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► **To cite this version:**

Fiona S. Poke, Brad M. Potts, René E. Vaillancourt, Carolyn A. Raymond. Genetic parameters for lignin, extractives and decay in *Eucalyptus globulus*. *Annals of Forest Science*, 2006, 63 (8), pp.813-821. hal-00884047

HAL Id: hal-00884047

<https://hal.science/hal-00884047>

Submitted on 11 May 2020

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Genetic parameters for lignin, extractives and decay in *Eucalyptus globulus*

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(Received 8 August 2005; accepted 1 February 2006)

Abstract – *Eucalyptus globulus* is grown in temperate regions of the world for pulp production. The chemical and physical properties of its wood make it highly suited to this purpose. This study analysed genetic variation in lignin content, extractives content and decay, for nine localities of *E. globulus*. Heritability estimates were also obtained, and the relationships between these traits and physical wood traits and growth were examined. Significant genetic variation was found between localities for lignin content (Klason lignin and acid-soluble lignin contents) and decay. The only trait for which significant variation between families within locality was detected was acid-soluble lignin content, which resulted in this trait also having the highest narrow-sense heritability (0.51 ± 0.26). However, family means heritabilities were high for lignin content, extractives content and decay (0.42–0.64). The chemical wood traits were strongly correlated with each other both phenotypically and genetically, with important correlations found with density and microfibril angle. Correlations suggested that during selection for the breeding objective traits, it is likely that favourable states in the chemical wood traits, decay resistance and fibre properties are concurrently being selected, whereas growth may be selected for independently. This initial study provides a stepping stone for future studies where particular localities of the breeding population may be characterised further.

correlation / eucalypt / genetic variation / heritability / lignin

Résumé – Paramètres génétiques pour la lignine, les extractibles et la pourriture chez *Eucalyptus globulus*. *Eucalyptus globulus* est cultivé dans de nombreuses régions tempérées pour la production de pâte à papier. Les propriétés physiques et chimiques de ce bois en font un matériau très apprécié pour cette utilisation. Cette étude analyse les variations génétiques de la teneur en lignine, en composés extractibles et de la sensibilité à la décomposition du bois de neuf provenances de *Eucalyptus globulus*. L'héritabilité de ces propriétés ainsi que leurs relations avec les caractéristiques physiques du bois et la croissance ont été examinées. Des différences inter-provenances significatives ont été détectées pour les teneurs en lignine (lignines dosées par la méthode de Klason, ou lignines soluble en solution acide) et la vitesse de décomposition. Le seul caractère qui a présenté une variation significative entre familles dans les provenances a été la teneur en lignines solubles en solution acide. De ce fait, une forte héritabilité (au sens strict) a été détectée pour ce caractère ($0,51 \pm 0,26$). Cependant, les héritabilités moyennes dans les familles étaient élevées pour la teneur en lignine, les teneurs en composés extractibles et la vitesse de décomposition (0,42–0,64). Les caractéristiques chimiques du bois étaient fortement inter-corrélées au niveau phénotypique et génétique, avec des corrélations importantes également avec la densité et l'angle des microfibrilles. Les corrélations suggèrent que durant la sélection de caractères objectifs d'amélioration, des traits favorables associant caractéristiques chimiques du bois, résistance à la décomposition et propriétés des fibres puissent être sélectionnés simultanément, alors que la croissance doit faire l'objet d'une sélection indépendante. Cette étude constitue une base pour de futurs travaux permettant une caractérisation plus fine de provenances particulières dans cette population de sélection.

corrélation / eucalyptus / variation génétique / héritabilité / lignine

1. INTRODUCTION

Eucalyptus globulus is grown for pulp production in temperate Australia and other parts of the world, including South America, southern Europe, Africa and Asia. Considerable genetic variation has been identified between the subraces of *E. globulus* for a wide range of traits, including growth and both physical and chemical wood properties [1, 10, 23, 24]. Some of this variation has been exploited in breeding programs for the selection of superior trees. When selecting trees for pulp production, only a few traits are currently examined in Australia, with the focus on increases in volume per hectare, basic density and pulp yield [1, 16, 17]. Although selection for these traits gives an increase in the pulp yield per hectare, many

other physical and chemical wood properties are important to kraft pulping, and variations in these can be conducive to minimising the costs or environmental impacts of the process.

