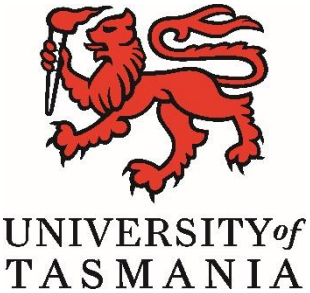

Genetic architecture of economic traits in *Eucalyptus globulus*

by

Henry Nickolas

B.Sc. (Hons), M.Sc.

School of Natural Sciences

Submitted in partial fulfilment of the requirements for the Doctor of Philosophy University of Tasmania November, 2020

Declarations

This thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the thesis, and to the best of my knowledge and belief no material previously published or written by another person except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.
This thesis may be made available for loan and limited copying and communication in accordance with the Copyright Act 1968.

The publishers of the papers comprising Chapters 2 and 3 hold the copyright for that content and access to the material should be sought from the respective journals. The remaining non-published content of the thesis may be made available for loan and limited copying and communication in accordance with the Copyright Act 1968.

Date: 17/03/2020

Henry Nickolas

We the undersigned agree with the above declarations and approve the submission of this thesis:

Brad Potts
Supervisor
School of Natural Sciences and ARC Training Centre for Forest Value
University of Tasmania, Hobart, Australia

Signed:
Date: 17/03/2020

René Vaillancourt
Supervisor
School of Natural Sciences and ARC Training Centre for Forest Value
University of Tasmania, Hobart, Australia

Signed:
Date: 17/03/2020

Peter Harrison
Supervisor
School of Natural Sciences and ARC Training Centre for Forest Value
University of Tasmania, Hobart, Australia

Signed:
Date: 17/03/2020

Chris Burridge
Acting Head of discipline
Discipline of Biological Sciences
University of Tasmania

Signed:
Date: 18/03/2020

Statement of Co-Authorship

The following people and institutions contributed to the publication of work undertaken as part of this thesis:

Candidate	Henry Nickolas, School of Natural Sciences and ARC Training Centre for Forest Value, University of Tasmania, Hobart, Australia
Author 1	Brad Potts, School of Natural Sciences and ARC Training Centre for Forest Value, University of Tasmania, Hobart, Australia
Author 2	René Vaillancourt, School of Natural Sciences and ARC Training Centre for Forest Value, University of Tasmania, Hobart, Australia
Author 3	Peter Harrison, School of Natural Sciences and ARC Training Centre for Forest Value, University of Tasmania, Hobart, Australia
Author 4	Paul Tilyard, School of Natural Sciences and ARC Training Centre for Forest Value, University of Tasmania, Hobart, Australia
Author 5	Dean Williams, Sustainable Timber Tasmania (STT), Hobart, TAS, Australia
Author 6	Geoff Downes, Forest Quality Pty. Ltd., Huonville, TAS, Australia
Author 7	David Pilbeam, Tree Breeding Australia Limited

Contribution of work by co-authors for each paper:

PAPER 1: Located in Chapter 2
Nickolas, H., Harrison, P.A., Tilyard, P., Vaillancourt, R.E. & Potts, B.M. (2019) Inbreeding depression and differential maladaptation shape the fitness trajectory of two co-occurring *Eucalyptus* species. Annals of Forest Science, 76, 10.

Author contributions:

The Candidate drafted the manuscript and was the primary author. All authors critically reviewed the manuscript. The Candidate, Author 1 and Author 2 were responsible for conceptualization. The field trial was established by Author 1. The Candidate collected the 28-year data from the trial and was responsible for collation and integration with the historic data. Data analyses were done by the Candidate with technical advice from Author 1 and Author 3.

PAPER 2: Located in Chapter 3
Nickolas H., Williams D., Downes G., Tilyard P., Harrison P.A., Vaillancourt R.E., Potts B.M. (2020) Genetic correlations among pulpwood and solid-wood selection traits in *Eucalyptus globulus*. New Forests 51(1): 137-158.

Author contributions:

The Candidate drafted the manuscript and was the primary author. All authors critically reviewed the manuscript. The Candidate, Author 1 and Author 2 were responsible for conceptualization. Author 5 was responsible for the establishment and management of the trial, as well as collecting growth, AWV and Straightness data. Author 6 was responsible for the wood density and pulp yield data. The Candidate was responsible for collation of the various datasets. Data analyses were done by the Candidate with technical advice from Author 1 and Author 3.

PAPER 3: Located in Chapter 4
Nickolas H., Williams D., Downes G., Harrison P.A., Vaillancourt R.E., Potts B.M. (2020) Application of resistance drilling to genetic studies of growth, wood basic density and bark thickness in *Eucalyptus globulus.* Australian Forestry, pp.1-8

Author contributions:

The candidate drafted the manuscript and will be the primary author. All authors critically reviewed the manuscript. The Candidate, Author 1 and Author 2 conceptualized the chapter. Author 5 was responsible for the establishment and management of field trial studied. Author 6 was responsible for the collection of the resistance drilling data. The Candidate was responsible for the data analysis, with technical advice provided by Author 1 and Author 3.

PAPER 4: Located in Chapter 5
In preparation for publication as: Nickolas H., Downes G., Pilbeam D., Harrison P. A., Vaillancourt R. E., Potts B. M. (2019) Heterosis and genetic architecture of growth and wood properties in an inter-race diallel of *Eucalyptus globulus.*

Author contributions:

The candidate drafted the manuscript and will be the primary author. All authors critically reviewed the manuscript. The Candidate, Author 1 and Author 2 conceptualized the chapter. Author 1 and Author 2 were responsible for the crossing design and crossing program. Author 7 was responsible for the establishment and management of the field trial. Author 6 was responsible for the resistance drilling data and Author 7 was responsible for the validation coring. The Candidate and Author 6 were responsible for the collection of the pulp yield data. The Candidate was responsible for the data analysis, with technical advice provided by Author 1, Author 2 and Author 3.

We, the undersigned, endorse the above stated contribution of work undertaken for each of the published (or submitted) peer-reviewed manuscripts contributing to this thesis:

Signed:

	Henry Nickolas Candidate School of Natural Sciences and ARC Training Centre for Forest Value, University of Tasmania	**Brad Potts** Primary Supervisor School of Natural Sciences and ARC Training Centre for Forest Value, University of Tasmania	**René Vaillancourt** Supervisor School of Natural Sciences and ARC Training Centre for Forest Value University of Tasmania
Date:	17/03/2020	17/03/2020	17/03/2020

Signed:

	Peter Harrison Supervisor School of Natural Sciences and ARC Training Centre for Forest Value University of Tasmania	**Chris Burridge** Acting Head of discipline Discipline of Biological Sciences University of Tasmania
Date:	17/03/2020	18/03/2020

Acknowledgement

I would like to express my sincere gratitude to my supervisory team of Brad Potts, René Vaillancourt and Peter Harrison for their guidance, practical suggestions, patience, and encouragement throughout the PhD journey. I am also thankful for their continuous and timely advice and constructive criticisms at all stages of research work. This work would not have been completed without their invaluable help and support.

I would like to forward special thanks to Paul Tilyard and Thomas Baker for their assistance with fieldwork.

This project was supported by an Australian Research Council Linkage Grant (LP140100506) awarded to the University of Tasmania in partnership with the Southern Tree Breeding Association (now Tree Breeding Australia). I thank our collaborators and industrial partners, including Tree Breeding Australia Limited, Sustainable Timber Tasmania and Forest Quality. In particular, I thank David Pilbeam, Dean Williams and Geoff Downes for their enormous support in managing the field trials and data collection.

I wish to also acknowledge the financial support provided by the Tasmanian Graduate Research Scholarship, and the ARC Training Centre for Forest Value for their contributions to conference and workshop travel costs.

My heartfelt thanks to my peers, colleagues and officemates for the love, affection, confidence, moral support and so much more that they gave me. Their constant encouragement has gone a long way in helping me throughout my studies.

I am most indebted to my loving Mom, Dad, Sister and Brother for their constant encouragement and blessings without which I would not have completed this research.

Special thanks to my wife Merin, whose overwhelming love, support and attempts at understanding my work situations helped me to make the last year of my PhD life comfortable.

Above all, I bow before the God Almighty, for all the bountiful blessings he is showering on me every moment, without which this study would never have seen light.

List of publications and presentations arising from this thesis

Refereed journal articles:

Nickolas, H., Harrison, P.A., Tilyard, P., Vaillancourt, R.E. & Potts, B.M. (2019) Inbreeding depression and differential maladaptation shape the fitness trajectory of two co-occurring *Eucalyptus* species. Annals of Forest Science, 76, 10.

Nickolas H., Williams D., Downes G., Tilyard P., Harrison P.A., Vaillancourt R.E., Potts B.M. (2020) Genetic correlations among pulpwood and solid-wood selection traits in *Eucalyptus globulus*. New Forests 51(1): 137-158.

Nickolas H., Williams D., Downes G., Harrison P.A., Vaillancourt R.E., Potts B.M. (2020) Application of resistance drilling to genetic studies of growth, wood basic density and bark thickness in Eucalyptus globulus. Australian Forestry, pp.1-8

Posters

Nickolas H, Vaillancourt RE, Potts B (2017) Differences in inbreeding depression and adaptation between two co-occurring eucalyptus species. In '11th Annual Graduate Research Conference', 7-8 September, Tasmania, Australia, p. 94

Nickolas H, Williams D, Downes G, Tilyard P, Harrison PA, Vaillancourt R, Potts B (2019) Genetic evidence for the alignment of pulpwood and solid-wood breeding in *Eucalyptus globulus*. In 'Eucalypt genetics: Fundamental and Applied Research in a Post-genome era ', 18-21 February, Tasmania, Australia. (Eds R Jones, D Steane, P Tilyard, R Vaillancourt, R Andrew, P Rymer and B Potts), p. 64

Table of content

Abstract

This thesis addresses several key issues related to the genetic architecture of traits relevant to the genetic improvement of *Eucalypts globulus* for pulpwood and solid wood production. It comprises three main studies, that address (i) the long-term effect of inbreeding, (ii) the genetic association between pulp-wood and solid wood selection traits and (iii) the importance of non-additive genetic effects.

