

# Independent lines of evidence of a genetic relationship between acoustic wave velocity and kraft pulp yield in *Eucalyptus globulus*

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## Abstract

• **Key message** Multiple lines of evidence suggest acoustic wave velocity (AWV) would provide a rapid and efficient method to indirectly select for superior pulp yield in *Eucalyptus globulus* breeding programs.

• **Context** *Eucalyptus globulus* is one of the most widely planted hardwood species in temperate regions of the world and is primarily grown for pulpwood.

• **Aims** To determine if acoustic wave velocity (AWV) can be used to indirectly select for kraft pulp yield in *E. globulus*.

• **Methods** Genetic group effects, additive and non-additive variance components, and genetic correlations were estimated for AWV and pulpwood traits, including Kraft pulp yield. In a separate trial, the relative position of quantitative trait loci (QTL) for these traits was compared.

• **Results** Estimated narrow-sense heritabilities for AWV and pulp yield were both 0.26, and these traits were strongly genetically correlated (0.84). Furthermore, co-located QTL for these traits were identified. Further evidence that AWV could be used to indirectly select for pulp yield was provided by the ranking of genetic groups—Otways and King Island had the highest AWV and pulp yield and Strzelecki and Tasmania the lowest. There was no evidence of dominance variation in wood property traits.

• **Conclusion** Together, these findings suggest that AWV could be used as a selection criterion for kraft pulp yield in *E. globulus* breeding programs.

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**Contribution of the co-authors** Matthew G. Hamilton, Jules S. Freeman, David P. Blackburn, Geoffrey M. Downes, David J. Pilbeam and Brad M Potts undertook writing/editing the manuscript and all, except Matthew Hamilton and Brad Potts were involved in the data collection. Matthew Hamilton undertook the bulk of the quantitative genetic analyses. Jules S. Freeman undertook the bulk of the QTL analyses. Matthew Hamilton and Jules Freeman wrote the first draft of the manuscript. Brad Potts assisted in the genetic analyses and manuscript preparation. David J. Pilbeam was actively involved in the management of the trials over their life.

**Keywords** Genetic correlation · Quantitative trait loci (QTL) · Wood property · Heritability · Dominance

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## 1 Introduction

*Eucalyptus globulus* is one of the most widely planted hardwood species in temperate regions of the world, with extensive commercial plantations in Australia, Chile, Uruguay, Spain and Portugal. The species has excellent kraft pulping properties and is primarily grown for this purpose. However, there is an increasing interest in growing the species for solid-wood products, including sawn timber and engineered wood products (Hamilton et al. 2015b; Potts et al. 2013). Growth rate and pulp yield are economically important tree characteristics for vertically integrated kraft pulpwood growers and, along with basic density, are key targets for improvement in *E. globulus* breeding programs (Potts et al. 2013). These traits are usually assessed in breeding populations at between 4 and 8 years of age using a variety of tools including near infrared reflectance spectroscopy (NIR), which is a non-destructive means of estimating wood chemical properties (Downes et al. 2009).

Positive genetic correlations among acoustic wave velocity (AWV), pulp yield and basic density have been identified in *E. nitens* (Blackburn et al. 2012), a species closely related to *E. globulus*, suggesting that AWV may represent a cheaper alternative to NIR or be used as a means of identifying candidates for NIR in that species (Blackburn et al. 2014; Raymond et al. 2010). Acoustic velocity assessment can be undertaken directly using a microsecond timing device or with a resonance tool (refer to Raymond et al. 2010). Traditional interest in the assessment of AWV has been as an indirect measure of wood stiffness as it is positively correlated with modulus of elasticity (MOE), a key structural characteristic indicative of end-product stiffness (Blackburn et al. 2012; Blackburn et al. 2010). Furthermore, favourable phenotypic correlations among AWV, MOE and pulp yield in eucalypts (Dickson et al. 2003; Downes et al. 2008; Raymond et al. 2010) suggest acoustic assessments could be used to segregate logs into stiffness and/or pulp yield classes at mills, log landings or at harvest, by incorporating sensors into processing heads (Amishev et al. 2010). Such segregation would allow logs to be allocated to their most profitable use, can reduce the cost of handling, transporting and processing unsuitable logs and/or can improve the efficiency of processing (Raymond et al. 2010).

The primary objective of this study was to examine the potential to use standing tree AWV to indirectly select for kraft pulp yield in *E. globulus*. We used outcrossed full-sib families and quantitative genetic approaches to quantify the additive and non-additive genetic variation in, and estimate genetic correlations among, these traits as well as diameter and cellulose content. To further characterise the genetic relationships between AWV and these traits, quantitative trait loci (QTL) for acoustic wave velocity were also detected and compared with previously reported QTL for growth and wood properties in the same mapping families.