Kraft pulping generally involves the removal of most of the extractives, approximately 80% of the lignin and approximately 50% of the hemicellulose from the cellulose fibres using alkali [35]. For the production of high quality paper, the pulp is further bleached to remove the residual lignin, which is responsible for turning the paper yellow through oxidation and light absorption [35]. The lignin and extractives contents of wood, are traits that are fast being recognised as having importance in minimising the costs and environmental impacts of kraft pulping. As lignin and extractives are the primary waste products of the pulping process, lower levels in the wood will result in faster delignification and/or a reduction in the use of

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chemicals and energy. This will help minimise the production of pollutants from the pulping process.

Studies into the genetic variation and heritability of lignin and extractives have been limited in *Eucalyptus* until recently. This was mainly due to the expensive and time-consuming nature of the chemical assay used to measure these traits [2, 3]. More recently, simple and cost-effective techniques have been developed for predicting these traits, involving near infrared reflectance (NIR) analysis on ground wood cores [4, 30]. This has been found to be an effective technique for reliably predicting these traits in large numbers of samples. A small number of studies have suggested that there is genetic variation in lignin and extractives contents in *E. globulus*, although these involved only three or four provenances and a small number of individuals [24, 41]. Studies investigating the correlations between the chemical wood traits and other wood properties in *E. globulus*, have also been limited by small sample sizes and to small numbers of traits, and have involved phenotypic correlations only [23, 39]. Genetic correlations have not been reported for these traits in *E. globulus*. To fully explore the scope of variation within the chemical traits and their genetic control, large numbers of individuals, families and provenances are required which encompass the range of *E. globulus*.

The susceptibility of trees to wood decay is important as it may impact on pulp yield in two ways: firstly, the plant defence response may lead to an increase in the amount of lignin and extractives present in the wood, which will reduce the pulp yield; secondly, decay leads to degradation of the wood causing a breakdown of the cellular structure [29]. This decay can be caused by pathogen infection of exposed, vulnerable tissue following wounding, or through attack of the heartwood (heart rot), which is incapable of an active response due to the lack of living cells [29]. The impact of decay will also depend upon whether the decay organisms are feeding on cellulose or lignin. Two types of fungi generally are responsible for decay, brown rot fungi which degrade cellulose and white rot fungi which degrade lignin. Decay can be observed in “pockets” that are compartmentalised by a reaction zone (purple/pink coloured boundary between healthy and decayed wood) and discolouration of the surrounding wood, or as rotting of the heartwood [29, 40]. Fungal attack has been found to be associated with increases in lignin due to its resistance to degradation by pathogens, in *E. gunnii* [18], and increases in extractives which contain antimicrobial compounds, in *E. nitens* [5]. In *E. globulus* genetic variation in decay susceptibility and its relationship to other wood properties has not been examined.

Improving the chemical wood properties of tree species through breeding requires genetic variation to be present for selection. It is also necessary to understand how the traits to be improved are related to one another and to other traits that are not currently being selected, so that when an increase in one trait is selected for, the potential effects on other traits may be predicted. A study conducted by Apiolaza et al. [1] examined the variation in growth and wood traits as well as their correlations using 188 individuals of *E. globulus* from 35 families and eight subraces, which are currently part of the single breeding population of the Australian national breeding program. The traits examined included diameter at breast height

over bark (DBH), basic density (BD), mean fibre length (FL), mean microfibril angle (MFA) which is the average angle of the cellulose microfibrillar helix relative to the longitudinal fibre axis [11], predicted pulp yield (PY) and cellulose content (CELL). The current study aimed to build on that of Apiolaza et al. [1] with a particular focus on the chemical wood properties. Using the same open-pollinated progenies grown in a field trial, we examined the variation in and the heritability of lignin content (LIG), extractives content (EXTR) and extent of decay (DEC) between and within nine localities of *E. globulus* originating from around Tasmania and south-east Victoria. Phenotypic and genetic correlations were also determined amongst these traits and with the growth and wood traits of Apiolaza et al. [1]. The relationship between these chemical wood traits and with the physical wood traits, wood decay and growth has not been examined before in *E. globulus* and will provide an indication of how multiple traits are affected during the selection of superior trees.

2. MATERIALS AND METHODS

2.1. Plant material

Wood samples of *Eucalyptus globulus* were collected from a base population field trial located at West Ridgley, Tasmania (Gunns Ltd). This trial was established in 1989 based on the CSIRO Australian Tree Seed Centre collection and is comprised of open-pollinated families [12, 13]. The trial was an incomplete block design with 451 families in five replicates, each with 17 incomplete blocks, and two-tree row plots [1]. A total of 177 trees from 37 families (Tab. I) were sampled to cover the same range of eight subraces sampled by Apiolaza et al. [1], with one tree or occasionally two trees per plot sampled. Due to the fact that only a subset of families was sampled the trial was treated as a randomised complete block design for analysis. The locality denoted North-east Tasmania comprises two localities, Royal George and German Town, which were merged because of small sample sizes and their close proximity. Two bark-to-bark wood cores were taken from each tree approximately 10 cm above the previous core sites taken by Apiolaza et al. [1] two years before, according to the method described by Raymond et al. [33].