The long-term effect of inbreeding was studied using a 28 year-old field trial comprising outcrossed, selfed and open-pollinated (OP) families of *Eucalyptus globulus* and a commonly co-occurring species *E. ovata*. These species have a mixed mating system, where open-pollinated (OP) progenies are expected to include selfs and outcrosses. Inbreeding depression for early age growth due to selfing was high (age 4: 27% for *E. globulus* and 49% for *E. ovata*) but diminished with age, as size-dependent mortality resulted in the purging of smaller inbred progenies. Most mortality occurred between the ages 4 and 13 years, and size-dependent mortality resulted in a shift in inbreeding depression from growth to survival with age. OP progenies exhibited intermediate levels of inbreeding depression, but later-age survivors exhibited no evidence of inbreeding depression, consistent with the purging of selfs. General higher mortality rate in all cross-types of *E. ovata* compared with *E. globulus* was suggested to be due to climatic maladaptation, arising from the onset of drought conditions after 10 years of comparable growth, with the inbred progeny of both species appearing more susceptible.

To further explore the genetics of growth and wood properties of *E. globulus*, two OP progeny trials were studied and the genetic association of selection traits important to pulpwood and solid wood breeding objectives were examined. These base population trials comprised of 135 families derived from native stand seed collections of the 13 races from which the Australian National *E. globulus* Breeding Population was founded. Significant additive genetic variation was found for all traits (stem diameter at breast height [DBH], stem straightness, acoustic wave velocity, wood basic density and pulp yield). There was no adverse race or additive level genetic correlation of DBH with any of the other traits studied. While race and additive genetic correlations were usually aligned, significant opposing genetic correlations were evident at these different genetic scales for pulp yield and wood basic density. Furthermore, key breeding objective-specific traits were either favourably (pulp yield - acoustic wave velocity) or not significantly (pulp yield - straightness) genetically correlated, arguing that genetic improvement between pulpwood and solid wood are well aligned and that breeding for one objective will have no adverse impact on the other.

Wood basic density in the previous study was assessed using wood cores taken from standing trees using a mechanical corer. This is a relatively expensive assessment approach and has limited the large-scale assessment of wood density in breeding trials. A hand-held IML Power Drill series instrument (RESI) is starting to be used in the forest industry to measure wood basic density. The RESI trace also can be used to extract measures of stem diameter and bark thickness. This study quantified and confirmed the genetic association between RESI resistance values and core basic density (≥0.95). It also confirmed high genetic correlations (>0.90) of bark thickness and diameter (DBH) estimated from RESI to the analogous traditional methods. Significant family and subrace differentiation were detected for the three RESI-derived traits, with the subrace differentiation for bark thickness exhibited among the highest subrace differentiation (QST > 0.63) reported to date for *E. globulus*, signalling divergent selection. This study confirmed that traditional measurements of wood density, DBH and bark thickness can be replaced with RESI measurements, for the genetic studies of *Eucalyptus globulus*.

While OP trials have been useful to demonstrate significant racial variation within *E. globulus* and provide the initial estimates of the levels of additive genetic variation for selection traits, they do not allow the estimation of non-additive genetic effects. This is a key issue now that the advanced generations of the Australian National *E. globulus* Breeding Program comprise full-sib families and there are deployment options for exploiting non-additive genetic effects through full-sib family deployment. The relative importance of non-additive genetic effects, including inter-race heterosis, was examined using a trial derived from first generation selections from the breeding program. The trial was 9 year-old and established from 515 full-sib families derived from a diallel crossing design involving intra- and inter-race hybrids of the three most widely used races in the breeding program. Growth (diameter at breast height; DBH) was assessed at ages 2, 4, 6, and 8 years, allowing the detection and monitoring of changes in additive, dominance/heterosis as well as maternal and reciprocal effects with age. Key findings include the generally insignificant maternal and reciprocal effects, significant dominance variance (22 to 34% of the additive variance) and significant inter-race heterosis which increased with age (2.2% to 6.5%). While not significant, all inter-race combinations were better than the best of their intra-race crosses (‘better-parent’ heterosis), consistent with low levels of inbreeding in intra-race crosses. Three replicates of the trial were non-destructively assessed for pulp yield using NIR spectroscopy, and resistance drilling (RESI) used to assess wood basic density as well as bark thickness. The reliability of the RESI basic density estimates were validated, and in contrast to growth, these traits were shown to be predominantly under additive genetic control.

In summary, the studies of both open-pollinated (OP) and control-pollinated progeny trials have provided novel insights into the genetic architecture of growth, wood property traits and bark thickness in *Eucalyptus globulus*. The demonstration of significant non-additive genetic effects for growth demonstrates the advantage of identifying the best heterotic full-sib families for deployment through mass-supplementary pollination, which also gives the additional benefit of avoiding inbreeding. In addition, the study confirms the neutral or favourable association between pulpwood and solid wood traits in this species, arguing that with the breeding so far focused on pulpwood, pulpwood-selected germplasm and current plantations will not be degraded in terms of their genetic suitability to use them for solid-wood products.

Chapter 1 - General introduction

1.1. Eucalypts

Eucalypts are a large group of woody plants in the family Myrtaceae (Grattapaglia *et al.* 2012). This predominantly tree group of plants consists of seven genera, including *Eucalyptus*, *Corymbia*, *Angophora*, *Arillastrum*, *Allosyncarpia*, *Eucalyptosis* and *Stockwellia* (Ladiges *et al.* 2003); of which, *Eucalyptus* is the largest genus, consisting of more than 750 species (Nicolle 2019). *Eucalyptus* is indigenous to Australia and some of the islands to its north, including Papua New Guinea (Ladiges *et al.* 2003). They are also the main hardwood plantation species grown in tropical, sub-tropical and temperate regions of the world (Eldridge *et al.* 1993; Odoom 2001). Altogether, there is over 20 million ha of *Eucalyptus* plantations worldwide (Harwood 2011), of which 835,000 ha are in Australia (Downham and Gavran 2019). They provide the raw material for several industrial products, for example - eucalypt oil and gum, medicinal products, timber and most importantly, pulp for the paper industry (Coppen 2002; Batish *et al.* 2008; McGavin *et al.* 2014; Hart and Santos 2015; ABARES 2016b). Although *Eucalyptus* is economically important and species rich, only a handful (approximately 1 %) of species have been used at an industrial scale (Cotterill and Macrae 1997). Industrial plantations of *Eucalyptus* in the world are dominated by nine species or their hybrids - *Eucalyptus camaldulensis, E. dunnii, E. globulus, E. grandis, E. nitens, E. pellita, E. saligna, E. tereticornis, and E. urophylla* (Harwood 2011). The most planted eucalypt species in Australia is *E. globulus* followed by *E. nitens* (ABARES 2016b). In 2017-'18, there was 457,000 ha of *E. globulus* plantations in Australia, which is 51% of the total hardwood estate (869,000 ha; Downham and Gavran 2019).

Eucalyptus globulus is native to south-eastern Australia, including the islands of Tasmania, and is commonly called the 'Tasmanian blue gum' (Nicolle 2006). Studies identified *E. globulus* as part of a complex of four closely related species *E. maidenii, E. pseudoglobulus, E. bicostata and E. globulus* (Jordan *et al.* 1993; Brooker 2000). Core populations of these species are morphologically and geographically different to each other but they are linked by intergrade populations (Jordan *et al.* 1993; Jones *et al.* 2012). This has resulted in these taxa often being taxonomically treated as subspecies (Kirkpatrick 1975) but, following the latest informal taxonomy of the genus (Nicolle 2019), the species-level terminology is adopted here. What is referred to as *E. globulus* in most breeding/genetic studies, including the present study, is core *E. globulus* and its intergrades (Dutkowski and Potts 1999), which is hereafter referred to as *E. globulus*.

Eucalyptus globulus is one of the top ten planted forest tree species around the world (Potts *et al.* 2014) and one of the three main species used by the eucalypt Kraft pulp industry, worldwide (Cotterill and Macrae 1997). Countries such as Australia, Chile, Spain and Portugal grow *E. globulus* mainly for pulpwood, for paper making. In Australia, during 2017-2018, 82% of the hardwood plantations (where *E. globulus* is dominant) were cultivated for the production of pulp logs for producing woodchips, pulp and eventually paper (Downham and Gavran 2019). However, there is an increasing interest in using plantations of this species for higher valued solid-wood products such as sawn timber, veneer and composites (Nolan *et al.* 2005; Hamilton *et al.* 2007; Derikvand *et al.* 2016). While the export of logs harvested from pulpwood plantations for rotary peeled veneer production is expanding (McGavin *et al.* 2015), plantations from which sawlogs or veneer logs are extracted usually require different silvicultural practices to that used in pulpwood plantations. Plantations managed for such solid wood production need to be thinned and pruned to produce clear wood, and they have a longer rotation age than pulpwood plantations, to produce bigger logs (Nolan *et al.* 2005; Beadle *et al.* 2008). In Australia, the establishment and management of commercial hardwood plantations for solid wood products have increased (McGavin *et al.* 2014), with 17.9% of plantations managed for sawlog production in 2019 (Downham and Gavran 2019). The total hardwood log (saw and veneer logs) production from plantations in Australia has increased from 0.19 million m^3 in 2015-16 to 0.48 million m^3 in 2016-17 (ABARES 2018) and is forecasted to increase to approximately 1 million m^3 per year by 2055-2059 (ABARES 2016a).

1.2. Breeding system of *Eucalyptus globulus*

Similar to many forest trees (White *et al.* 2007) and eucalypts in general (Byrne 2008), *E. globulus* has a mixed mating system, thus seeds produced through open pollination (OP) may contain outcrossed and inbred progenies. The reported outcrossing rates of individual trees range from 0.13 to 1.00 (Hardner *et al.* 1996; Patterson *et al.* 2004b; Potts *et al.* 2008; Rao *et al.* 2008; Mimura *et al.* 2009). Outcrossing rate is positively correlated to the level of self-incompatibility of the tree (Patterson *et al.* 2004b), a trait which is genetically controlled with genotypes ranging from nearly fully self-compatible to fully self-incompatible (McGowen *et al.* 2004; Patterson *et al.* 2004b). The outcrossing rate can be up to 0.47 for self-compatible trees, with higher outcrossing rate in the upper- than the lower-canopy (Patterson *et al.* 2001; Patterson *et al.* 2004b), which potentially reflects the type of pollinator (Hingston *et al.* 2004). The outcrossing rate in native stands decreases with fragmentation (Mimura *et al.* 2009) and decreasing stand density (Borralho and Potts 1996; Hardner *et al.* 1996). For example, the

average outcrossing rates estimated using microsatellite loci from continuous native populations range from 86 to 89% but was reduced to 65 to 79% in a fragmented forest in agricultural landscapes (Mimura *et al.* 2009). In addition, there is evidence for bi-parental inbreeding in seed collected from native stands (Mimura *et al.* 2009), no doubt arising from the family group structuring which occurs within the forests (Hardner *et al.* 1998; Jones *et al.* 2007). Variable outcrossing rates have also been reported from trees in seed orchards of *E. globulus*, with average rates ranging from 77 to 92% reported for seedling seed orchards (Potts *et al.* 2008) and 60% for a grafted clonal seed orchard (Patterson *et al.* 2004b). The study by Rao *et al.* (2008), of a grafted *Eucalyptus globulus* breeding arboretum where genotypes were planted as single line plots, revealed that outcrossing rate for individual mothers varied from 15-95%, and averaged 48%. These studies clearly show that open-pollinated seed of *E. globulus* derived from native stands and production facilities will contain variable amounts of inbred progenies, either through self-pollination or bi-parental inbreeding.

