.001	0.56	0.07
Klason lignin (%)	18.0	22.2	21.0	0.68	3.2	3.74	<0.001	0.52	0.07
PPY (%)	51.3	58.7	54.1	1.21	2.2	4.62	<0.001	0.50	0.07
DBH2 (cm)	2.9	8.1	5.4	0.93	17.2	1.85	0.033	0.16	0.09
DBH6 (cm)	7.0	17.6	12.6	1.76	14.0	1.79	0.036	0.15	0.09

level (type I error rate <0.05; Table 3). Two regions of the genome affected multiple traits, hence the QTL mapped to only nine discrete regions (defined as having QTL peaks separated by more than 5 cM) on six linkage groups (Table 3). Specifically, reflecting the strong correlations between the chemical wood property traits, a genomic region (spanning 4.1 cM) on linkage group 5 was significantly associated with cellulose, klason and total lignin content as well as pulp yield, with the QTL effects at each locus inherited from the female parent (Table 3). A QTL region on linkage group 10 was associated with both total lignin and wood density, although in this case the QTL effects for each trait segregated from different parents (Table 3) and the traits were not correlated (Table 2).

Wood density was associated with three QTL in total, located on linkage groups 1, 6 and 10, one of which was highly significant at the genome-wide level, and two were significant at the chromosome-wide level (Table 3). In combination, these QTL explained an estimated 38.8% of the genotypic variation (the variation between genotype means) and 26% of the phenotypic variation for wood density (Table 3). Cellulose was associated with four QTL in total, on linkage groups 3, 5, 6 and 9. Three of these were significant at the genome-wide level, while one was significant at the chromosome-wide level. In combination, these QTL explained an estimated 57.3% of the genotypic variation and 34.9% of the phenotypic variation for cellulose content (Table 3). Two QTL were identified for total lignin content, one of which was significant at the genome-wide level and the other at the chromosome-wide level, located on linkage groups 5 and 10, respectively (Table 3). In combination these QTL explained 28.4% of

the genotypic variation and 20.2% of the phenotypic variation for total lignin content (Table 3). Klason lignin was affected by a single QTL, which mapped to exactly the same location as the QTL for total lignin on linkage group 5, but with a slightly lower LOD value (Table 3). A single QTL for pulp yield with genome-wide significance was located on linkage group 5, explaining 18.5% of the genotypic variation, and 12.2% of the phenotypic variation for this trait (Table 3). Two separate QTL were identified for growth, one at age 2 years (DBH2) and one at age 6 years (DBH6; Table 3). These QTL were both significant at the chromosome-wide level and were located on linkage groups 5 and 10, respectively (Fig. 1). The pair-wise interactions between all combinations of QTL within each trait were non-significant, suggesting that there was no interaction between them.

Discussion

Analysis of variation in quantitative traits

The genetic variation within the mapping pedigree includes the segregation of variance due to differences between King Island and Taranna provenances of *E. globulus*, from which the grandparents of the mapping pedigree originate, and differences within these provenances. Taranna and King Island provenances of *E. globulus* are known to differ significantly in wood density and growth rate (Volker et al. 2008). Accordingly, these traits and all others considered in this study exhibited significant genetic variation. The higher clonal repeatabilities of wood property traits ($H^2 =$

Table 2 Matrix of Pearson correlation coefficients between the genotype means for the traits analysed in this study

	Density	Cellulose	Total lignin	Klason lignin	PPY	DBH2
Cellulose	0.19*					
Total lignin	−0.04	−0.72***				
Klason lignin	−0.04	−0.71***	0.90***			
PPY	0.16	0.83***	−0.87***	−0.81***		
DBH2	−0.10	−0.10	0.20	0.24*	−0.10	
DBH6	−0.08	−0.06	0.23*	0.27**	−0.10	0.84***

*P<0.05, **P<0.01, ***P<0.001

Table 3 Genotype and overall trait means for markers adjacent to the significant QTL, identified by MQM mapping in *Eucalyptus globulus*