2.2. Wood and growth measurements

Measurements for BD, MFA, FL, PY and CELL already existed for these trees at age 11 years [1]. Additional measurements were taken for BD and DBH, and measurements were obtained for DEC, LIG and EXTR all at age 13 years.

DEC was recorded for each core as the percentage of the core with heart rot, pocket decay and/or discolouration and results were averaged for the tree. For statistical analysis the different types of decay data were grouped, with 0 indicating no decay followed by 10% intervals thereafter, and class midpoints were used for analysis. Due to the presence of decay in the pith for many of the cores, partial cores (the outer quarters of each core free of decay) were used for further BD, LIG and EXTR measurements.

BD was determined for one core from each tree using the water displacement method [37], by submerging the partial cores in cold water for approximately two days, removal of remaining bark and

Table I. Breakdown of subraces (as classified by Dutkowski and Potts [10]), localities and families of *E. globulus* used in this study from the base population trial at West Ridgley, Tasmania.

Subrace	Locality	Number of families	Number of individuals
Flinders Island, Tasmania	Central Flinders Island	4	18
King Island, Tasmania	South King Island	5	27
North-eastern Tasmania	North-east Tasmania	4	16
South-eastern Tasmania	Moogara	4	14
South-eastern Tasmania	North Maria Island	3	14
Southern Tasmania	South Geeveston	4	18
Strzelecki Foothills, Victoria	Madalya Road	4	20
Strzelecki Ranges, Victoria	Bowden Road	4	22
Western Otways, Victoria	Cannan Spur	5	28
Total	9	37	177

excess water followed by volume (V) measurements. The mass (M) of each core was taken after drying at 105 °C for approximately two days. BD was calculated using the following formula:

$$BD_{(kg/m^3)} = \frac{M}{V} \times 1000$$

The remaining partial cores were used to develop the NIR calibrations reported in Poke et al. [30] for total lignin (TLIG), acid-soluble lignin (ASLIG) and Klason lignin (KLIB) contents (TLIG = ASLIG + KLIB) plus EXTR. These calibrations were based on chemical measurements for 54 to 61 samples and had good correlation coefficients (0.62–0.93), and predicted and laboratory values for the validation set of samples were highly correlated (0.83–0.99) [30]. The calibrations were used to predict these traits for the remainder of the individuals in the data set.

2.3. Statistical analysis

Variance components for BD, DBH, ASLIG, KLIB, TLIG, EXTR and DEC were estimated using the MIXED procedure in SAS (Version 9.1, SAS Institute Inc.), with locality fitted as a fixed effect, and family within localities, replicate and residual within localities as random effects. Locality least square means and the differences between them were also calculated using the MIXED procedure in SAS, with a Tukey-Kramer adjustment applied for multiple comparisons.

The individual narrow-sense (h_{op}^2) and family mean (H_{fm}^2) heritabilities of BD, DBH, ASLIG, KLIB, TLIG, EXTR and DEC were estimated using ASREML [14], with the fixed locality term removed from the model in the latter case. h_{op}^2 refers to the narrow-sense heritability within localities which is used operationally to predict genetic gains from within locality selection. H_{fm}^2 is the family means heritability which indicates the gain that would be made from selecting the best families across all localities for deployment. h_{op}^2 and H_{fm}^2 were estimated as [19]:

$$h_{op}^2 = \frac{\sigma_{add(loc)}^2}{(\sigma_{add(loc)}^2 + \sigma_e^2)}$$

$$H_{fm}^2 = \frac{\sigma_f^2}{\sigma_f^2 + \sigma_e^2/k}$$

where: $\sigma_{add(loc)}^2$ = additive genetic variation within locality variance component estimated assuming a coefficient of relatedness within open-pollinated families of 0.4, after first adjusting the additive relationship matrix for a 30% selfing rate [9];

σ_f^2 = family variance component calculated across localities;

σ_e^2 = residual variance component;

k = harmonic mean number of trees per family.