## 2 Materials and methods

### 2.1 Genetic material and trial designs

Two adjoining unthinned and unpruned *E. globulus* genetic trials, herein referred to as Trial 1 and Trial 2, were studied. These trials were the subject of a previous study of growth and harvesting traits (Hamilton et al. 2015a). Data from Trial 1 were used to estimate quantitative genetic parameters, and data from Trial 2 were used for QTL analyses. These trials were established on an ex-pasture site with a slight west-north-westerly aspect ( $\sim 4.5^\circ$ ) by the Southern Tree Breeding Association (STBA) on a Western Australian Plantation Resources (WAPRES) property near Manjimup, Western Australia ( $34^\circ 14' 52''$  S,  $116^\circ 3' 32''$  E) in 1991. Manjimup experiences a Mediterranean climate with an average annual rainfall of 1007 mm.

Trial 1 was comprised of 11 replicates, each with six  $4 \times 6$  tree blocks of 'cross-type' treatments with different levels of inbreeding: self-pollinated (one block per replicate), open-pollinated seed-orchard (one block per replicate) and full-sib out-crossed families (four blocks per replicate). Within replicates, cross-type treatments were randomly allocated to blocks and, within blocks, families were planted as single-tree plots. In the case of full-sib out-crossed families, an incomplete-block trial design was imposed (i.e. 'blocks' were treated as 'incomplete-blocks' within replicates). To estimate genetic parameters unbiased by variable levels of inbreeding and inbreeding depression (Costa e Silva et al. 2010), only data from full-sib out-crossed families were used in the current study. Insufficient seedlings of some out-crossed families were available at the time of planting and their plantation positions were filled with families with excess individuals.

In total, 166 full-sib out-crossed families from 178 parents were represented in Trial 1. The parents were from 12 subraces (Dutkowski and Potts 1999) which were consolidated into five genetic groups due to the low genetic contribution of some subraces (Table 1). Survival at the time of assessment was 91%.

Trial 2 comprised nine full-sib out-crossed  $F_1$  families, three of which were used for QTL analysis. From each family, a total of 92 individuals from 4 to 5 replicate  $5 \times 5$  plots were used for QTL analysis, which were the same individuals as used in an earlier QTL study (Freeman et al. 2013). In combination, these families sampled a diverse section of the natural distribution of *E. globulus*. The trial was designed according to a modified row-column design at the plot level, where over-represented families were randomly assigned to plot positions of under-represented families. (see Freeman et al. 2013 and Hamilton et al. 2015a, for more detailed descriptions of pedigrees and trial designs).

**Table 1** Genetic contributions from full-sib out-crossed families in Trial 1

Genetic group	Families <sup>a</sup>	Genetic contribution to planted trees (%)	Subrace	Families <sup>a</sup>	Genetic contribution to planted trees (%)
Furneaux	117	41.6	Flinders Island	93	33.1
Kings Island	16	5.6	Southern Furneaux	31	8.5
Otways	81	25.5	Kings Island	16	5.6
			Cape Patton	14	3.6
			Eastern Otways	2	0.3
			Western Otways	67	21.7
Strzelecki	28	7.0	Strzelecki Foothills	1	0.1
			Strzelecki Ranges	27	6.9
Tasmania	57	20.2	Northeastern Tasmania	12	4.4
			Southeastern Tasmania	25	7.4
			Southern Tasmania	10	3.3
			Tasman Peninsula	15	5.1

Note that the families represented are not additive across the genetic groups and subrace columns whereas the percentage contributions at the tree level are.

<sup>a</sup> Number of families which have any contribution from the listed genetic group or subrace.

## 2.2 Traits assessed

At Trial 1, standing tree AWV was assessed with a single-pass Fakopp Microsecond Timer prior to harvest 10 years after planting. Fakopp probes were placed at 0.5 and 1.7 m, one directly above the other, on the eastern side of each stem. Probes were positioned to minimise the number of branches and/or branch stubs between them and driven into the wood to a depth of approximately 12 mm below the bark—the upper probe pointing down and the lower probe pointing up at approximately 45° to the stem. The Fakopp Microsecond Timer measures the time taken for an acoustic stress wave to pass from one probe to the other, allowing estimation of AWV based on the distance between the probes. Outer-wood breast height drill swarf samples (approximately 40 mm depth; Meder et al. 2010) were then taken for NIR prediction of Kraft pulp yield (Kappa 18) and cellulose content (Downes et al. 2011). Swarf samples were air-dried and ground in a Wiley mill to provide a 16-mesh woodmeal. This gave a sample sufficiently fine enough to ensure the portion from which spectra were collected was representative of the whole sample. Ground samples were stored and analysed at ambient laboratory conditions. Woodmeal from each of these samples were used to obtain NIR spectra between 4000 and 10,000 wave-numbers (1000–2500 nm) on a Bruker MPA FT-NIR instrument. Predictions of pulp yield and cellulose content were then made using previously published calibrations (Downes et al. 2011). Stem diameter over bark of the main stem of each tree was measured at breast height (1.3 m; DBH).