Trait	LG	Map position cM	Adjacent marker	LOD[a]	% exp gen[b]	% exp phen[c]	Parental genotypes ♀	♂	Seg.	Genotype means			
Density (kg/m³)	1	74.6	p06.b04	3.01	10.8	7.2	nn	np	M	nn (471)	np (486)		
	6	44.2	p18.b06	4.07	12.5	8.4	lm	ll	F	lm (484)	ll (470)		
	10	28.0	p11.b02	4.96**	15.5	10.4	nn	np	M	nn (469)	np (486)		
Cellulose (%)	3	38.4	p01.b01	3.47	18.7	11.4	nn	np	M	nn (43.4)	np (43.6)		
	5	41.8	p11.b04	5.35***	9.2	5.6	lm	ll	F	lm (43.8)	ll (43.3)		
	6	15.2	p12.b06	4.89**	20.2	12.3	nn	np	F	nn (43.4)	np (43.7)		
	9	0.0	Emb204	4.40*	9.2	5.6	ef	eg	F	ef (44.0)	ee (43.2)	eg (43.3)	fg (43.7)
Total lignin (%)	5	40.5	p06.b11	4.30*	15.0	10.7	nn	np	F	nn (27.2)	np (27.5)		
	5[d]	41.8	p11.b04				lm	ll	F	lm (27.2)	ll (27.5)		
	10	30.0	Emb127	3.89	13.4	9.5	ab	cd	F	ac (27.7)	ad (27.5)	bc (27.1)	bd (27.1)
Klason lignin (%)	5	40.5	P06.b11	4.10*	16.4	11.3	nn	np	F	nn (20.8)	np (21.1)		
	5[d]	41.8	p11.b04				lm	ll	F	lm (20.8)	ll (21.1)		
PPY (%)	5	44.6	P21.b02	4.58*	18.5	12.2	lm	ll	F	lm (54.6)	ll (53.7)		
	5[d]	41.8	p11.b04				lm	ll	F	lm (54.5)	ll (53.8)		
DBH2 (cm)	5	105.6	Emb37	3.06	14.2	3.8	ab	cd	M	ac (5.2)	ad (5.8)	bc (5.6)	bd (5.0)
DBH6 (cm)	10	1.9	p18.b10	3.31	19.3	5.2	hk	hk	M	hh (11.9)	k- (12.7)		

LG linkage group, *Seg.* indicates whether segregation of the QTL effect is from the *M* male or *F* female parent

*P<0.05, **P<0.01, ***P<0.001 genome-wide significance level. All other QTL shown were significant at the chromosome-wide level

[a] The peak LOD score at each QTL.

[b] The proportion of the total genotypic variation explained by each QTL peak

[c] The proportion of the total phenotypic variation explained by each QTL peak

[d] For the co-located QTL segregating from the female parent on linkage group 5, the genotype means at a common marker are shown to enable a comparison of QTL effects, in addition to the genotype means at the QTL peak in order to show the maximum effect at each QTL

0.44–0.56) compared to growth traits (H^2=0.15–0.16) in this study was consistent with general findings in the species (Raymond 2002; Volker et al. 2008). These differences in genetic parameters between the two trait types were generally reflected in the greater number and significance of QTL for wood property traits in comparison to growth traits.