Trait correlations were determined amongst the age 13 measurements of BD, DBH, ASLIG, KLIB, TLIG, EXTR and DEC and with the traits of Apiolaza et al. [1]. Phenotypic correlations (Pearsons correlation matrix) amongst individuals were determined in SAS using the CORR procedure. Additive genetic correlations could not be estimated using ASREML [14] directly, as bivariate models failed to converge due to the small sample size. However, as an approximation of the genetic correlations, Pearsons correlation matrices were obtained using family means adjusted for locality differences and for the nine locality means using the CORR procedure in SAS.

3. RESULTS

3.1. Trait statistics and variances

The number of individuals measured for each trait and the statistics for each trait are detailed in Table II and include the subset of measurements from Apiolaza et al. [1]. Sixty-nine percent of samples were found to have decay symptoms. Variation in the traits measured in this study, between replicates, localities and family within localities, are detailed in Table III. No significant variation was detected at any level for BD, TLIG and EXTR (Tab. III). Locality was a significant source of variation for DEC, DBH, KLIB and ASLIG (Tab. III). Of these four traits, only two had significant differences between the locality least square means following Tukey-Kramer adjustment (Tab. IV). For DEC, South King Island had significantly more decay than five other localities including Bowden Road ($P < 0.001$), Madalya Road ($P < 0.002$), Central Flinders Island ($P < 0.003$), North-east Tasmania ($P < 0.004$) and Cannan Spur ($P < 0.023$). For ASLIG, South Geeveston and South King Island were significantly different to each other ($P < 0.01$). Significant variation between families within locality was detected for ASLIG only.

Table II. Statistics for growth and wood measurements of individual trees for the *E. globulus* base population trial at West Ridgley, Tasmania.

Trait (Abbreviation)	Unit	<i>n</i>	Mean	Standard deviation	Minimum	Maximum
Mean fibre length at age 11 (FL)	mm	141	0.77	0.06	0.59	0.95
Mean microfibril angle at age 11 (MFA)	°	149	16.9	2.9	11.7	27.5
Predicted pulp yield at age 11 (PY)	%	157	51.8	1.6	42.5	57.0
Cellulose content at age 11 (CELL)	%	157	42.4	1.5	37.8	46.6
Basic density at age 11 (BD)	kg/m ³	161	494.5	40.5	395.8	589.4
Basic density at age 13 (BD)	kg/m ³	133	522.9	44.9	412.1	667.7
Diameter at breast height at age 13 (DBH)	cm	177	24.1	5.4	13.4	37.5
Klason lignin content at age 13 (KLIG)	%	155	22.38	1.21	18.97	25.45
Acid-soluble lignin content at age 13 (ASLIG)	%	155	6.12	0.52	4.42	8.11
Total lignin content at age 13 (TLIG)	%	155	28.48	1.26	24.72	31.23
Extractives content at age 13 (EXTR)	%	155	6.00	1.84	2.12	12.73
Extent of decay at age 13 (DEC)	%	143	35.9	32.4	0	95.0

Table III. Analyses of variance for growth and wood traits at age 13 years between replicates, localities, and families within localities, plus estimates of the heritability of within locality variation, and family means heritability, for these traits in the samples from the *E. globulus* base population trial at West Ridgley, Tasmania. Probability values are denoted *** $P < 0.001$, * $P < 0.05$ and ns = non-significant.

Trait	df	Basic density (BD)	Diameter at breast height (DBH)	Klason lignin content (KLIG)	Acid-soluble lignin content (ASLIG)	Total lignin content (TLIG)	Extractives content (EXTR)	Extent of decay (DEC)
Replicate Z value (probability value)	4	0 ^a (-)	0.57 (0.284) ns	1.26 (0.105) ns	0.92 (0.178) ns	1.24 (0.107) ns	0.64 (0.261) ns	0.40 (0.345) ns
Locality F value (probability value)	8	1.83 (0.114) ns	2.73 (0.023) *	2.52 (0.033) *	2.52 (0.034) *	2.03 (0.079) ns	1.80 (0.120) ns	5.40 (0.0004) ***
Family [locality] Z value (probability value)	28	0.89 (0.186) ns	0 ^a (-)	0.688 (0.249) ns	1.90 (0.028) *	1.2 (0.115) ns	1.52 (0.064) ns	0 ^a (-)
Narrow-sense heritability (standard error)		0.24 (0.26)	0 ^a	0.13 (0.20)	0.51 (0.26)	0.29 (0.23)	0.35 (0.23)	0 ^a
Family means heritability (standard error)		0.42 (0.19)	0.19 (0.19)	0.42 (0.16)	0.64 (0.10)	0.50 (0.14)	0.48 (0.14)	0.50 (0.14)

Z values are random terms and F values depict fixed terms.