Inbreeding is a significant issue in evolutionary biology and breeding, that can lead to the loss of heterozygosity in the populations and reduced fitness or productivity, termed inbreeding depression (Husband and Schemske 1997). Inbreeding depression is common in many plant species and is opposite to the phenomenon termed hybrid vigour/heterosis which reflects the improved performance of crossbred progeny (Lippman and Zamir 2007). Inbreeding changes the genetic relatedness among progeny and, when combined with inbreeding depression, may bias breeding value predictions and quantitative genetic parameters calculated from OP progeny tests, including estimates of the additive genetic variance (Namkoong 1966) and heritability (Hodge *et al.* 1996; Costa e Silva *et al.* 2010b). Therefore, understanding the consequences of inbreeding on performance is important for tree improvement, as forest trees including *Eucalyptus* are often subject to high levels of inbreeding depression (Petit and Hampe 2006; Ginwal 2010; Hedrick *et al.* 2016).

Eucalyptus globulus is the eucalypt species in which the consequences of inbreeding has been most studied. Hardner and Potts (1995), Hardner *et al.* (1998) and López *et al.* (2000b) showed a marked reduction in seed set or seed viability as a result of self-pollination in this species. Most of the reduced seed set is believed to be due to post-zygotic abortion of self-fertilised seed, which is an early form of inbreeding depression (Pound *et al.* 2003; McGowen *et al.* 2010). *E. globulus* has also shown severe inbreeding depression for later age growth and survival (Hardner and Potts 1995; Hardner *et al.* 1998; Costa e Silva *et al.* 2010b). For example, Costa e Silva *et al.* (2011) reported inbreeding depression for survival and stem diameter at age 14 years as 49% and 36% respectively, when selfed progenies were planted in competition with

other cross-types (OP and outcross). This study also included four degrees of inbreeding (outcrosses, crosses among half-sibs, crosses among full sibs and selfing) and showed a linear relationship of inbreeding depression for survival and growth with the inbreeding coefficient. However, the long-term (>15 years) effects of inbreeding depression have not been studied in this species. In general, the degree of inbreeding depression exhibited in open-pollinated progeny will depend upon the level of outcrossing and tendency of the mother tree to exhibit inbreeding depression (Hardner *et al.* 1996; Costa e Silva *et al.* 2010a).

While many plant species show improved progeny performance following outcrossing, in some cases outbreeding can lead to a reduction in progeny performance, and this phenomenon is termed outbreeding depression. Increased population divergence through geographic or environmental isolation (among other processes) has been implicated in outbreeding depression following inter-population crosses (Figure 1.1) (Waser 1993; Frankham *et al.* 2011). In some cases, reduced fitness may not be apparent in the first generation progeny following inter-population crossing, but rather expressed in the later generation progeny (Fenster and Galloway 2000; Edmands 2007). Indeed, inter-population crossing may result in genetic rescue of inbreed populations, but later it may result in outbreeding depression in subsequent generations (Edmands 2007). Studies suggest that there is an optimal degree of genetic divergence between parents for the expression of heterosis (Figure 1.1), which represents a balance between inbreeding and outbreeding depression (Waddington 1983; Waser and Price 1989; Waser and Price 1994; Tallmon *et al.* 2004; Ayre *et al.* 2019).

In forest trees, inter-provenance/race hybridisation has been reported to result in better progeny performance for growth traits compared to the average of the crosses within the parental provenances/races (Ying 1978; Harfouche *et al.* 1995b; Harfouche *et al.* 2000; Joseph *et al.* 2000). This is certainly the case in *E. globulus*, the main eucalypt species in which the consequences of inter-provenance/race crossing has been studied (Vaillancourt *et al.* 1995; Volker *et al.* 2008; Costa e Silva *et al.* 2014). However, while outbreeding depression has been reported in inter-specific hybrids of eucalypts (Potts *et al.* 1992; López *et al.* 2000b; Costa e Silva *et al.* 2012; Larcombe *et al.* 2014), to date there are no reports of outbreeding depression at lower levels of genetic divergence associated with, for example, inter-provenance/race hybridisation within species.

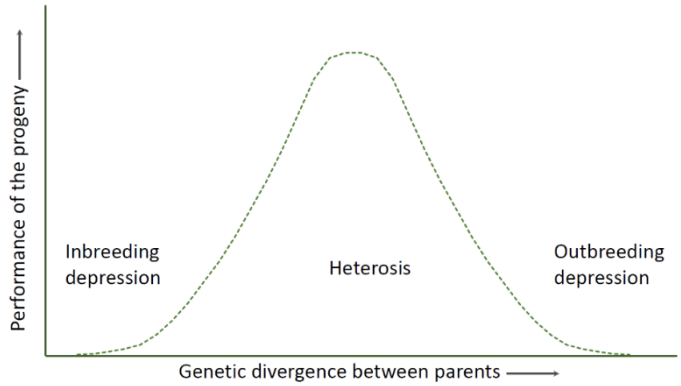

Figure 1.1. A hypothetical curve representing the relationship between parental genetic divergence and progeny fitness (conceptualized from Waddington 1983; Waser 1993)

1.3. *Eucalyptus globulus* breeding programme

Deployment of genetically superior stock in plantations is one of the strategies used to increase the production of high-quality logs for both pulpwood and solid wood products. Many traits that affect these end-products, e.g. plantation productivity and wood quality, are under moderate to strong genetic control (Greaves *et al.* 2004; Kube and Raymond 2005), hence it is possible to improve these 'economic traits' to increase profitability for forest growers/processors through tree breeding (Potts *et al.* 2011). Tree breeding is a long-term process and the selection of parents mostly will have its impact on profitability after up to 20 years (Greaves *et al.* 2004). However, the importance of tree improvement has been shown by the increase in yield made possible by various breeding programmes since the 1950's (Cotterill and Macrae 1997; Namkoong *et al.* 2012), including those in *Eucalyptus* (Hart and Santos 2015).

The domestication of *Eucalyptus globulus* started in the 1960's in Portugal (Ribeiro *et al.* 2011), and there are now breeding programmes in many countries including Australia, Chile, Portugal, and Spain (Potts *et al.* 2004). In Australia, domestication of *E. globulus* was started in 1971 (Potts *et al.* 2014) and by 1976 the first range-wide seed collection of multiple provenances was done which resulted in the planting of trials in various countries, including Australia (Eldridge *et al.* 1993). This collection included provenances of *E. pseudoglobulus*, *E. bicostata* and *E. maidenii*, as well as *E. globulus* (Volker and Orme 1988; Miranda *et al.* 2001), and resulted in breeding programmes focusing on *E. globulus* (Eldridge *et al.* 1993). This was followed by a large range-wide native-stand seed collection of the core and intergrade populations of *E. globulus* by the Australian Tree Seed Centre (ATSC) of CSIRO in 1987 and 1988 (Potts *et al.* 2014). This collection formed the main component of the base population of many breeding programmes around the world, including Australia (Potts *et al.* 2014). The most complete trials

established from this collection were in Tasmania, Australia, and formed the basis of many quantitative genetic studies of this species (reviewed in Potts *et al.* 2004; 2011; 2014). The study of the provenance variation in five Tasmanian trials by Dutkowski and Potts (1999) identified significant geographically based genetic variation in multiple quantitative traits which were summarised by classifying the gene-pool (i.e. *E. globulus* and intergrade populations) into 13 races and 20 sub-races.

The Southern Tree Breeding Association (now Tree Breeding Association Inc; TBA) combined the pre-existing genetic resources for *E. globulus* in Australia to establish the Australian National *E. globulus* Breeding Programme in 1994, which aimed at the long-term population improvement of the species (McRae *et al.* 2001; Potts *et al.* 2014). The original genetic resource for the base population of this breeding programme was the open-pollinated (OP) families collected from native stands which were established in field trials across Australia by members of the programme. Some of these base population trials were culled to convert them into open-pollinated seed orchards for producing seeds for deployment (Volker *et al.* 1990). However, subsequent generations of breeding have been undertaken using manual pollination, allowing tracking of both the male and female pedigrees (Potts *et al.* 2014). *Eucalyptus globulus* possesses large flower (Jordan *et al.* 1993; Jones *et al.* 2002), which has facilitated the adoption of advancements in the pollination techniques called 'one-stop pollination' (Harbard *et al.* 2000) or 'single-visit pollination' (Williams *et al.* 1999). This ease of crossing has allowed the implementation of large-scale controlled crossing to generate full-sib families for breeding purposes, which is difficult and costly with smaller-flowered species such as *E. nitens* (Potts *et al.* 2014). In addition, modification of this control crossing technique has been utilised by many companies for the production of *E. globulus* seeds of specific parental combinations for deployment using the method called mass supplementary pollination (Patterson *et al.* 2004a; Potts *et al.* 2008). In response to earlier studies on forest trees that had shown positive heterosis for inter-race/population crosses (Ying 1978; Harfouche *et al.* 1995b; Joseph *et al.* 2000; Volker *et al.* 2008), coupled with the desire to reduce the flowering time differences inherent among the different races of *E. globulus* (Gore and Potts 1995; Jones *et al.* 2011), the Australian National *E. globulus* Breeding Programme adopted a strategy of bi-parental crossing of selections from the base population with a focus on inter-race crossing. As of 2014, more than 1400 full-sib families had been established in field trials, 76% of which were inter-race combinations with the dominant races being Furneaux [F], Strzelecki Ranges [S] and Western Otways [W] (McRae *et al.* 2001; Potts *et al.* 2014). Hereafter the term provenance is used in a general sense for populations/collections originating from a specific geographic area,

but where a specific intra-species classification has been defined and published, such as the hierarchal classification of races and subraces of *E. globulus* (Dutkowski and Potts 1999), the published terminology has been followed.