QTL number and effects

For each of the traits in this study, between one and four QTL were located, jointly explaining between 14.2% and 57.3% of the genotypic variation and between 3.8% and 34.9% of the phenotypic variation for each trait. Direct comparison of QTL number and effects between studies is not possible, because most studies have employed different experimental designs, markers and analysis techniques. In general, practical limitations on sample sizes in QTL studies of forest trees are likely to bias their results, by under-estimating the number of QTL for each trait, and over-estimating their effects (Beavis 1998). However, using genotype means from two ramets as the data for QTL analysis in this study would have increased the power to detect QTL (Bradshaw and Foster 1992). Regardless of the
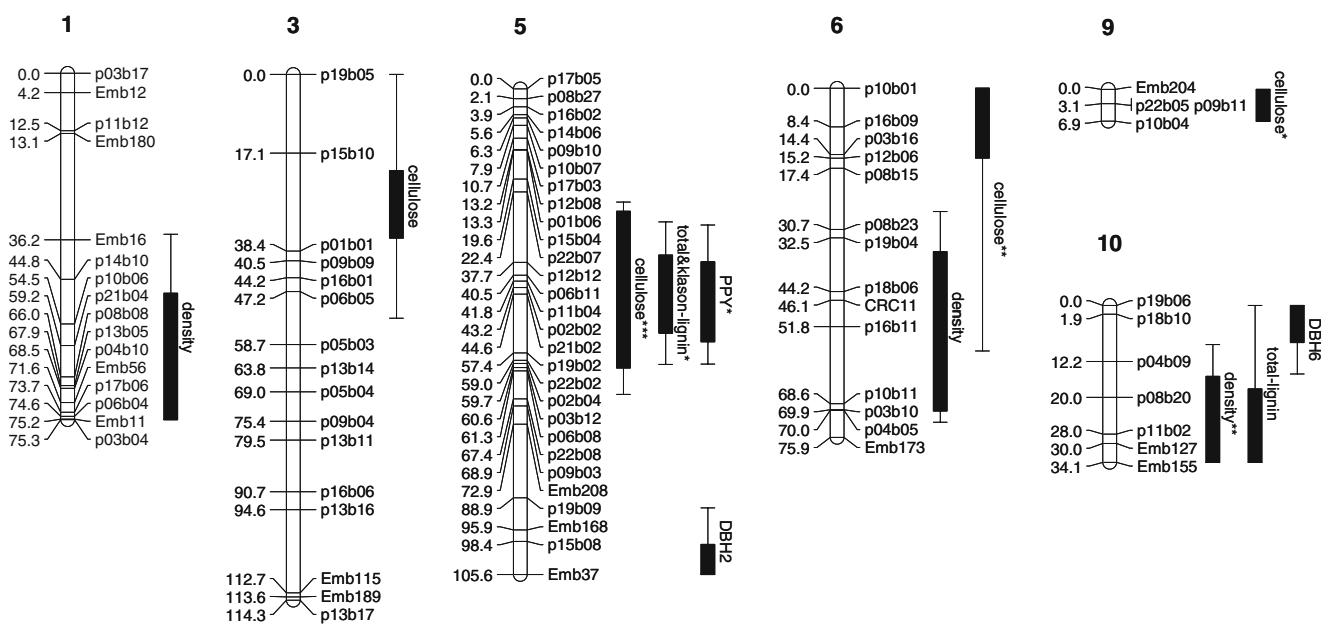
1

0.0	p03b17
4.2	Emb12
12.5	p11b12
13.1	Emb180
36.2	Emb16
44.8	p14b10
54.5	p10b06
59.2	p21b04
66.0	p08b08
67.9	p13b05
68.5	p04b10
71.6	Emb56
73.7	p17b06
74.6	p06b04
75.2	Emb11
75.3	p03b04

density

3

0.0	p19b05
17.1	p15b10
38.4	p01b01
40.5	p09b09
44.2	p16b01
47.2	p06b05
58.7	p05b03
63.8	p13b14
69.0	p05b04
75.4	p09b04
79.5	p13b11
90.7	p16b06
94.6	p13b16
112.7	Emb115
113.6	Emb189
114.3	p13b17

cellulose

5

0.0	p17b05
2.1	p08b27
3.9	p16b02
5.6	p14b06
6.3	p09b10
7.9	p10b07
10.7	p17b03
13.2	p12b08
13.3	p01b06
19.6	p15b04
22.4	p22b07
37.7	p12b12
40.5	p06b11
41.8	p11b04
43.2	p02b02
44.6	p21b02
57.4	p19b02
59.0	p22b02
59.7	p02b04
60.6	p03b12
61.3	p06b08
67.4	p22b08
68.9	p09b03
72.9	Emb208
88.9	p19b09
95.9	Emb168
98.4	p15b08
105.6	Emb37