^a Variance component was at the boundary of the parameter space.

3.2. Heritability estimates

Narrow-sense heritability estimates had large standard errors due to the lack of significant variation between families within localities for most traits, no doubt reflecting the small sample size (Tab. III). Moderately high heritability values were obtained for ASLIG (0.51 ± 0.26) and EXTR (0.35 ± 0.23), although ASLIG was the only trait where significant variation between families within localities was detected. Both BD (0.24 ± 0.26) and TLIG (0.29 ± 0.23) showed moderate heritabilities, with KLIG (0.13 ± 0.20) showing little heritability. Within locality variation in DEC and DBH was non-heritable. The heritabilities of family means integrated both

within and between locality variation, and were somewhat higher than the narrow-sense heritabilities due to the inclusion of locality effects in the differences between families. ASLIG (0.64 ± 0.10), EXTR (0.48 ± 0.14), TLIG (0.50 ± 0.14) and DEC (0.50 ± 0.14) showed high estimates. BD (0.42 ± 0.19) and KLIG (0.42 ± 0.16) had moderately high estimates, and DBH a moderate estimate (0.19 ± 0.19) (Tab. III).

3.3. Trait correlations

Strong correlations were identified between the wood and growth traits at locality, family and individual (phenotypic)

Table IV. Locality least square means and standard errors (in parenthesis) for growth and wood traits at age 13 years for samples from the *E. globulus* base population trial at West Ridgley, Tasmania.

Locality	Basic density (BD) (kg/m ³)	Diameter at breast height (DBH) (cm)	Klason lignin content (KLIB) (%)	Acid-soluble lignin content (ASLIG) (%)	Total lignin content (TLIG) (%)	Extractives content (EXTR) (%)	Extent of decay (DEC) (%)
Central Flinders Island	520 (12) a	26.0 (1.2) a	22.6 (0.4) a	6.0 (0.2) ab	28.6 (0.4) a	6.4 (0.6) a	23.4 (7.2) a
South King Island	486 (13) a	25.3 (1.0) a	21.9 (0.4) a	5.7 (0.1) a	27.7 (0.4) a	5.4 (0.5) a	65.6 (6.5) b
North-east Tasmania	512 (14) a	20.9 (1.3) a	22.7 (0.4) a	6.1 (0.2) ab	28.8 (0.4) a	7.1 (0.6) a	22.1 (7.9) a
Moogara	511 (14) a	22.2 (1.4) a	22.6 (0.4) a	6.3 (0.2) ab	28.9 (0.4) a	6.1 (0.6) a	48.8 (8.9) ab
North Maria Island	537 (16) a	23.2 (1.4) a	22.5 (0.4) a	6.0 (0.2) ab	28.5 (0.5) a	6.0 (0.7) a	40.2 (8.5) ab
South Geeveston	532 (14) a	25.6 (1.2) a	21.8 (0.4) a	6.6 (0.2) b	28.2 (0.4) a	5.0 (0.6) a	48.6 (8.5) ab
Madalya Road	535 (12) a	22.9 (1.2) a	22.8 (0.4) a	6.0 (0.2) ab	28.8 (0.4) a	6.7 (0.5) a	21.3 (7.4) a
Bowden Road	542 (12) a	22.7 (1.1) a	22.8 (0.4) a	6.2 (0.1) ab	28.9 (0.4) a	6.2 (0.5) a	18.6 (7.4) a
Cannan Spur	527 (11) a	26.3 (1.0) a	21.8 (0.3) a	6.2 (0.1) ab	27.9 (0.4) a	5.3 (0.5) a	33.7 (6.1) a

Localities with common letters for the same trait are not significantly different at $P < 0.05$ following Tukey-Kramer adjustment for multiple comparisons.

levels (Tab. V). Correlations between locality means and family means with locality differences removed, represented genetic based correlations. As expected, a strong, positive relationship was identified between TLIG and its components (ASLIG and KLIB) at most levels, although KLIB and ASLIG were not significantly correlated. EXTR was strongly correlated with lignin content for individuals, but the correlations were positive with KLIB and TLIG, and negative with ASLIG. Genetic correlations were observed between EXTR and both KLIB (families and localities) and TLIG (localities). KLIB, TLIG and EXTR all had significant, negative, phenotypic and genetic correlations with both CELL and PY, although these were sometimes not significant at the locality level. Lignin content showed significant negative correlations with BD at the family level supported at both ages 11 and 13 years. TLIG was also positively correlated with MFA at the individual and family level, with ASLIG and KLIB correlated with MFA at the individual level only. TLIG and DBH showed a weak negative correlation at the locality level only.