The Australian National *E. globulus* Breeding Programme has historically focused on improving Australian plantations for short-rotation pulpwood production, where the plant traits affecting profitability are mainly growth (volume area per hectare), wood basic density and pulp yield (Borralho *et al.* 1993; Greaves *et al.* 1997; Raymond 2002; Hart and Santos 2015). However, in combination with the appropriate silvicultural management, genetic improvement is also an option for increasing the production of high-quality logs with significant clear wood for solid wood products (Nolan *et al.* 2005). While traits such as growth and wood density are shared between solid wood and pulpwood breeding objectives (Raymond 2002; Hamilton *et al.* 2007; Hamilton *et al.* 2009a; Hamilton *et al.* 2010), the profitability of plantations grown for solid wood products are also potentially affected by different traits compared to pulpwood plantations, including stem straightness, branch shedding, wood stiffness [MOE], log-end splitting and log bowing, decay, shrinkage and collapse (Potts *et al.* 2011). However, relative to the pulp-wood selection traits, there are relatively few studies of these solid wood traits in *E. globulus* (Hamilton *et al.* 2010; Callister *et al.* 2011; Potts *et al.* 2014). This is due to various reasons such as lack of an established eucalypt solid-wood processing industry based on *E. globulus* plantations, longer rotation intervals, wide range of products and the scarcity of genetic field trials grown under solid-wood silvicultural regimes (Hamilton *et al.* 2010; Beadle *et al.* 2011; Potts *et al.* 2014).

1.4. Estimation of genetic parameters

The genetic gain achieved through a breeding programme is in part influenced by the accuracy of the predicted breeding values and other genetic parameters (Cappa *et al.* 2017). In the case of *E. globulus*, Best Linear Unbiased Predictions (BLUP) implemented with multi-trait individual tree mixed models have long been used for the estimation of breeding values (Jarvis *et al.* 1995; Kerr *et al.* 2001). These models initially could not account for the male pedigree due to the use of open-pollinated progeny (Jarvis *et al.* 1995). However, as full-sib families dominate the later generations of the breeding programme, full pedigree information back to the base maternal parent is increasingly available.. More recently, this pedigree information is starting to be supplemented with estimates of genomic relatedness among individuals to further improve the accuracy of genetic parameters and breeding values (Jonas and de Koning 2013) and decrease the length of the breeding cycle, as is occurring in many forest trees species (Ratcliffe

et al. 2017). Key to BLUP analyses is the genetic parameters that define the genetic architecture of the species from a quantitative genetic perspective. Such parameters include race variation, the proportion of additive genetic variance (narrow-sense heritability), dominance effects (including inbreeding depression), maternal and reciprocal effects, epistasis, genotype x environment interactions (GxE) and genetic correlations among traits (Lynch and Walsh 1998; White *et al.* 2007). Population improvement mainly focuses on exploiting additive genetic variance, which is a key factor in determining the response of each trait to selection (Falconer and Mackay 1996). In many forest tree programmes, this is undertaken using open-pollinated progenies (White *et al.* 2007). However, non-additive genetic effects are also found to be important in some cases, hence they affect the accuracy of the models used for genetic evaluation as well as breeding and deployment decisions (Costa e Silva *et al.* 2004; White *et al.* 2007).

In the case of *E. globulus*, some of the first signals of the inaccuracies in estimating breeding values from models that don't account for non-additive effects came from studies comparing the growth performance of open-pollinated and control pollinated progenies. Hodge *et al.* (1996) reported that the parental breeding values for growth estimated from open-pollinated families and full-sib families from the same parents crossed in a factorial mating design were not significantly correlated. Using the same trials, Volker (2002) also found inflated heritabilities for all growth traits from open-pollinated progenies compared to controlled pollinated progenies. In another experiment with different parents, Costa e Silva *et al.* (2010a) subsequently showed that the growth of open-pollinated progenies was more correlated with that of selfed progenies than with outcrossed polymix progenies from the same parents. In addition, the inflation of heritability when the non-additive effects are not considered in the model was also empirically demonstrated by Araújo *et al.* (2012). These findings suggested a stronger influence of non-additive effects, including inbreeding depression, than parental additive effects on open-pollinated progeny performance. A study dominated by selections from the Portuguese landrace of *E. globulus*, for example, suggests that non-additive genetic effects on growth may be nearly (80%) as large as the additive genetic effects (Araújo *et al.* 2012). The few available estimates for *E. globulus* of the relative importance of additive and dominance genetic variance under outcrossing have generally confirmed this result, showing significant dominance genetic variation for growth traits (Li *et al.* 2007; Araújo *et al.* 2012; Callister *et al.* 2013), although there are exceptions (Costa e Silva *et al.* 2004). However, the limited size and breadth of genetic material used in most of these studies could have affected extrapolation to the entire breeding population.

Another consideration is the role of maternal and non-maternal reciprocal effects on progeny performance, which can be important components of the non-additive genetic variance (Misztal 1997), but may also have a non-genetic basis (Roach and Wulff 1987). *E. globulus* trees differ markedly in their reproductive potential as female parents (Suitor *et al.* 2009b; 2009c), and if reciprocal effects on progeny performance are important, then the directionality of the crossing will need to be considered in both breeding and deployment. While reciprocal effects have been reported in seed traits and early-age performance in plants, including forest trees (Roach and Wulff 1987; Lindgren and Wei 1994; López *et al.* 2003; Rix *et al.* 2012; Vivas *et al.* 2017; Vivas *et al.* 2019), there are only a few published studies addressing this issue in eucalypts in general (Wyk 1977; Vivas *et al.* 2017; Vivas *et al.* 2019), and only one field study in *E. globulus* (López *et al.* 2003). The study by López *et al.* (2003) showed statistically significant reciprocal effects on early growth of *E. globulus* seedlings, but these rapidly diminished after field planting. However, the significance of these reciprocal effects has not been tested in experiments involving the main races and selections in the Australian National *E. globulus* Breeding Programme. In contrast to growth, many other traits studied in *E. globulus*, including disease resistance (Dungey *et al.* 1997) and wood density (Volker 2002), are mainly under additive genetic control, and parental breeding values are relatively well predicted from open-pollinated progenies.

Non-additive genetic effects such as dominance can only be captured with deployment strategies such as cloning or deployment of elite full-sib families [family forestry] (Foster and Shaw 1988; Lynch and Walsh 1998; White *et al.* 2007). While some plantations of *E. globulus* are established through clonal propagation of selected genotypes in South America and the Iberian Peninsula (Gaspar *et al.* 2005; López *et al.* 2010; Griffin 2014), clonal propagation is not economic in Australia, and most plantations are established with seedling propagules, mainly from open-pollinated seed orchards (Potts *et al.* 2004; Potts *et al.* 2008; Griffin 2014). Nevertheless, in *E. globulus* there is the family forestry option of deploying full-sib families through mass supplementary pollination, although such seed is more expensive to produce than open-pollination seed. Apart from avoiding inbreeding depression through the mixed mating which occurs under open-pollination (Patterson *et al.* 2004b), a key issue in understanding the advantages of deploying full sib-families is the relative importance of non-additive genetic effects as explained above, including inter-race heterosis (Potts *et al.* 2008; Araújo *et al.* 2012).

While non-additive effects such as dominance have been estimated in *E. globulus* from relatively sparse mating designs used in operational breeding programmes (Li *et al.* 2007; Araújo *et al.* 2012) or more structured, denser crossing designs such as factorials (Vaillancourt *et al.*

1995; Hodge *et al.* 1996; Volker *et al.* 2008), complete or incomplete diallel mating designs are needed to accurately estimate reciprocal effects (López *et al.* 2003; Costa e Silva *et al.* 2014), although these may compromise other parameter estimates due to the smaller number of parents involved. A complete diallel involves crossing all the parents with one another in both directions (i.e. as male and female) in all possible combinations. This compares with a factorial design where the males and females are different individuals (Acquaah 2009). Both designs allow estimation of additive and dominance variances assuming no epistasis (Misztal 1997), but diallel schemes are advantageous as they can estimate maternal and non-maternal reciprocal effects. To estimate the dominance and additive variance with equal accuracies, about 20 times more data is required than when only measuring additive variance (Misztal 1997). Realizing such crossing on a large scale has practical limitations, there are thus relatively few quantitative genetic studies of non-additive genetic effects in forest trees (Paul *et al.* 1997; Isik *et al.* 2003; Costa e Silva *et al.* 2004; Callister and Collins 2008; Miguez-Soto *et al.* 2016). There are even fewer studies involving diallel crossing designs at a population (Harfouche and Kremer 2000) or full-sib family (Blada 1999; Isik *et al.* 2003; Mihai *et al.* 2014; Russell *et al.* 2015; Dong *et al.* 2019) level in forest trees. Accordingly, there is little appreciation of the extent to which non-additive genetic effects, particularly reciprocal and maternal effects, influence the genetic architecture of traits of biological and economic significance in forest trees.

The present study utilises a combination of open-pollinated and control-pollinated progeny trials of *E. globulus* to address the various issues raised above. Five previously established and monitored trials were studied, with new growth and wood property assessments undertaken and combined with historic data. The first study (Chapter 2), regarding the inbreeding depression, was done using a 28-year old field trial which was planted in 1988 at Ridgley in north-western Tasmania. This trial included *E. globulus* and *E. ovata*, with three cross-types each (outcrosses, open-pollinated and selfs). The studies on the genetic correlations (Chapter 3 & 4) were done using three *E. globulus* field trials established with open pollinated seed lots collected from wild trees from throughout the geographic range of *E. globulus*. Two of these trials were established in north-west (NW) Tasmania (Salmon River [SR] and Togari [TO]) in 2005 and the other in northern Tasmania (Latrobe [LA]) in 1989. For the diallel study (Chapter 5), a trial established in 2007 at Manjimup, Western Australia was used. This trial was established using full-sib families from a diallel mating of *E. globulus* parents from three different races.