At Trial 2, standing tree AWV was assessed at the same time and using the same procedure as Trial 1. These data were used in combination with historic wood chemistry, wood density and growth data collected at age 7 years for genetic mapping and QTL analysis (refer to Freeman et al. 2013).

## 2.3 Estimation of quantitative genetic parameters

To estimate variance components, univariate restricted maximum likelihood (REML) analyses were undertaken separately for each trait using data from out-crossed families in Trial 1 and the following linear mixed model:

$$y = \mathbf{Xb} + \mathbf{Zu} + \mathbf{e} \quad (1)$$

where  $y$  is the vector of trait observations,  $b$  is the vector of fixed effects with its design matrix  $\mathbf{X}$ ,  $u$  is the vector of random effects with its design matrix  $\mathbf{Z}$  and  $e$  is the vector of random residual terms. The models included as fixed effects in  $b$  the overall mean and replicate. The random effects in  $u$  were incomplete block within replicate, genetic group general combining ability (GCA), genetic group specific combining ability (SCA), the additive genetic component within genetic group and full-sib family within genetic group (refer to Hamilton et al. 2015a). For each trait, the narrow-sense heritability ( $h^2$ ), coefficient of additive genetic variance ( $CV_a$ ), dominance variance  $\sigma_d^2$  and dominance ratio ( $d^2$ ) were estimated from univariate analyses as follows:

$$\hat{h}^2 = \frac{\hat{\sigma}_a^2}{\hat{\sigma}_a^2 + \hat{\sigma}_f^2 + \hat{\sigma}_e^2} \quad (2)$$

$$\widehat{CV}_a = \frac{\sqrt{\hat{\sigma}_a^2}}{\bar{x}} \quad (3)$$

$$\hat{\sigma}_d^2 = 4\hat{\sigma}_f^2 \quad (4)$$

$$\hat{d}^2 = \frac{4\hat{\sigma}_f^2}{\hat{\sigma}_a^2 + \hat{\sigma}_f^2 + \hat{\sigma}_e^2} \quad (5)$$

where  $\bar{x}$  is the trait mean,  $\sigma_a^2$  is the additive genetic variance,  $\sigma_f^2$  is the non-additive full-sib family-specific variance and  $\sigma_e^2$

is the residual variance. A bivariate model was used to estimate inter-trait genetic correlations (refer to Hamilton et al. 2015a). Variances for random effects that were not significantly different from zero at the  $P = 0.10$  level in univariate analyses were fixed to zero in bivariate analyses. Two-tailed likelihood ratio tests were used to test if genetic correlations were significantly different from zero and one-tailed likelihood ratio tests were used to determine if these correlations were significantly different from one (Gilmour et al. 2009). Standard errors of parameters were estimated from the average information matrix, using a standard truncated Taylor series approximation (Gilmour et al. 2009).

Pearson correlation-coefficients among individual-tree phenotypic values, herein referred to as phenotypic correlations, were also estimated. Two-tailed  $t$  tests were used to test if phenotypic correlations were significantly different from zero. Analyses were conducted using ASReml™ Version 3.0 (Gilmour et al. 2009) and SAS™ (version 9.1).

#### 2.4 Analysis of quantitative trait loci

This study expanded on previous QTL analyses for growth and wood properties in the same  $F_1$  families (Freeman et al. 2013). Analyses of AWV QTL were conducted with MapQTL 6.0 (Van Ooijen and Kyazma 2009), using the consensus linkage map described in Freeman et al. (2013). MapQTL 6.0 (Van Ooijen and Kyazma 2009) default parameters were used for all analyses. Putative QTL were declared at a logarithm of odds (LOD) threshold of 3, which equated to an average chromosome-wide Type I error rate of  $<0.05$ , determined by permutation testing in each family (1000 replications; Churchill and Doerge 1994). It should be noted, this threshold is more conservative than the threshold for

‘suggestive QTL’ (i.e. chromosome-wide Type I error rate of  $<0.1$ ) adopted by Freeman et al. (2013). Interval and multiple-QTL model (MQM) mapping were performed as described by Freeman et al. (2013) but used the regression algorithm now available in MapQTL 6.0 (Van Ooijen and Kyazma, 2009).

To compare the genetic architecture underlying variation in AWV, growth and wood properties, the QTL for AWV were positioned on the map presented by Freeman et al. (2013) (Table 2, Fig. 1). This included QTL for chemical wood properties (kraft pulp yield, cellulose, Klason lignin, lignin S:G ratio and extractives content; each predicted using near-infrared spectroscopy), basic density and diameter at breast height, measured at 7 years of age. To aid the comparison of QTL from the present study with those previously detected, QTL were mapped using 15 cM intervals, following Freeman et al. (2013). Co-located QTL are defined as those with overlapping 15 cM intervals (Fig. 1). In addition to Trial 2 at Manjimup, Freeman et al. (2013) also included trees from the same  $F_1$  families planted at Branxholme in western Victoria ( $n = 183$ – $184$  trees per family, half at each trial site) and conducted analyses within each  $F_1$  pedigree site by site, and combined across sites (Table 2).