cellulose***, total&klason-lignin*, PPY*, DBH2

6

0.0	p10b01
8.4	p16b09
14.4	p03b16
15.2	p12b06
17.4	p08b15
30.7	p08b23
32.5	p19b04
44.2	p18b06
46.1	CRC11
51.8	p16b11
68.6	p10b11
69.9	p03b10
70.0	p04b05
75.9	Emb173

cellulose**, density

9

0.0	Emb204
3.1	p22b05 p09b11
6.9	p10b04

cellulose*

10

0.0	p19b06
1.9	p18b10
12.2	p04b09
20.0	p08b20
28.0	p11b02
30.0	Emb127
34.1	Emb155

density**, total-lignin, DBH6

Fig. 1 The location of each putative QTL for wood properties and growth traits in *Eucalyptus globulus*. Linkage groups and loci are as described by Freeman et al. (2006), the names of microsatellite markers start with Emb or CRC, while the remainder are AFLP markers. The distance between markers is indicated to the *left* of linkage groups, in centimorgans. *Solid bars* and *lines* represent 1- and 2-LOD support intervals, respectively. The 2-LOD support interval corresponds to an approximate 95% confidence interval (Van Ooijen 1992). Genome-wide significance level is indicated as described in Table 3, all other QTL were significant at the chromosome-wide level (*P*<0.05)

differences between studies, previous QTL studies investigating wood property traits in forest trees such as eucalypt (Verhaegen et al. 1997; Thamarus et al. 2004; Bundock et al. 2008), pine (Markussen et al. 2003; Devey et al. 2004) and poplar (Zhang et al. 2006) have generally located several QTL with small to moderate effects for traits such as wood density, pulp yield and chemical properties, consistent with the present study. Therefore, given the above bias, it is likely that numerous QTL of small effect remain undetected, and that the genetic control of wood property traits in general is under polygenic control.

Although the effects of individual QTL are relatively small, and are likely to be inflated (Beavis 1998), the range between genotype means for each trait compare favourably to the phenotypic range found across native populations. For example, when compared to a diverse collection (nine different races) of *E. globulus* germplasm grown in a common environment (Poke et al. 2006), the difference between mean values of the QTL genotypes for pulp yield and klason lignin encompassed 6.1% and 5.1% of the range of values recorded (at 11 and 13 years), respectively. The proportion of the range of variation covered by the difference between QTL genotypes is even greater when considering the combined effects of multiple QTL (where present) for each trait. For instance, assuming completely additive effects, the QTL for lignin, cellulose and wood density in combination account for 13.8% (at 13 years),

19.5% (at 11 years) and 23.8% (at 11 years), respectively, of the range found in a base population trial of *E. globulus* (Poke et al. 2006).

In contrast to the QTL for wood properties, those for growth traits were weak. Growth is commonly thought to be under polygenic control, since it has relatively low narrow sense heritability in eucalypts (Volker et al. 2008). Hence, the fact that only a single QTL was detected for each of the two growth traits in this study (DBH2 and DBH6), probably reflects the limited power to detect QTL of small effect given our sample size. Furthermore, in light of the low clonal repeatability and relatively low LOD scores for the QTL for growth traits in this study, the possibility that the detected QTL are false positives cannot be ruled out.

QTL location

Of the 13 QTL identified in this study, six were co-located (i.e. had QTL peaks within 5 cM of each other) in two map regions, on linkage groups 5 and 10 (Table 3; Fig. 1). The co-location of QTL provides a genetic explanation for the correlation between traits. The co-location of QTL for cellulose, lignin (total and klason) and pulp yield on linkage group 5 was not surprising, since in both the present and previous studies, variation in cellulose content was strongly positively correlated with variation in pulp yield, both of

which were strongly negatively correlated with lignin levels (Table 2; Wallis et al. 1996; Thamarus et al. 2004; Poke et al. 2006). At each of the QTL in question, the significant allelic affects were segregating from the female parent only (Table 3) and were consistent with the direction of inter-correlations between the traits, suggesting the co-location may be due to the pleiotropic effects of a single locus (Sewell and Neale 2000). Of the remaining three QTL for cellulose, two of these (on linkage groups 3 and 9) also featured non-significant LOD peaks for PPY, total and klason lignin (results not shown). Thamarus et al. (2004), also found co-located QTL for pulp yield and cellulose content on two different linkage groups in *E. globulus*, distinct from those presented here (Table 4). Similarly, Rocha et al. (2007) found co-located QTL for pulp yield and lignin content in *Eucalyptus grandis × E. urophylla*. Taken together, these findings confirm suggestions that cellulose content is a useful proxy for pulp yield (Raymond and Schimleck 2002), and that selection for increased cellulose content or pulp yield are likely to result in a reduction in lignin content (Poke et al. 2006), which is a favourable outcome for a pulpwood breeding objective.