1.5. Thesis outline

This thesis addresses several key issues related to the genetic architecture of *E. globulus*, for various pulpwood and solid wood selection traits and their genetic association with each other, using the above open-pollinated and control-pollinated progeny trials. Chapter 2 studies the long-term effects of inbreeding on the survival, growth and reproduction of *E. globulus* and *E. ovata*, and compares the effects of inbreeding to the adaptive differences between the two species over 28 years. While there have been numerous studies undertaken on the quantitative genetics of various traits of *E. globulus* using the base population trials (Potts *et al.* 2004; Potts *et al.* 2014), there are still many outstanding issues, several of which are the focus of Chapter 3, especially validating and understanding the race variation and the genetic control and association of key selection traits in the pulpwood and solid wood breeding objectives. Wood basic density is generally assessed using direct or indirect techniques (Downes *et al.* 1997; Stackpole *et al.* 2010a). Previously, wood density was assessed using oven-dried 12 mm wood cores obtained with a mechanical coring machine or using a Pilodyn. However, there is a growing interest in using the IML Power Drill series instrument (RESI) as an indirect method to assess wood density in various tree species (Silva *et al.* 2017; Downes *et al.* 2018; Fundova *et al.* 2018; Sharapov *et al.* 2019). This method is often more cost effective and faster than other techniques, and generally better predicts wood density than the Pilodyn - an alternative option for high-speed non-destructive sampling (Downes *et al.* 2018; Fundova *et al.* 2018). The strong phenotypic level relationship between RESI resistance values with the core basic density has been previously reported (Downes *et al.* 2018). However, to use it for tree improvement programmes, genetic rather than phenotypic correlations need to be estimated. In Chapter 4, I test the utility of the RESI for the genetic assessment of various traits in standing trees. The genetic correlations between resistance drill measurements and the analogous traditional methods used to assess diameter, wood density and bark thickness was quantified. In Chapter 5, resistance drilling is used to assess wood density variation and bark thickness in a large-diallel trial based on selections of *E. globulus* from three races. These measurements are combined with pulp yield predictions and an age-series of stem diameter measurements to determine the magnitude of inter-race heterosis and within-race additive and non-additive genetic variances for the key pulpwood selection traits. The non-additive genetic variances estimated include dominance, as well as maternal and non-maternal reciprocal variances and these are compared to the additive genetic variance. It should be noted that the parameters presented are estimates based on the parental samples of the populations studied and their extrapolation to population level parameter estimates will obviously depend upon the number and representativeness of parents sampled. They also refer to the genetic architecture within

genetic groups (e.g. races or subraces) as is traditionally the case in eucalypt studies. Further, several of the studies are restricted to single site experiments and thus the genotype x environment interaction effects are not included, but the consistency of the results were gauged by comparison with other studies.

The four experimental chapters are presented in the format of scientific journal articles. Every chapter contains an introduction explaining the background of the study, the methodology used for that experiment, following the results and discussion. Since every chapter contains a discussion about the findings in the context of the present knowledge, Chapter 6 is a brief general discussion which draws together the findings of the thesis and highlighting the implications of these results to the Australian National *E. globulus* Breeding Programme.

Chapter 2 - Inbreeding depression and differential maladaptation shape the fitness trajectory of two co-occurring *Eucalyptus* species

2.1. Introduction

Inbreeding is an important consideration in evolutionary biology and genetic improvement. It affects the levels of genetic variability in populations through drift and can change the expression of quantitative genetic variation in populations (Charlesworth and Willis 2009). Additionally, it can reduce performance in fitness related traits (Charlesworth and Charlesworth 1987), often leading to size-dependent mortality (Koelewijn *et al.* 1999; Costa e Silva *et al.* 2011). This reduced performance is termed inbreeding depression (ID) and is thought to be caused by two main mechanisms - dominance and over-dominance (Roff 2002). Dominance is the most accepted mechanism (Hedrick and Garcia-Dorado 2016), positing that ID results from the expression of deleterious recessive or partially recessive alleles. Such alleles are generally rare and accumulate in large, particularly outcrossed populations giving rise to a 'genetic load' which is expressed upon inbreeding (Willi *et al.* 2006). In plants, inbreeding can occur through self-pollination or mating between related individuals; which may occur as a consequence of, for example, a mixed mating system and restricted opportunities for unrelated mating in small populations, respectively (Goodwillie *et al.* 2005). Under such conditions, ID may act to counter local adaptation in wild populations (Willi *et al.* 2006) and limit the response to artificial selection in breeding programs (Kardos *et al.* 2016).

Inbreeding has been implicated in the response of plant populations to climate change from several perspectives (Leimu *et al.* 2010). It has been suggested that stressful and deteriorating environments may increase levels of inbreeding through, for example, production of smaller flowers (Strauss and Whittall 2006) and loss of pollinators (Potts *et al.* 2010). Climate change may also increase inbreeding through reduced population sizes (i.e. population bottlenecks) arising from maladaptation and habitat fragmentation (Leimu *et al.* 2010; Levin 2011). Indeed, with range shifts associated with differential adaptation of species (Lenoir *et al.* 2008), an interplay between climatic maladaptation and ID is expected to arise from range fragmentation and founder effects at the trailing and leading edge of a species range (Hampe and Petit 2005; Leimu *et al.* 2010). Climate shifts may also lead to changes in the fitness impact of inbreeding, with ID reported to increase in more stressful environments (Armbruster and Reed 2005). Such issues are particularly relevant to forest trees, which dominate many of the world's terrestrial ecosystems and climate change is already impacting their populations

worldwide (Bertrand *et al.* 2016). Moreover, due to their often large population sizes and outbred mating systems, these long-lived organisms are particularly vulnerable to ID (Petit and Hampe 2006).

Trees of the genus *Eucalyptus* L'Hér. dominate many of Australia's forest and woodland ecosystems. Decline of eucalypt populations, likely linked to climate change, has already been reported (Matusick *et al.* 2013; Prober *et al.* 2016), and future climate projections suggest that a significant component of the Australian eucalypt flora will be outside of their historic climate envelopes by 2080's (González-Orozco *et al.* 2016). Given the often limited dispersal capabilities of eucalypt seed, there will likely be a heightened risk of maladaptation in these changing environments (Booth 2017). Any evolutionary change in eucalypts will depend upon selective filtering of the genetic diversity present in the dispersed seed (Martinsen *et al.* 2001). In the case of most eucalypt species, open-pollinated (OP) seed is derived from mixed mating and thus may contain various proportions of selfs, as well as related and unrelated outcrosses (Potts and Wiltshire 1997). It thus may, to various extent, reflect the additive genetic adaptations of the female (Hodge *et al.* 1996). Accordingly, selective filtering of each regenerating cohort will be expected to involve a dual process of selection against the products of inbreeding and environmentally maladapted genotypes.

We here study the dynamic interplay of adaptation and ID on the long-term composition of a common garden field trial. The trial comprised two eucalypt species - *Eucalyptus globulus* Labill. and *E. ovata* Labill. – whose ranges broadly overlap in the wild, but on a fine-scale occupy separate habitats, and form relatively sharp boundaries (Williams and Potts 1996). As both species are widespread with large populations and ecologically differentiated, we hypothesize that (i) selective filtering of the field trial will occur through a combination of ID and inter-specific differences in adaptation, (ii) the expression of inbreeding depression will vary through time, and (iii) differences in adaptation between species will be accentuated with inbreeding.

2.2. Materials and methods

2.2.1. Field trial and assessed traits

To compare the effect of inbreeding depression (ID) on *E. globulus* and *E. ovata*, three cross-types (outcrosses, open-pollinated and selfs) were generated, involving 23 *E. globulus* and 12 *E. ovata* undomesticated females (Table 2.1; crossing methodology is detailed in Hardner and Potts 1995; see also Lopez et al. 2000). The *E. globulus* trees used as females were mainly ornamentals growing in a linear road-side planting near Hobart, Tasmania. The *E. ovata* females were growing in a remnant native-forest south of Hobart. To generate outcrosses, trees were

crossed with unrelated pollen derived from either single-tree pollen collections from southeast Tasmania, Australia or polymixes (i.e. a mix of pollen collections). The *E. globulus* outcross population comprised 14 full-sib and 13 polymix families. These families represented 24 parents, (11 as females; four as pollen parents and four as both pollen parents and females in the full-sib crossing; and an additional five parents as components of the pollen mix). The *E. ovata* outcross population was less diverse, comprising nine full-sib families and 4 polymix families. These families represented 14 parents, (five as females; an additional four pollen parents in the full-sib crossing and five as components of the pollen mix). The effective representation of the five pollen parents in the polymix families is unknown. Seeds from each treatment were harvested and grown in a nursery (detailed in Hardner and Potts 1995), with healthy, seven-month-old progeny transplanted into a common garden field trial in 1988 at Ridgley in north-western Tasmania (S41°10'S, E145°46'E). The trial contained five replicates, within which progeny of each species were grown in separate blocks. To limit the potential competition effect between different progenies, each block consisted of two sub-blocks, one containing selfs and the other containing both outcrosses and open-pollinated (OP) progenies. Within each sub-block, families were allocated randomly in plots of up to three trees, with each tree planted at a spacing of 3 x 3m (Hardner and Potts 1995; López *et al.* 2000b).

Table 2.1. Summary of the genetic material used in the study. Shown are the number (n) of females (mothers), families, and the total number of seedlings planted in the trial for each cross-type (outcrossed *E. globulus* [GLxGL] and *E. ovata* [OVxOV], open-pollinated *E. globulus* [Glop] and *E. ovata* [OVop], and selfed *E. globulus* [GLself] and *E. ovata* [OVself]). In the case of the outcrosses, each female was crossed with multiple males resulting in more families than females.

Treatment	Females (n)	Families (n)	Seedlings planted (n)
GLxGL*	15	27	282
GLop	19	19	284
GLself	11	11	116
OVxOV*	5	13	216
OVop	12	12	206
OVself	6	6	35

*also include controlled outcrosses derived using a mixture of pollen (polymixes)

We monitored three fitness surrogates (survival, growth, and reproduction of survivors) over a 28-year period (1988-2016). Survival and growth were assessed seven times after planting (2 months, 1 year 8 months, 3 years 8 months, 10 years, 12 years 6 months, 20 years 7 months and 27 years 7 months; hereafter 0, 2, 4, 10, 13, 21 and 28 years respectively). Growth was assessed using height 2-months after planting and thereafter using stem diameter at breast height (DBH; 1.3m above ground). DBH was measured on all stems, but analyses were conducted only using the largest stem per individual. Reproduction was assessed at 4 years and 28 years as a whole-tree assessment for the presence/absence of buds, flowers or capsules. Data exploration identified a single vigorous tree in the *E. ovata* self-plot, which was alive and reproductive at the final assessment, as a clear outlier. This tree was excluded from analyses, as it was most likely a pedigree error.

2.2.2. *Estimating* inbreeding depression

The level of ID for survival (ID_{surv}) and growth (ID_{growth}) resulting from either selfing (ID_{self}) or open-pollination (ID_{op}) relative to the controlled outcross was calculated following Hardner and Potts (1995):

$$ID_{self} = \left(\frac{\text{outcross} - \text{self}}{\text{outcross}} \right) * 100 \quad [1]$$

$$ID_{op} = \left(\frac{\text{outcross} - \text{OP}}{\text{outcross}} \right) * 100 \quad [2]$$

where outcross, OP and self are the average value of the progeny respectively. A positive ID value thus indicates a negative deviation of the mean of the selfs and Ops, respectively, from that of the outcrosses, corresponding to a decrease in performance.