### 3 Results

In the quantitative genetic analyses (Trial 1), a significant ( $P < 0.05$ ) genetic group GCA effect was observed in AWV, pulp yield and cellulose content (Table 3). The genetic group SCA effect was significant in the case of AWV only. King Island exhibited the highest AWV, and Tasmania the lowest (Table 4).

**Table 2** Quantitative trait loci (QTL) for acoustic wave velocity, identified by interval (IM) and multiple-QTL (MQM) mapping, in two  $F_1$  families of *Eucalyptus globulus*. Co-located QTL and candidate genes from a previous study in these pedigrees are also presented (Freeman et al. 2013).

Linkage group	Position cM <sup>a</sup>	Family4 LOD		Family5 LOD		Closest co-located QTL <sup>b</sup> (distance cM)	Candidate gene co-location <sup>c</sup>
		IM	MQM	IM	MQM		
3	6.9	2.81*	4.82**				CSA3
3	56.9			3.75*	3.13*	K lig 5.7w*** (5.7)	
5	64.2	4.33**	6.10***			KPY 3.5* (1.1)	
10	50.9			4.51**	4.87**	Density 5.4w*** (0.8)	COBL
11	96.7	n.s.	3.24*				
11	48.6			n.s.	3.45*	Density 3.7v* (4.1)	

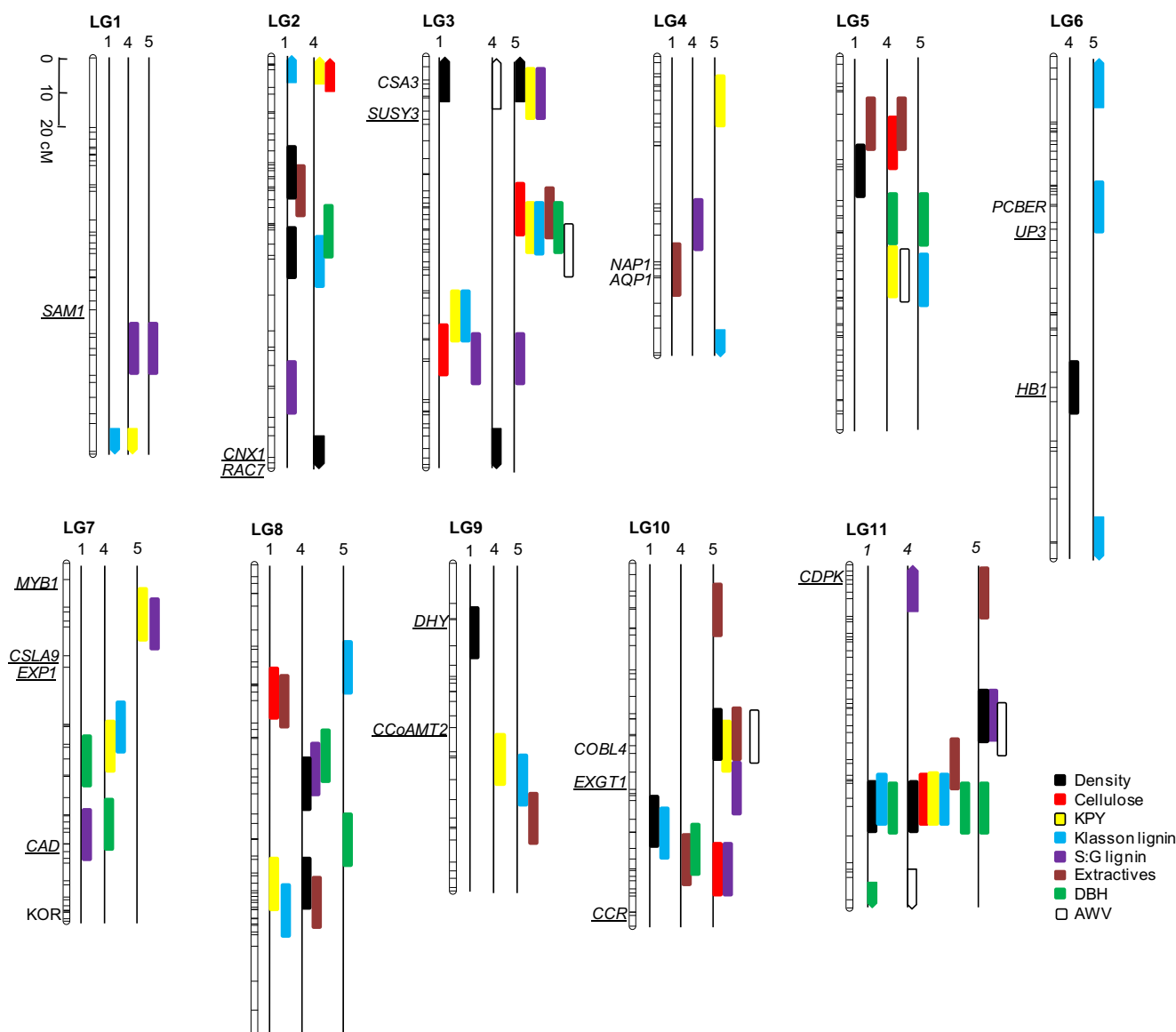
K lig klason lignin, KPY kraft pulp yield, Density basic density