In the case of the QTL for total lignin content and wood density on linkage group 10, the fact that the effects of each QTL segregate from a different parent (Table 3), and the two traits are uncorrelated (Table 2), would argue tight linkage is a more likely explanation to account for the co-location of QTL for these traits. The QTL for DBH6 and density on linkage group 10 have overlapping confidence intervals (Fig. 1) and could potentially represent the action of the same genes. In contrast, the QTL for DBH2 on linkage group 5 and density on linkage group 6 clearly represent independent loci. The presence of independent QTL for growth and wood density has important implications for tree breeding. Despite some reports of a negative phenotypic correlation between growth and wood density (MacDonald et al. 1997; Wei and Borralho 1997; Costa e Silva et al. 2009), the presence of independent QTL for these traits indicates that at least some components of their respective genetic control are unique to each trait. Similarly, past studies in *Pinus radiata* (Devey et al. 2004) and *E. globulus* (Bundock et al. 2008) each found one case of co-located QTL affecting both traits, but also several independent QTL for density (Devey et al. 2004) and growth (Bundock et al. 2008). Taken together,

Table 4 Homology between linkage groups, QTL as well as mapped EST's and candidate genes for wood properties identified on the basis of shared markers between studies in *Eucalyptus*

Linkage group[a]	This study	Bundock et al. (2008)[b]	Thamarus et al. (2004)[c]		Grattapaglia et al. (1996)[d]
	E. globulus	*E. globulus*	*E. globulus*		*E. grandis*
	QTL	QTL	Candidate genes and EST's	QTL	QTL
1	Density	Bud abundance (4 and 5)	COMT, SAMS (est), Laccase (est) (7)	MFA	
2			PAL (4)	Cellulose, pulp yield	Density, CBH
3	Cellulose		ECSI (8)	MFA, fibre length	
4			MsaS2 (11)	Density, cellulose	
5	Cellulose, lignin, PPY, DBH2	DBH6 (3)	(9)	Density	Density, CBH, % bark
6	Density, cellulose		EXS1 (6)		Density, % bark
7			CCOAOMT, ECAI, XET (est) (2)		Density, CBH
8		Density (6)	(1)	Density	
9	Cellulose	(1)	EXS2 (5)	Fibre length	
10	Density, lignin, DBH6		CCR (10)	Cellulose, pulp yield	

[a] The numbering of linkage groups follows that established in *E. grandis* by Grattapaglia et al. (1996) and also used by Brondani et al. (2002, 2006)

[b] Density measured using pilodyn penetration, # linkage group, coded as reported in Bundock et al. (2008)

[c] *MFA* micro fibril angle, # linkage group, coded as reported in Thamarus et al. (2004)

[d] *CBH* stem circumference at breast height, *% bark* percentage weight of bark wood

these findings suggest there is potential to achieve some genetic improvement in growth rate and wood property traits simultaneously.

An interesting finding was the lack of co-located QTL for DBH2 and DBH6, despite the positive correlations between them in the present study (Table 2). This finding probably reflects the previously mentioned relatively low clonal repeatability for growth traits (Table 1), indicating a large environmental component to variation in growth traits. A lack of temporal stability of QTL for growth traits has been commonly reported (e.g. Verhaegen et al. 1997; Bundock et al. 2008) consistent with different factors affecting growth throughout ontogeny. Nonetheless, in this study DBH6 had a non-significant LOD peak coinciding with the location of the QTL for DBH2, and vice versa (results not shown).