2.2.3. Statistical analysis

All statistical analyses were undertaken using R version 3.3.1 (R Core Team 2017). The differences in patterns of survival among the species and cross-type treatments were assessed using the *survival* package (Therneau and Lumley 2009). The analyses were undertaken by treating survival as a 'right-censored' trait whereby a tree that died between the time interval of t_1 and t_2 was recorded as dead at t_2. Non-parametric Kaplan-Meier survival curves were estimated to visualize the temporal decay in survival for each treatment using the 'survfit' function of the *survival* package. To statistically test whether the survival curves differed, an analysis of covariance (ANCOVA) was undertaken by fitting *a priori* pairwise contrasts using the 'coxph' function of the *survival* package following Crawley (2012). Significant ($P<0.05$) pairwise differences between survival curves were assessed using the log-rank score test, and the

probability of death (i.e. hazard ratio and its 95% confidence interval) were obtained for each contrast as the exponential of the beta coefficient.

Cross-type differences in the three traits were further explored by fitting the following model:

$$y = \mu + \textbf{cross-type} + \textbf{species} + \textbf{cross-type*species} + \textit{replicate} + \textit{replicate*cross-type} + \textit{replicate*species} + \textit{replicate*cross-type*species} + \varepsilon \quad [3]$$

where cross-type, species and their interaction were fitted as fixed effects (bold) and replicate and its interaction with cross-type and species fitted as random effects (italics), and ε was the random residual. Models for survival and reproduction were fitted using generalised linear mixed effects models (GLMM) assuming a Bernoulli error with a logit link function using the 'glmer' function of the *lme4* package (Bates *et al.* 2014), whereas DBH was fitted using a linear mixed effect model (LMM) undertaken with the 'lmer' function. Statistical significance of the fixed effects were assessed using either a (i) Type III Wald chi-square for the GLMM, or (ii) F test for the LMM where the denominator degrees of freedom were estimated using the Kenward-Roger approximation undertaken with the 'anova' function of the *lmerTest* package (Kuznetsova *et al.* 2015). Where appropriate, model over-dispersion and assumptions of normality and homoscedasticity were assessed following Zuur *et al.* (2010), with response traits transformed where necessary to meet these assumptions. When statistically significant fixed effects were detected, Tukey's multiple comparison tests were undertaken with the 'glht' function of the *multcomp* package (Hothorn *et al.* 2009).

To test the effect of ID on survival and growth for both species, we constructed *a priori* contrasts (outcross *versus* OP, outcross *versus* selfs) for each species and used a two-tailed z-score test to determine whether the observed mean difference was significantly different from zero. This was undertaken using the 'glht' function after fitting the following model:

$$y = \mu + \textbf{treatments} + \textit{replicate} + \textit{replicate*treatments} + \varepsilon \quad [4]$$

where treatments is the fixed effect of cross-type by species with six levels.

To understand the dynamic interplay between growth and survival over the course of the experiment, we tested whether mortality between assessment dates could be related to tree size, as smaller trees have been shown to have a greater mortality risk in plantation grown *E. globulus* (i.e. size-dependent mortality; Chambers *et al.* 1996). For each species, size-dependent mortality over the time interval t_1 to t_2 was tested for each cross-type by comparing the t_1 DBH of the surviving and dead cohorts at the t_2. This comparison was done using an analysis of variance undertaken with the 'lmer' and 'anova' functions of the *lmerTest* package in R as detailed above. As self-thinning within the stand may confound comparisons in DBH

among cross-types, we assessed whether there existed a relationship between the log-transformed values of the average DBH (e.g. tree size) of surviving trees and the number of surviving trees per hectare following Lonsdale (1990) using a simple linear regression model.

The historic and growing period climate variables for the trial site were calculated using long-term daily data obtained from the Australian Bureau of Meteorology (http://www.bom.gov.au/jsp/awap/, accessed 1st March 2017). Daily minimum and maximum temperature and precipitation from the 1st January 1911 to 31st December 2016 were extracted for the trial site using the 'getAWAP' function of the *AUSclim* package (unpublished R package). This function first downloads topography adjusted rasters at a spatial resolution of three minutes (ca. 5 km) (Jones *et al.* 2009) and extracts daily climate data for a set of given coordinates. This climate data was then used to calculate a multi-scalar drought index (standardised precipitation evapotranspiration index, SPEI; Vicente-Serrano *et al.* (2010)) by de-seasonalising 12 month accumulation of precipitation minus pan evapotranspiration (PET) to calculate standardised departures of soil moisture availability (Vicente-Serrano *et al.* 2010; Cook *et al.* 2014). Pan evapotranspiration was calculated using a modified Hargreaves (1994) equation to correct for variation in monthly precipitation, which has been shown to significantly improve estimates of PET in arid environments (Droogers and Allen 2002). The SPEI and PET variables were calculated using the 'spei' and 'hargraves' function of the *SPEI* package (Vicente-Serrano *et al.* 2010).

To determine whether patterns of differential survival of the two species were associated with maladaptation to extreme climate events, relative survival fitness of *E. ovata* compared to *E. globulus* was calculated using Kaplan-Meier curves (see above) as:

$$\text{Relative fitness} = \frac{OV_{surv(CT)}}{GL_{surv(CT)}} \quad [5]$$

where $OV_{surv(CT)}$ and $GL_{surv(CT)}$ are the proportion of surviving *E. ovata* and *E. globulus,* respectively, for each cross-type (CT). Relative survival curves were then overlain on a plot of a 5-year moving average window for a drought metric, the standardised precipitation evapotranspiration index (SPEI, Vicente-Serrano *et al.* 2010)

2.3. Results

2.3.1. Inbreeding depression due to selfing

In *E. globulus,* ID_{self} for growth was highest during the first 4 years (22 to 27%), thereafter it declined rapidly and became insignificant by age 13 years (3%) (Figure 2.1b, Table 2.2a). In contrast, ID_{self} for survival was not initially significant but increased rapidly after age 4 years. It

was significant by age 10 years (25%) and subsequently doubled in magnitude by age 13 years (51%) (Figure 2.1b & Table 2.2a). ID_{self} for survival slowly increased thereafter to reach a maximum of 64% by age 28 years at a time where ID_{self} for growth was effectively zero. Over the period studied, there was continuous size-dependent mortality in the self-population but this was less evident in the outcross population where size-dependent mortality was not significant over the 2 to 10-year time interval (shown by the lines for each cross-type in Figure 2.1d). Most of the expression of ID_{self} had been manifest by age 13 years thereafter changes for both growth and survival were relatively small. Indeed, by age 28 years the few surviving *E. globulus* selfs were of similar DBH to the outcrosses, although their competitive environment was obviously less due to low tree density in the self-plots (Figure 2.2). Overall, the *E. globulus* selfs had a 2.5 times greater risk of mortality than outcrosses (Table 2.3), and most of this risk was incurred over the 4 to 13-year time interval.

In the *E. ovata* population studied, ID_{self} for growth was nearly double that of *E. globulus* for the age 2 and 4 years but it was only after the age 10 years that the species by cross-type effect was significant (age 10 years $F_{1,14}$ =7.0, P=0.019; 13 years $F_{1,16}$ =21.7, P<0.001; 21 years $F_{1,46}$ =8.7, P=0.005). At this stage when the *E. globulus* ID_{self} for growth was dropping to effectively zero, the *E. ovata* ID_{self} remained high to age 21 years and significant up to age 13 years (Figure 2.1b, Table 2.2b). *E. ovata* ID_{self} for survival exhibited a similar but delayed change through time compared to *E. globulus,* with the exception that it continued to increase and reached a maximum of 100% by age 28 years (Figure 2.1b & Table 2.2b). Size-dependent mortality in *E. ovata* selfs was only significant between ages 4 and 13 years (Figure 2.1d), after which the same trends were evident. However, with few surviving plants, the statistical power to test for size-dependent mortality was reduced after age 13 years. Size-dependent mortality of the outcrossed *E. ovata* was significant for all but the 2 to 4-year time interval (Figure 2.1d), which could explain the maintenance of significant ID_{self} for growth (Figure 2.1b). Overall, the *E. ovata* selfs had a 3.4 times greater risk of mortality than outcrosses (Table 2.3) and, as with *E. globulus*, most of this risk was incurred over the 4 to 13-year time interval.