DEC was highly negatively correlated with BD only at age 11 years for individuals and localities, but not for families. Significant genetic variation has been reported for BD at age 11 at the subrace level [1]. When the South King Island locality (particularly susceptible to decay) was removed from the analysis, DEC and BD (age 11) were no longer correlated at the locality level, but a significant correlation still remained at the individual level ($r = -0.230$, $P < 0.017$). DEC had weak,

positive phenotypic correlations with PY and CELL, but there were no significant genetic relationships. DEC showed negative relationships with KLIB, TLIG and EXTR for localities, which were still significant for KLIB ($r = -0.714$, $P < 0.047$) and EXTR ($r = -0.717$, $P < 0.045$) when South King Island was removed from the analysis. DEC had a positive correlation with FL at the family level only.

4. DISCUSSION

4.1. Variation in and heritability of wood properties and growth

Two of the four chemical wood traits examined had significant variation at either the locality or family within locality level, indicating there is genetic variation within *E. globulus*. Useful heritability estimates were also obtained for several traits despite their relatively large standard errors due to the small sample size. Both Klason lignin and acid-soluble lignin contents showed significant variation among localities, which suggested improvement could be made through locality selection. Surprisingly no locality differences were found for total lignin content, although this is consistent with the study of Miranda and Pereira [24] using five trees of *E. globulus* from each of four provenances. Acid-soluble lignin content showed significant variation for families and the highest

Table V. Correlations (Pearsons correlation matrix) amongst growth and wood traits for the *E. globulus* base population trial at West Ridgley, Tasmania. L = correlations amongst the nine locality means (df = 7), F = correlations amongst family means (adjusted for locality differences; df = 25 to 27) and I = phenotypic correlations amongst individuals (df = 106 to 155). Significant probability values are denoted *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

Trait	Type	Klason lignin content (KLIG) (age 13)	Acid-soluble lignin content (ASLIG) (age 13)	Total lignin content (TLIG) (age 13)	Extractives content (EXTR) (age 13)	Extent of decay (DEC) (age 13)
Mean fibre length (FL)	L	-0.576	0.558	-0.308	-0.553	0.019
(FL)	F	-0.293	-0.329	-0.353	-0.179	0.386 *
(age 11)	I	-0.156	0.082	-0.124	-0.185 *	0.110
Mean microfibril angle (MFA)	L	0.109	0.549	0.397	0.140	0.049
(age 11)	F	0.372	0.281	0.408 *	0.312	-0.005
Predicted pulp yield (PY)	I	0.299 ***	0.198 *	0.361 ***	0.188 *	-0.024
(age 11)	L	-0.707 *	0.314	-0.552	-0.779 *	0.530
Cellulose content (CELL)	F	-0.690 ***	-0.175	-0.639 ***	-0.368	0.027
(age 11)	I	-0.426 ***	-0.023	-0.421 ***	-0.379 ***	0.221 *
Basic density (BD)	L	-0.640	0.204	-0.550	-0.744 *	0.183
(age 11)	F	-0.592 **	-0.199	-0.553 **	-0.451 *	0.006
Basic density (BD)	I	-0.401 ***	-0.028	-0.394 ***	-0.399 ***	0.183 *
(age 11)	L	0.367	0.284	0.495	0.200	-0.704 *
Basic density (BD)	F	-0.417 *	-0.500 **	-0.540 **	-0.195	-0.091
(age 11)	I	-0.007	-0.079	-0.055	0.057	-0.339 ***
Basic density (BD)	L	0.235	0.153	0.307	0.152	-0.460
(age 13)	F	-0.415 *	-0.419 *	-0.510 **	0.227	0.264
Diameter at breast height (DBH)	I	-0.183 *	-0.074	-0.225 **	0.189 *	-0.032
(age 13)	L	-0.637	-0.075	-0.674 *	-0.618	0.286
Klason lignin content (KLIG)	F	0.279	0.107	0.283	0.101	-0.055
(age 13)	I	0.069	0.047	0.103	-0.003	0.087
Acid-soluble lignin content (ASLIG)	L	-0.275	-0.275	0.874 **	0.907 ***	-0.706 *
(age 13)	F	0.226	0.226	0.939 ***	0.442 *	-0.251
Total lignin content (TLIG)	I	-0.141	-0.141	0.930 ***	0.533 ***	-0.044
(age 13)	L			0.226	-0.321	-0.115
Extractives content (EXTR)	F			0.543 **	-0.162	-0.295
(age 13)	I			0.226 **	-0.312 ***	-0.089
Extractives content (EXTR)	L				0.752 *	-0.763 *
(age 13)	F				0.306	-0.322
Extent of decay (DEC)	I				0.396 ***	-0.067
(age 13)	L					-0.703 *
Extent of decay (DEC)	F					0.167
(age 13)	I					-0.069