Chromosome-wide significance levels for each QTL: \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$

<sup>a</sup> Position of the closest marker to the peak of each QTL. Where significant, the position from MQM mapping is reported

<sup>b</sup> Co-located QTL for physical and chemical wood properties from Freeman et al. (2013). The closest co-located QTL identified in the same  $F_1$  family is shown, followed by its maximum LOD value from interval mapping and, in parenthesis, the distance from the QTL for acoustic wave velocity in cM. ‘v’ and ‘w’ indicate the maximum LOD was from the analysis solely in the Victorian or Western Australian trial, respectively; otherwise, the LOD score is from analysis across trial sites.

<sup>c</sup> Candidate genes (from Freeman et al. 2013) within the QTL intervals



**Fig. 1** Quantitative trait loci (QTL) for acoustic wave velocity, as well as QTL previously identified for wood properties and growth, in three F<sub>1</sub> families of *Eucalyptus globulus*. Each QTL is represented by an arbitrary 15 cM bar surrounding the QTL peak, where QTL maps within 7.5 cM of

the end of a linkage group the end is truncated. ‘w’ and ‘v’ indicate QTL significant only in the Western Australian and Victorian trial sites, respectively. Candidate gene positions are indicated to the left of each LG, see Freeman et al. (2013) for more details

For pulp yield and cellulose content, Otways and King Island ranked highest, and Strzelecki lowest. The within genetic group additive genetic variance was significantly different from zero for all traits but the dominance variance was significant for diameter only (Table 2; Hamilton et al. 2015a). Narrow-sense heritabilities ranged from 0.15 for diameter to 0.26 for both AWW and pulp yield and coefficients of additive genetic variation ranged from 1.9% for pulp yield to 12.6% for diameter.

Both phenotypic and additive genetic correlations among traits were positive and significantly different from zero in all cases except for the additive genetic correlation between pulp yield and diameter (Table 5). At the phenotypic level, diameter

was significantly positively correlated with AWW, pulp yield and cellulose content (0.55–0.63). The additive genetic correlations among wood properties were very strong ( $\geq 0.84$ ), including that between pulp yield and AWW. There was no evidence to indicate that the strength of this correlation was an artefact of a common relationship with diameter as: (i) the genetic correlation between pulp yield and diameter, although moderate (0.38), was not significantly different from zero; and (ii) when the analysis used to estimate the genetic correlation between AWW and pulp yield was modified to include diameter as a covariate, the additive genetic correlation remained strong and significantly different from zero (0.80;  $P < 0.001$ ).



**Table 3** Number of trees sampled ( $N$ ), mean, estimated genetic group general combining ability ( $\sigma_g^2$ ), genetic group specific combining ability ( $\sigma_{gc}^2$ ), within-race additive ( $\sigma_a^2$ ), within-race dominance ( $\sigma_d^2$ ) and residual ( $\sigma_e^2$ ) variance components as well as the narrow-sense heritability ( $h^2$ ), within-race additive genetic coefficient of variation ( $CV_a$ ) and dominance ratio ( $d^2$ ) for each of the traits studied. Standard errors are shown in parentheses, and the significance of variances from zero is indicated based on a one-tailed likelihood ratio test

Trait	$N$	Mean	$\hat{\sigma}_g^2$	$\hat{\sigma}_{gc}^2$	$\hat{\sigma}_a^2$	$\hat{\sigma}_d^2$	$\hat{\sigma}_e^2$	$\hat{h}^2$	$\widehat{CV}_a$	$\hat{d}^2$
Acoustic wave velocity ( $\text{km s}^{-1}$ )	942	4.84 (0.086)	0.014 (0.014)*	0.007 (0.007)*	0.081 (0.023)***	NE	0.23 (0.017)	0.26 (0.07)	5.90	NE
Kraft pulp yield (%)	929	56.1 (0.34)	0.24 (0.22)*	0.01 (0.05) <sup>ns</sup>	1.15 (0.37)***	0.32 (0.48) <sup>ns</sup>	3.27 (0.26)	0.26 (0.07)	1.91	0.07 (0.11)
Cellulose content (%)	929	45.3 (0.31)	0.19 (0.19)*	0.06 (0.09) <sup>ns</sup>	0.99 (0.32)***	0.23 (0.42) <sup>ns</sup>	2.95 (0.23)	0.25 (0.07)	2.20	0.06 (0.1)
Diameter (mm)	959	209 (8.1)	100 (118) <sup>ns</sup>	67 (91) <sup>ns</sup>	684 (323)**	859 (554)*	3573 (245)	0.15 (0.07)	12.47	0.19 (0.12)