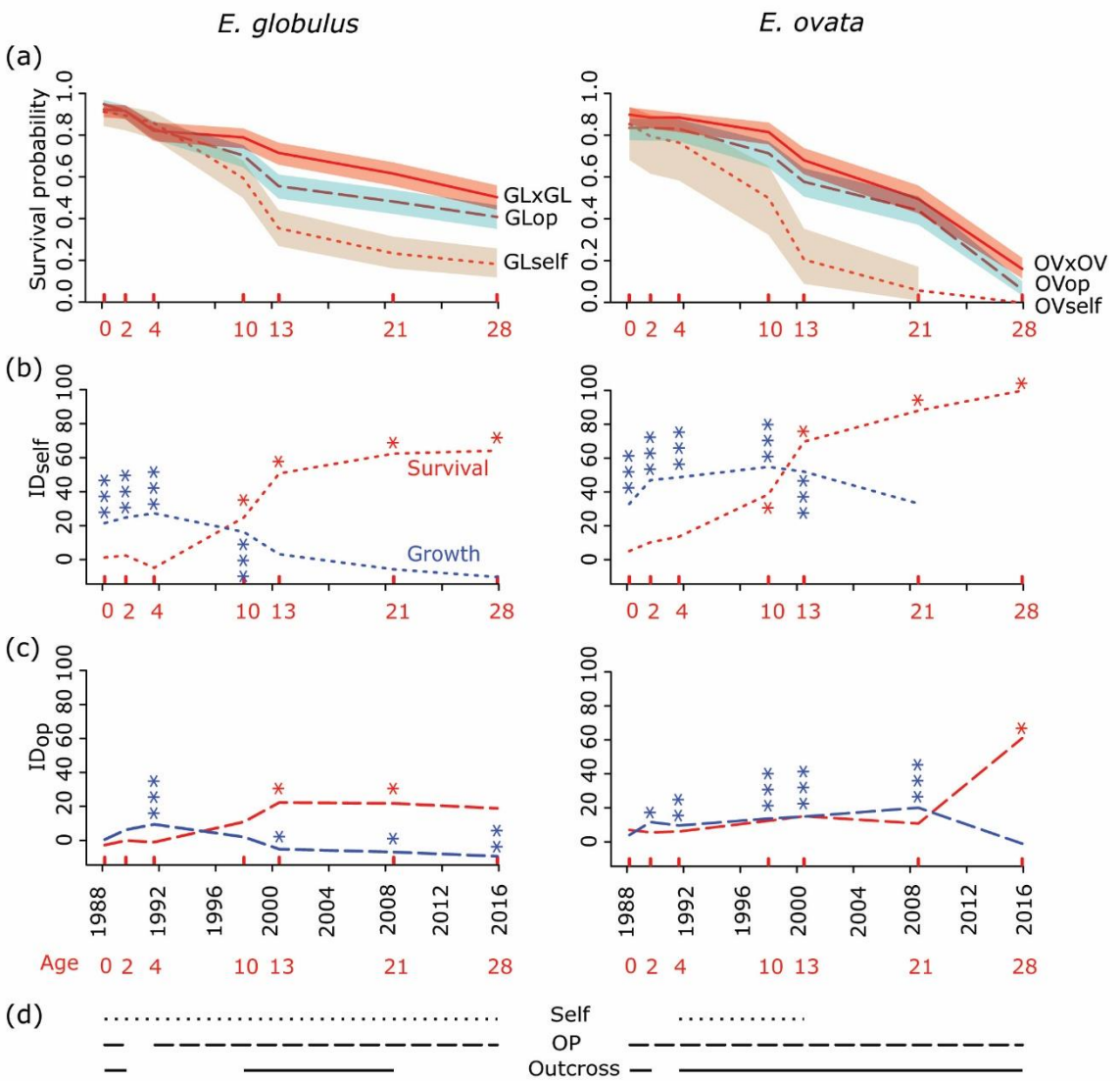

Figure 2.1. Temporal changes in (a) survival, (b) inbreeding depression (ID) due to selfing and (c) ID in open-pollinated (OP) progenies, and (d) size-dependent mortality for *E. globulus* and *E. ovata*. Survival data is shown using red lines and growth (height and DBH) data using blue lines. Dotted lines correspond to selfs, dashed lines to OPs and solid lines to outcross progenies. 95% of confidence intervals (CI) in (a) are indicated as colour bands with non-overlapping bands signalling significant difference among cross-types. CIs were not calculated for the last scoring of *E. ovata* selfs as none survived to 28 years in (a), and ID for DBH cannot be calculated in (b). (b) and (c) show the temporal transition of ID from growth to survival due to size-dependent mortality (summarised in d), with asterisks indicating levels of significance (P<0.05*; P<0.01**; P<0.001***; P>0.05 blank). (d) Lines indicate the intervals over which significant (P<0.05) size-dependent mortality occurred in each cross-type, and gaps indicate the intervals when size-dependent mortality was not occurring (where trees died during the assessment interval were not significantly different in initial size to the surviving trees). In all intervals where significant differences were detected, trees that died were smaller than surviving trees at the beginning of the assessment interval indicated. The statistical tests for growth were based on DBH in all intervals except the first assessment at 2 months, which was based on height. The x-axis represents the growth period of the trial (1988 to 2016) and the red tick marks indicate tree age since planting in the trial when assessments were undertaken (to the nearest year). Changes in cross-type growth over the same period are indicated in Table 2.2.

Table 2.2. Least-square means (LSM) and estimates of inbreeding depression (ID) for each cross-type and monitoring period for *E. globulus* (a) and *E. ovata* (b). A two-tailed z-score test was used to determine whether observed mean effects of ID on survival and growth for both species were significantly different from zero (P<0.05*; P<0.01**; P<0.001***; P>0.05ns). As no *E. ovata* ID_{self} survived to age 28 years, pairwise comparisons were not undertaken. Year 0 age for growth represents height of the plant at 2 months, since they were not tall enough to measure DBH.

(a) *E. globulus*

Age	Survival					Height[1]/DBH				
	LSM %			ID_{self} %	ID_{OP} %	LSM (cm)			ID_{self} %	ID_{OP} %
	Outcross	OP	Self			Outcross	OP	Self		
0[1]	93.2	95.6	92.1	1^{ns}	-3^{ns}	123.5	122.8	97.0	22^{***}	1^{ns}
2	92.4	92.6	90.5	2^{ns}	0^{ns}	67.5	63.3	50.7	25^{***}	6^{ns}
4	83.4	84.1	87.4	-5^{ns}	-1^{ns}	135.6	122.8	98.6	27^{***}	10^{***}
10	80.0	71.2	60.2	25^{*}	11^{ns}	219.7	215.2	184.6	16^{***}	2^{ns}
13	72.3	55.8	34.7	51^{*}	22^{*}	241.0	253.3	233.5	3^{ns}	-5^{*}
21	62.1	48.0	22.7	62^{*}	22^{*}	325.5	348.0	345.2	-6^{ns}	-7^{*}
28	50.3	40.5	17.6	64^{*}	19^{ns}	379.9	414.2	423.3	-10^{ns}	-9^{**}

(b) *E. ovata*

Age	Survival					Height[1]/DBH				
	LSM %			ID_{self} %	ID_{OP} %	LSM (cm)			ID_{self} %	ID_{OP} %
	Outcross	OP	Self			Outcross	OP	Self		
0[1]	90.6	84.0	86.1	5^{ns}	7^{ns}	97.5	93.5	65.8	33^{***}	4^{ns}
2	89.1	84.0	80.3	10^{ns}	6^{ns}	52.2	45.9	27.6	47^{***}	12^{*}
4	89.2	83.5	77.3	14^{ns}	6^{ns}	112.0	101.0	57.4	49^{***}	10^{**}
10	82.0	71.8	48.7	39^{*}	12^{ns}	192.1	165.7	86.0	55^{***}	14^{***}
13	68.3	58.3	21.2	70^{*}	15^{ns}	218.6	186.0	104.5	52^{***}	15^{***}
21	49.4	44.0	5.8	88^{*}	11^{ns}	315.0	251.8	207.7	33^{ns}	20^{***}
28	15.9	6.2	0.0	100	61^{*}	429.7	437.3	-	-	-1^{ns}

In the present case, ID_{self} for DBH of surviving *E. ovata* and *E. globulus* at later ages is likely under-estimated as the differential survival between selfs and outcrosses reflects a change in the competitive environment (Figure 2.2*).* Nevertheless, this does not account for species differences in the ID_{self} for DBH as when compared using the self-thinning growth curves at a common stand density, the species differences in ID_{self} are maintained. For example, at a common tree density of 600 trees per ha, the estimated inbreeding depression for selfs of *E. globulus* and *E. ovata* was 52% and 71%, respectively. The selfed estimates for both species were more than two-fold greater than the estimated inbreeding depression in the OP progenies which was 20% and 25%, respectively (Figure 2.2).

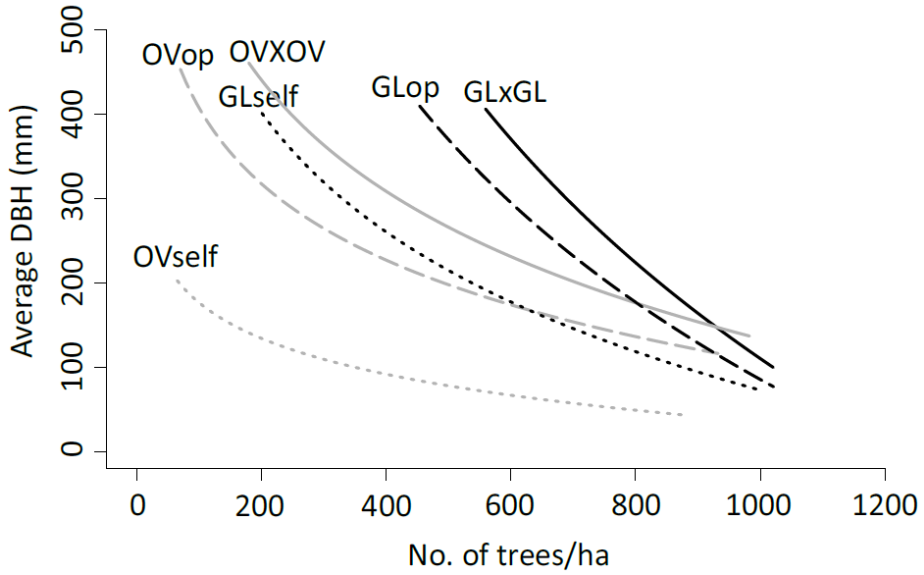

Figure 2.2. The relationship between tree size (diameter at breast height (DBH) at 1.3m) and the density of alive trees (number/per hectare) for outcrossed *E. globulus* (GLxGL) and *E. ovata* (OVxOV), open-pollinated *E. globulus* (GLop) and *E. ovata* (OVop), and selfed *E. globulus* (GLself) and *E. ovata* (OVself), as assessed from planting to 28 years of age. The fitted line shows the trajectory for each cross-type by species combination using log-transformed values of DBH.

Table 2.3. Pairwise contrasts between Cox's proportional hazard ratio estimates (95% confidence intervals) for mortality over a 28-year period. Hazard ratios were estimated for outcrossed *E. globulus* (GLxGL) and *E. ovata* (OVxOV), open-pollinated *E. globulus* (GLop) and *E. ovata* (OVop), and selfed *E. globulus* (GLself) and *E. ovata* (OVself). The Hazard ratio is an indication of the relative probability of mortality of the first treatment group per unit of time compared to the second control group in each comparison. The significance of the observed Hazard ratio was tested using the non-parametric log-rank test between the pairwise contrasts.

Contrast	Hazard ratio	Confidence interval	Log-rank test (df=1)	P value
GLself - GLxGL	2.48	1.90 - 3.23	48.0	<0.001
GLop - GLxGL	1.35	1.07 - 1.68	6.72	0.010
OVself - OVxOV	3.43	2.33 - 5.05	44.2	<0.001
OVop - OVxOV	1.36	1.11 - 1.67	8.9	0.003
OVxOV - GLxGL	2.09	1.67 - 2.61	43.9	<0.001
OVop – Glop	1.92	1.55 - 2.37	38.1	<0.001
OVself - GLself	1.70	1.15 - 2.53	7.1	0.008

2.3.2. Inbreeding depression due to open-pollination

Consistent with a small component of the OP population being selfs, ID_{OP} for DBH of *E. globulus* declined to insignificance and ID_{OP} for survival increased between the age 4 and 13 years (Figure 2.1c). In *E. globulus*, significant ID_{OP} for survival was evident by age 13 years at 22% and was maintained thereafter. In contrast, the ID_{OP} for *E. ovata* was not significant for survival until the final assessment but was evident for DBH over the 2 to 21-year growth period (Figure 2.1c). The difference in the *growth and survival* patterns of *E. ovata* and *E. globulus* OP populations may in part reflect the delayed onset of size-dependent mortality in *E. globulus* outcrosses compared with *E. ovata* outcrosses (Figure 2.1d). Mortality in the *E. globulus* OP population over the 4 to 10-year period likely reflects the removal of smaller selfs, as size-dependent mortality was not evident in the outcrosses during this period. In contrast, as size-dependent mortality was evident in both the selfs and outcrosses of *E. ovata* over the 4 to 10-year period, the mortality in the *E. ovata* OP population likely reflected the combined mortality of both smaller outcrosses and smaller selfs. Such mortality would counter an increase in ID_{op} for survival as would be expected if selfs alone were being selected against. The mortality risk from open-pollination was 1.4 times higher than that for outcrossing for both *E. globulus* and *E. ovata* (Table 2.3), which was less than half the mortality risk from selfing.