estimated narrow-sense (0.51) and family means (0.64) heritabilities. The only published narrow-sense heritability estimate for lignin traits in *E. globulus* is for total lignin content which was estimated to be very low at 0.09 ± 0.21 [8]. The moderate narrow-sense heritability estimate for total lignin content from the current study (0.29), together with a high family means heritability (0.50), suggest that lignin may be under stronger genetic control than previously thought. Sup-

porting this is an estimate for the clonal heritability of lignin content in *E. globulus* of 0.83 from Gominho et al. [15].

Extractives content was found to have a moderate narrow-sense heritability (0.35 ± 0.23), but no statistically significant differences were found between or within localities. Significant provenance effects for extractives content have been found previously in *E. globulus* [24, 41], suggesting provenance selection could be used to improve this trait. The

lack of variation in the current study may be due to different provenances being used, or may be attributed to possible site by genotype interactions affecting this trait. Kube [20] found strong genotype by site interactions for extractives among 434 *E. nitens* trees from 40 families grown over three sites, with heritability estimates found to vary between sites from low to very high, suggesting that the factors causing extractives production in some genotypes are very site specific. Miranda and Pereira [24] found no site effects for extractives while the current study found no replicate effects in *E. globulus*.

The heritability of and the variation in basic density and diameter for *E. globulus* has been examined extensively [22] and so will not be discussed in detail here. Basic density (age 13) did not have significant variation at the family or locality level, although the trends in locality means (King Island low and the Strzelecki localities high) were consistent with previous studies that have reported significant differences [1, 10, 26]. This suggested that the small sample size and the use of only the outer part of the core reduced the power of the current study and therefore significance would generally be underestimated.

All of the trees used in this study had been cored previously which meant tissue had potential exposure to infection by wood decaying organisms. The West Ridgley site is also a wet site which has been found to be a factor leading to an increase in the incidence of decay [25]. Localities differed significantly in the extent of decay with South King Island found to be particularly susceptible. This was the first evidence of genetic variation for decay resistance in *E. globulus*. The two main races of *E. globulus* that have been used for plantation growth in Australia, Strzelecki and King Island [32], were placed at either end of the range in decay as they have been previously for basic density [10]. The fast growing but low density King Island trees were originally grown for pulp production, however, Strzelecki and Western Otways became preferred [32]. It appears that high basic density trees now selected in the breeding program may be more resistant to decay. Although the narrow-sense heritability for decay was estimated here as zero, a high family means heritability was obtained (0.50 ± 0.14). Narrow-sense heritability estimates in *E. nitens* have been found to vary between studies from 0.13 to 0.41 [20, 42], and also between sites in a single study ranging from 0.04 to 0.63 [20]. The successful exclusion of decay is likely the result of a number of traits including lignin and extractives contents, and therefore environmental and site influences are likely to be strong [20].

4.2. Correlations amongst wood properties

Phenotypic correlations indicate the presence of relationships between traits that may be due to a similar response to environmental conditions or to genetic associations. Genetic correlations are important for determining the potential for concurrent or independent selection of traits. Correlations between family means (adjusted for locality differences) and between locality means, were used to give an indication of the genetic associations for this dataset. No study has yet identified the genetic correlations among the chemical wood traits

(excluding pulp yield and cellulose content) and their correlated effects on the physical wood traits and growth in *E. globulus*.

Correlations amongst the chemical wood traits were often strong and as expected in terms of kraft pulping properties [35]. A high pulp yield and cellulose content was associated with low extractives, Klason lignin and total lignin contents at both the phenotypic and genetic levels. This was consistent with the phenotypic correlations reported by Wallis et al. [39] for 11 individuals of *E. globulus*. Miranda and Pereira [23] examined 37 provenances of *E. globulus* and reported a similar correlation between pulp yield and extractives content at the provenance level, but not with total lignin content. No significant correlations were identified between acid-soluble lignin content and Klason lignin content, consistent with the findings of Miranda and Pereira [23] who suggested differences in the lignin composition may be responsible. Lignin and extractives contents were generally positively correlated here and Ona et al. [28] found similar relationships in a within-tree study of two *E. globulus* individuals. In *E. nitens* Kube and Raymond [21] reported a very high negative genetic correlation between extractives and cellulose contents. These studies collectively suggest that selection for increased pulp yield or cellulose content are likely to result in a reduction in lignin and extractives contents, which are favourable responses for a pulpwood breeding objective.