NE not estimated (hit parameter boundary and assumed to be zero)

<sup>ns</sup> not significant

\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$

In the QTL analysis (Trial 2), six discrete QTL were identified for AWV in total, three in each of the Families 4 and 5 (Table 2 and Fig. 1). No significant QTL were detected in Family 1. The QTL were distributed across four linkage groups (LG) and included those of moderate to high significance ( $P < 0.05$ – $P < 0.001$ ) on linkage groups 3, 5 and 10. Three of the six QTL for AWV co-located with those for pulp yield (Fig. 1—Family 4 LG5 and Family 5 LG3 and LG10), two of which involved co-location within the same family (Family 4 LG5 and Family 5 LG10). In both cases, the co-located QTL for AWV segregated from the same parent as the QTL for pulp yield, and one of these (Family 4 LG5) was independent of QTL for other wood properties. Two of the six AWV QTL (Family 4 LG3 and Family 5 LG10) co-located with wood property candidate genes (Table 2). While AWV QTL also co-located with other wood property traits, such as density and Klason lignin, the overall highest consistent Pearson phenotypic correlation observed within each family was with pulp yield (Table 6).

### 4 Discussion

Two lines of evidence indicated a genetic relationship between acoustic wave velocity (AWV) and NIR-predicted kraft pulp yield in *E. globulus*. Firstly, quantitative genetic analyses revealed that AWV and pulp yield were both under additive genetic control, and the additive genetic correlation between these traits was strongly positive (0.84). This correlation estimate appeared to be robust, because it was based on control pollinated seedlots, and its standard error was small. Furthermore, a similarly strong and positive additive genetic correlation between AWV and pulp yield was found in *E. nitens* (0.73; Blackburn et al. 2012). However, estimates of genetic correlations are notoriously variable across studies (Hamilton and Potts 2008), and further investigation is required to determine if this relationship is consistent across populations, environments, ages and different methods of measurement in *E. globulus* and other pulpwood species. Nevertheless, a positive genetic relationship between AWV and pulp yield was also reflected in the ranking of genetic groups—Otways and King Island had the highest AWV and pulp yield, and Strzelecki and Tasmania the lowest.

Secondly, AWV and pulp yield QTL were co-located in two independent families. Co-located QTL may reflect the influence of pleiotropic regulators influencing many developmental and/or biosynthetic pathways (Kirst et al. 2005) or linked clusters of genes (Breitling et al. 2008). However, segregation from the same parent in each case was consistent with pleiotropy. An additional QTL, on LG 3, was exactly co-located with a cellulose synthase gene *CSA3* (Fig. 1), which has been linked to variation in density in *E. globulus* in a previous study

**Table 4** Genetic group means by trait. Standard errors are shown in parentheses

Trait	Otways	Tasmania	Furneaux	Kings Island	Strzelecki
Acoustic wave velocity (km s <sup>-1</sup> )	5.0 (0.12)	4.5 (0.13)	4.9 (0.10)	5.2 (0.20)	4.6 (0.18)
Kraft pulp yield (%)	57.3 (0.37)	55.2 (0.42)	56.4 (0.32)	57.1 (0.70)	54.7 (0.61)
Cellulose content (%)	46.5 (0.40)	44.4 (0.42)	45.5 (0.32)	46.1 (0.69)	44.1 (0.61)
Diameter (mm)	244 (12.6)	178 (13.2)	200 (9.9)	211 (22.2)	220 (19.4)

(Thavamanikumar et al. 2014), could reflect a pleiotropic influence of this locus upon cellulose/pulp yield and AWW.

Taken together, our QTL and quantitative genetic analyses suggest acoustic wave velocity could be used as a selection criterion in breeding programs or as a screening tool to identify selection candidates to be sampled using more-precise but more expensive assessment tools such as NIR (Downes et al. 2009) and marker assisted selection (Thavamanikumar et al. 2011).