QTL homology

Putative QTL in outcrossed forest trees are often restricted to the pedigrees in which they are located, and therefore require validation in independent pedigrees to demonstrate their broad applicability (Sewell and Neale 2000). Hence, linkage and QTL studies in *Eucalyptus* are increasingly employing transferable markers such as SSR and candidate genes (see references below). Indeed, the inclusion of SSR markers from a variety of sources in the linkage map used to conduct this QTL study (Freeman et al. 2006), allows comparison of QTL, candidate gene and EST location at the linkage group level with several previous studies (e.g. Grattapaglia et al. 1996; Thamarus et al. 2004; Bundock et al. 2008; Table 4). However, due to the few markers that are common across the various linkage maps published in *Eucalyptus* to date, in most cases the resolution is lacking to determine homology between different studies beyond the linkage group level.

Several QTL for wood property traits in this study mapped to the equivalent linkage groups as previously mapped QTL and candidate genes in *E. globulus* (Thamarus et al. 2004; Bundock et al. 2008) and *E. grandis* (Grattapaglia et al. 1996; Table 4). However, more definitive information comes from QTL and candidate genes which map to different linkage groups between studies. For example, although linkage group 5 in this study featured QTL with the highest significance for cellulose, lignin and pulp yield, no candidate genes for wood properties were located on the equivalent linkage group in Thamarus et al. (2004), suggesting there are candidate genes with significant effects on wood properties yet to be mapped. Furthermore, a comparison of homology at the linkage group level demonstrates that within *E. globulus* alone, there are at least six independent QTL influencing density and seven influencing cellulose content (Table 4).

Such variation in QTL discovered between studies is to be expected, since it is well known that QTL expression, particularly in forest trees, will vary in different genetic backgrounds, environments and throughout ontogeny (Sewell and Neale 2000). Thus, QTL validation would best be approached through simultaneous QTL studies in multiple pedigrees and sites.

Previously mentioned limitations of the QTL technique, such as the broad confidence intervals associated with QTL and high degrees of linkage equilibrium in tree species (Strauss et al. 1992; Wu 2002), dictate that QTL are unlikely to be widely used for direct selection in existing temperate *Eucalyptus* breeding programmes. Nonetheless, QTL studies provide the basis for identifying important genomic regions which can then be further studied using techniques such as map-based cloning and functional analysis, as well as providing candidate genes for association studies (Thumma et al. 2005; Myburg et al. 2007). These techniques are more likely to provide information directly applicable to breeding. However, it will be beneficial to first narrow the number of genomic regions for further study. For example, the identification of wood property QTL in almost every linkage group across the four studies considered (Table 4), clearly indicates that further comparative QTL studies would be beneficial to pinpoint fewer genomic regions for further study. QTL that are stable in different genetic backgrounds and environments will be prime targets for such techniques.

The identification of homology, although broad at present, between QTL in this study and those located in different pedigrees and species, is an important step toward identifying genomic regions, and ultimately genes, underlying variation in traits of ecological and economic importance across a diverse range of germplasm in *E. globulus* and the genus. Our ongoing research is focussed on further linkage mapping and QTL studies across multiple pedigrees of *E. globulus*, using substantially increased sample sizes and high-throughput markers, allowing far greater marker densities. Such studies will provide a basis for validating existing QTL, and locating additional QTL, particularly those of relatively small effect (Beavis 1998). These studies will allow a reduction of confidence intervals associated with QTL to relatively small regions of the genome, which we can use to interrogate the genome sequence of *E. grandis* (when it becomes available in the near future; www.eucagen.org), in order to identify positional candidate genes (Myburg et al. 2007). Such positional candidates can then be used in association studies, to verify that they underlie phenotypic variation in the traits in question, across a greater range of genetic backgrounds and environments (Myburg et al. 2007; Grattapaglia and Kirst 2008). Verification across different settings will identify genes suitable to use for

indirect selection. Selecting upon the gene itself rather than QTL regions, would circumvent the problem of recombination between QTL markers and the genes of interest, which has been a major obstacle to marker-based selection in forest trees (Strauss et al. 1992; Wu 2002).

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