The correlated effect of lignin on wood density is interesting as density is one of the main selection traits in the *E. globulus* breeding program. Basic density at ages 11 and 13 were significantly positively correlated at most levels ($r = 0.56$, $P < 0.01$ for families and $r = 0.65$, $P < 0.0001$ for individuals), and both were negatively correlated with lignin content at the family level. No other studies have looked at the relationship between lignin and basic density for larger sample sizes in *Eucalyptus*. However, a negative genetic correlation has also been found between density and lignin content in *Pinus pinaster* [31]. It is therefore likely that favourable lignin profiles are being indirectly selected along with high basic density. Similar to other studies in *E. globulus* [23, 28] no apparent relationship was found between basic density and extractives content, although there are reports of positive associations in both *E. globulus* [41] and *E. nitens* [21].

Positive phenotypic and genetic correlations were found between microfibril angle and lignin content which is consistent with observations for coniferous wood [34]. This relationship is thought to be due to the distribution of the microfibrils about their preferred orientation being large when the microfibril angle is large, therefore creating an imperfect alignment with more room for lignin deposition [38]. These results suggest that a reduced microfibril angle (which gives the fibre a greater tensile strength and decreases its shrinkage [7]) may be associated with improved lignin profiles for pulping.

Decay resistance is unlikely to become a major focus for selection in breeding programs for pulpwood, however, it is an important issue in the production of solid wood [20]. Understanding the genetic relationships between decay and the chemical and physical wood traits, as well as growth, is therefore important. When examining relationships between decay

incidence or extent with wood chemistry, it is important to distinguish between the chemistry found for normal healthy wood, and that found in diseased wood or in the reaction zone between healthy and diseased wood. It has been reported previously that the extractives and lignin contents are elevated in response to decay in eucalypts [5, 18], with the extractives content found to be six times greater in the reaction zone compared to healthy sapwood [6]. Only negative locality level correlations were found in the current study between the extent of decay and both extractives and lignin contents. No correlations have been found between extent of decay and extractives content in *E. nitens* [20], however, a negative relationship has been found in *E. delegatensis* [43]. Together these studies indicate that increases in extractives and lignin contents may only occur for diseased wood or in the reaction zone (both of which were removed in the current study), and the surrounding, healthy wood has normal extractives and lignin levels.

A negative correlation between the extent of decay and basic density (age 11) was observed at the locality level, which seemed to be the result of one locality (South King Island) that appeared to be particularly susceptible to decay and is known for its low basic density [10]. However, the extent of decay showed significant phenotypic correlations with basic density (age 11, negative), even with the South King Island locality removed from the analysis. Similar negative correlations have also been found in *E. delegatensis* and *E. grandis* [27, 43]. It has been proposed that lower density wood has wider cell lumina, and therefore a larger surface area is exposed to the enzymes of decay micro-organisms, and also the water and air content in the wood may be at a level that promotes fungal growth [36]. A positive genetic correlation between the extent of decay and mean fibre length was also found, and may support this idea. The lack of a significant correlation between the extent of decay and basic density at age 13, may be because the decayed area of the core was removed prior to basic density measurements and only partial cores were used. The age 11 measures of wood density may have been taken before the formation of the decay and may be more indicative of wood susceptibility to decay.

The combination of the chemical wood properties with the physical wood properties of Apiolaza et al. [1], allows a primary analysis of the genetic variation of the most important traits associated with pulp production, and how they are correlated with one another. This is the first study incorporating such a large number of traits for *E. globulus*, although the results must be treated with some caution due to the small sample size. The results indicate that when selecting for the current breeding objective traits of high basic density and pulp yield [17], other traits beneficial to the pulping process may concurrently be selected, including low lignin and extractives contents, and a high cellulose content, as well as improved fibre properties. Selection for high basic density may also result in increased resistance to decay. Growth may be selected for independently of most of the chemical wood properties and decay resistance.

Acknowledgements: The authors would like to thank Gunns Ltd for access to field trials, Leon Savage for assistance with field sampling, and also the Australian Research Council for support.

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