Selection based on AWW is likely to result in positive indirect responses in other wood properties, including MOE and basic density, as suggested by genetic correlation estimates from *E. nitens* (Blackburn et al. 2012; Blackburn et al. 2010; Blackburn et al. 2014). Similarly, in addition to pulp yield, QTL for AWW were co-located with numerous growth and other wood property traits within and among families, consistent with strong correlations among chemical wood properties in *E. globulus* (e.g. Stackpole et al. 2011). Although common breeding objective traits for pulpwood include pulp yield, wood density and diameter growth (Potts et al. 2013), chemical traits, such as the content of cellulose, lignin and extractives and the ratio of syringyl to guaiacyl subunits of lignin (S:G ratio), are also of economic importance, as they are either related to pulp yield or affect the cost and efficiency of pulping (Stackpole et al. 2011). Hence, co-location of QTL with density and chemical traits other than pulp yield coupled with the positive inter-correlations of AWW with pulp yield, cellulose and density bode well for the prospects of using AWW to select trees, not only for solid wood but also for pulpwood breeding objectives.

The phenotypic correlation between AWW and pulp yield ( $r_p = 0.71$ ,  $R^2 = 0.50$ ) in the quantitative genetic study implies that segregation of *E. globulus* logs into pulpwood classes

based on acoustic properties is possible during or post harvest (Amishev et al. 2010). This correlation was stronger than previous estimates for *E. nitens* (Blackburn et al. 2012;  $r_p = 0.39$ ,  $R^2 = 0.15$ ; Downes et al. 2008;  $R^2 = 0.25$ ) and *E. dunnii* (Raymond et al. 2010;  $R^2 = 0.25$ ), possibly due to different assessment methods used. However, in all studies a significant and positive correlation between these traits was observed, conceivably driven by a positive correlation between AWW and fibre length and increased cellulose content and decreased lignin content corresponding with increased fibre length (refer to Raymond et al. 2010). Acoustic wave velocity has also been shown to correlate with cell wall microfibril angle in *Eucalyptus dunnii* (Dickson et al. 2003) and *Pinus radiata* (Sharma et al. 2015). Along with density, microfibril angle is a key trait affecting modulus of elasticity and thus stiffness, and has been shown to be affected by polymorphisms in the CCR gene (Thumma et al. 2005). The absence of co-location of AWW QTL with the CCR gene in the present case is noteworthy given the findings of Thumma et al. (2005).

The additive genetic correlation between AWW and diameter (0.58) was within the range of estimates for *E. nitens* reported by Blackburn et al. (2014; 0.18, 0.20, 0.51 and 0.71). Furthermore, the positive additive genetic correlations of diameter with cellulose content (0.48) and pulp yield (0.38) were favourable and in keeping with recent studies by Stackpole et al. (2010; 0.52 between diameter and pulp yield), Costa e Silva et al. (2009); 0.12 between diameter and pulp yield across multiple trials) and Apiolaza et al. (2005; 0.61 between diameter and cellulose content). However, these positive correlations were not significantly different from zero, except in the case of diameter with cellulose content in the current study. Furthermore, these results

**Table 5** Additive genetic (below diagonal) and phenotypic (above diagonal) correlations among studied traits. Dominance genetic correlations were not estimated as only the dominance variance for diameter was significantly different from zero ( $P < 0.05$ ). Standard errors are shown in parentheses, and the significance of correlations from zero are indicated. All additive genetic correlations were significantly different from one ( $P < 0.01$ )

	Acoustic wave velocity (km s <sup>-1</sup> )	Kraft pulp yield (%)	Cellulose content (%)	Diameter (mm)
Acoustic wave velocity (km s <sup>-1</sup> )		0.71 (0.02)***	0.75 (0.02)***	0.55 (0.03)***
Kraft pulp yield (%)	0.84 (0.07)***		0.96 (0.01)***	0.55 (0.03)***
Cellulose content (%)	0.90 (0.05)***	0.97 (0.01)***		0.63 (0.03)***
Diameter (mm)	0.58 (0.17)**	0.38 (0.19) <sup>ns</sup>	0.48 (0.17)*	

<sup>ns</sup> not significant

\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$

**Table 6** Phenotypic correlations of acoustic wave velocity (AWV) with core basic density, predicted kraft pulp yield, cellulose content and Klason lignin for the three mapping families studied

	Family 1	Family 4	Family 5
	( <i>n</i> = 95 to 96)	( <i>n</i> = 95)	( <i>n</i> = 92)
Density (kg m <sup>-3</sup> )	0.28**	0.27**	0.14 <sup>ns</sup>
Kraft pulp yield (%)	0.35***	0.38***	0.36***
Cellulose content (%)	0.27**	0.33***	0.29**
Klason lignin (%)	-0.21*	-0.06 <sup>ns</sup>	-0.34***

<sup>ns</sup> not significant\**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001

contrast with negative, albeit also non-significant, estimates published prior to 2006 (refer to Stackpole et al. 2010). Clarification of the direction and strength of correlations between diameter and these key pulpwood traits is required.

The strong genetic correlation between pulp yield and cellulose content (0.97) supports the findings of Apiolaza et al. (2005; 0.82) and Stackpole et al. (2010; 0.91). This indicates that tree breeders can make selections based on either NIR-predicted pulp yield or cellulose content with little impact on genetic gain.

The estimated narrow-sense heritability for AWV (0.26) was lower than most estimates from *E. nitens* open-pollinated base-population trials (0.16, 0.39, 0.44 and 0.74; Blackburn et al. 2014). Furthermore, NIR-predicted pulp yield and cellulose content heritabilities and CV<sub>a</sub> were lower than past estimates in *E. globulus* from open-pollinated trials (Apiolaza et al. 2005; Cotterill and Brolin 1997; Dean et al. 1990; Raymond and Schimleck 2002; Raymond et al. 2001a; Stackpole et al. 2010). It is not known if this low level of additive variation was an artefact of the assessment method (NIR on drill swarf samples extracted from near the cambium), which has not been extensively used. Lower heritability estimates for wood properties (e.g. pilodyn penetration, an indirect measure of basic density; Costa e Silva et al. 2009) in control-pollinated trials compared with open pollinated trials has previously been noted. However, in contrast to growth (Costa e Silva et al. 2010), there is no direct evidence from past studies of *E. globulus* wood properties for inbreeding depression affecting open-pollinated estimates of heritability (Volker et al. 2008) or of significant dominance variation. In the only other study to report a dominance variance for pulp yield, Costa e Silva et al. (2009) found that it hit the boundary of the parameter space at zero and that the epistatic variance was not significantly different from zero. Small and, again, non-significant dominance variances have been observed in the more widely studied wood property basic density (measured directly on wood cores or indirectly as pilodyn penetration; Costa e Silva et al. 2009; Li et al. 2007; Volker 2002). Furthermore, strong correlations observed between breeding values estimated from open-pollinated and control-pollinated trials (Volker 2002) and the absence of inbreeding depression (Hardner et al. 1998) also

suggest that dominance genetic effects are of low importance relative to additive effects in this trait. In contrast, the near unity ratio of dominance to additive variance for diameter, reflected the findings of some past studies, most notably Li et al. (2007; refer to Hamilton et al. 2015a).

The ranking of genetic groups for pulp yield was not consistent with the findings of Stackpole et al. (2010), the largest study of the genetics of this trait in *E. globulus*, in which pulp yield of all mainland Australian subraces was lower than the pulp yield of the Bass Strait Islands and Tasmanian subraces, with the exception of the three subraces from northeastern Tasmania. In the present study, the Strezlecki genetic group from the mainland had the lowest pulp yield and cellulose content, and King Island (a Bass Strait island) ranked second highest. However, the Otways genetic group, from the mainland, had the highest pulp yield, and Tasmania ranked the second lowest. The low pulp yield of the Tasmanian genetic group was possibly explained by the inclusion of a number of northeastern Tasmanian families (Table 1). Furthermore, studies other than Stackpole et al. (2010) found the western Otways subrace (the subrace with the greatest contribution to our Otways genetic group; Table 1) to rank highly for pulp yield (Apiolaza et al. 2005; Raymond et al. 2001a). Cape Patton and Eastern Otways were also represented in the Otways genetic group, but there are no published pulp yield estimates for these subraces beyond the Stackpole et al. (2010) study. The stability of the ranking of subraces across sites for this key pulpwood trait requires further investigation.

It is possible that NIR predicted pulp yield and cellulose content in the current study were upwardly biased, as samples were extracted from the outer wood and both pulp yield and cellulose content increase from pith to cambium (Downes et al. 2012). Average NIR-predicted kraft pulp yield (54.7% for Strzelecki to 57.3% for Otways) was greater than those reported in other genetic studies (e.g. 51.6% from Apiolaza et al. 2005; 52.2%, pooled estimate, from Costa e Silva et al. 2009; 54.1% from Dean et al. 1990; 51.9, 52.2 and 51.9% from Raymond et al. 2001b; and 53.1% from Stackpole et al. 2010). The same was true for cellulose content (42.2% from Apiolaza et al. 2005; 46% from Cotterill and Brolin 1997; and 42.9, 42.1, and 40.4% from Raymond and Schimleck 2002).

## 5 Conclusion

We found that AWV is significantly genetically positively correlated with predicted pulp yield in *E. globulus*, consistent with the previous studies in *E. nitens*. Standing-tree AWV is used as an indirect measure of modulus of elasticity, a key trait for structural veneer and sawn timber. Selection on the basis of AWV is thus expected to provide genetic gains in wood properties favourable to both pulpwood and solidwood breeding objectives. However, prior to widespread adoption of AWV as



a selection criterion in breeding programs, genetic correlations require verification across the species, breeding populations, environments, ages and methods of measurement.

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#### Compliance with ethical standards

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