2.3.3. Adaptive differences between species and climate impact

Growth and survival differences between the two species were evident at all assessment ages, except age 28 years, regardless of cross-type, with *E. ovata* tending to grow more slowly than *E. globulus* (Table 2.2). This was first detected two months after planting when there was greater mortality of *E. ovata* (88% survival) compared with *E. globulus* (95% survival) (Wald's $\chi^2_1=7.5$, $P=0.006$). While the survival of *E. ovata* tended to be lower than *E. globulus*, there was no significant species difference at most ages (age 2 years $\chi^2_1=3.4$, $P=0.063$; age 4 years, $\chi^2_1=0.0$, P=0.906; age 10 years, $\chi^2_1=0.0$, P=0.998; age 13 years, $\chi^2_1=0.2$, P=0.624), and the species x cross-type interaction was not significant over this time (P>0.05). However, there was a marked increase in the mortality of *E. ovata* relative to *E. globulus* over the 21 to 28-year period (Figure 2.1a & 2.3a), and by age 28 years *E. ovata* showed significantly higher mortality than *E. globulus* ($\chi^2_1=40.3$, $P<0.001$), regardless of cross-type (interaction $\chi^2_2=3.0$, $P=0.222$). The differential mortality of *E. ovata* over this period was first evident in the selfs (Figure 2.3a) and coincided with a peak in maximum summer temperatures in 1997 and the beginning of over a decade of prolonged drought (Figure 2.3b). While signalled in the 21-year assessment in the outcross and OP populations, it was the high *E. ovata* mortality over the 21 to 28-year interval that resulted in highly significant differential mortality in these cross-types (Figure 2.3a). This

peak in *E. ovata* mortality coincided with two consecutive years of high summer maximum temperatures following a decade of *drought* (Figure 2.3b), and the lowest water deficit (e.g. SPEI) calculated for the site since 1911 (Figure 2.4c).

Over the 28 years of monitoring, *E. ovata* had nearly a twofold greater risk of mortality than *E. globulus*, irrespective of cross-type (Table 2.3). While the mortality risks associated with the inter-specific differences are slightly lower than due to selfing, the timing of these risks does not coincide. The higher risk of mortality of *E. ovata* compared to *E. globulus* is evident at the establishment and during the 21 to 28-year interval, whereas the main risk of mortality due to selfing was most evident in the 4 to 13-year interval.

The lower fitness of *E. ovata* at this site compared to *E. globulus* is not only indicated by differences in survival (Figure 2.3a) and growth (Table 2.2), but also reproduction. At age 4 years, the proportion of the surviving trees which were reproductive (GLxGL-36%, Glop-38%, GLself-28%, OVxOV-19%, OVop-12% and OVself-0%) differed significantly between species (χ^2_1= 26.4, P<0.001) but not among cross-types (χ^2_2=2.8, P=0.247; interaction χ^2_2=2.9, P=0.232), with *E. ovata* trees less reproductive than *E. globulus.* However, at age 28 years, there were no significant differences in the proportion of surviving trees that were reproductive (GLxGL-63%, Glop-72%, GLself-75%, OVxOV-60%, OVop-63% and OVself-0%), between species (χ^2_1=0.38, P=0.539) or among cross-types (excluding *E. ovata* selfs due to 100% mortality, Fig 1a; χ^2_2=3.46, P=0.177). Indeed, over 50% of trees which were alive at 28 years of age were reproductive in all treatments, including the surviving selfs of *E. globulus.*

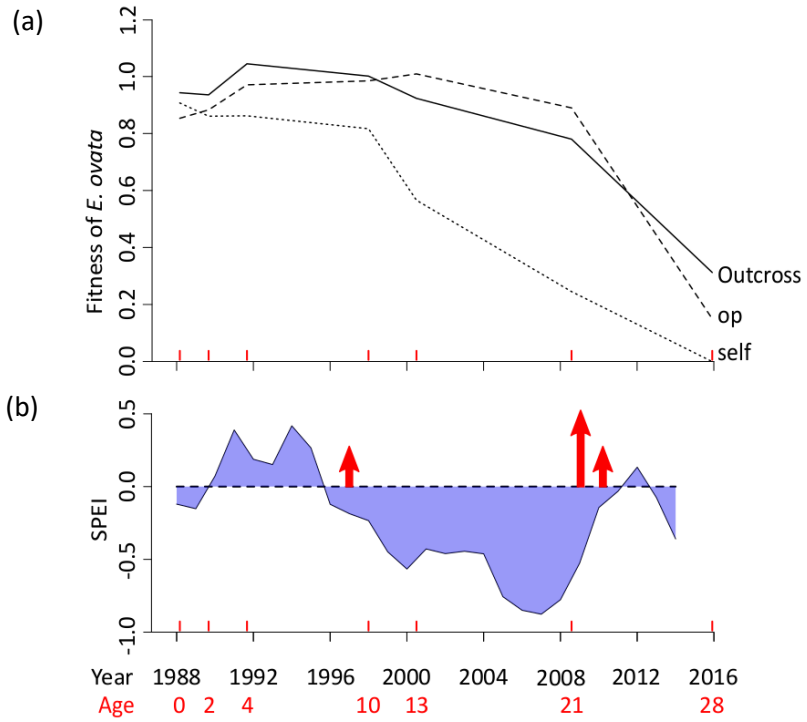

Figure 2.3. The fitness of *E. ovata* relative to *E. globulus* (a) and the standardised precipitation-evapotranspiration index (SPEI) curve (b) over the study period. (a) The fitness of all cross-types of *E. ovata* - outcrossed (OVxOV), open-pollinated (OVop), and selfed (OVself), relative to that of the respective *E. globulus* cross-types (GLxGL, GLop & GLself) at each age. Fitness values below one indicate *E. ovata* survived less than *E. globulus*. (b) The solid black line represents the trend in SPEI based on a 5-year moving average window. SPEI values below zero indicate water deficit and above zero indicate water surplus. Red arrows indicate the number of days per year above 30°C (short arrow represent one day and long arrow represent two days), which were calculated from the daily climate data surface obtained from the Australian Bureau of Meteorology.

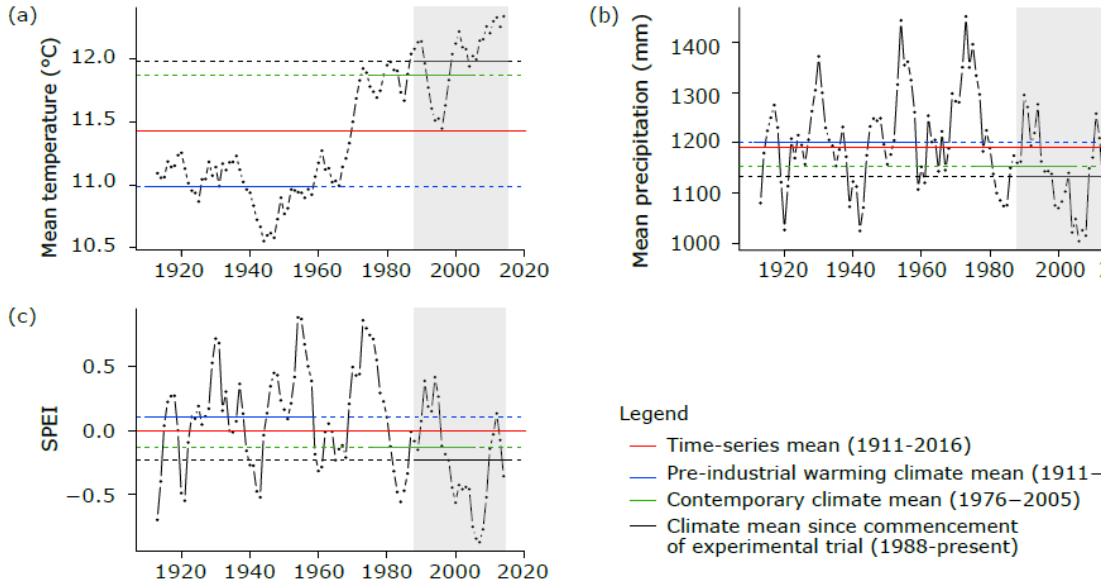

Figure 2.4. Long-term climate patterns for the West Ridgely trial site. Shown are the five-year moving average curves for (a) mean annual temperature, (b) mean annual precipitation, and (c) standardised precipitation evapotranspiration index (SPEI). The grey shading corresponds to the growth period of trial (1988-2016). The red line represents the overall average for the trial site (1911-2016), the blue line represents the historical climate average prior to the detectable signature of climate change in the southern hemisphere (Abram *et al.* 2016) (1911-1959), the green line corresponds to the climate average often used to represent the contemporary climate (1976-2005), and the grey line corresponds to the climate average during the growth period at the trial (1988-2016).

2.4. Discussion

Our 28-year study is one of the few long-term empirical studies of inbreeding depression (ID) due to selfing in eucalypts. While previous studies of eucalypts in general (Hardner and Tibbits 1998; Bison *et al.* 2004) and *E. globulus* in particular (Costa e Silva *et al.* 2010b; Costa e Silva *et al.* 2011) have reported severe ID for early growth (<13 years), the long-term consequences have not been well documented. In this study, we show that very few selfs survive to reproductive maturity compared with outcrosses. All *E. ovata* selfs were dead by 28 years resulting in an ID_{self} of 100% for survival. In *E. globulus,* ID_{self} reached 64% for survival as a small number of selfs did survive and were reproductive in the low competition environment of the 'self-plots'. Such levels of ID_{self} are comparable to that of 80% reported for Douglas fir (26 years, Stoehr *et al.* 2015) and 75% for Scots pine (23 years, Koelewijn *et al.* 1999). While our progeny testing was undertaken in a field trial, such high ID_{self} for survival argues that most selfs will be purged from the population early in stand development and they will contribute little to the build-up of inbreeding in wild eucalypt populations. In wild populations, trees including *E. globulus* may live for several centuries (Hickey *et al.* 2000) and be subject to severe competition during all stages of stand development (Florence 2004). Indeed, such purging of selfs may in-part explain the homozygote deficiency (i.e. F<0) often observed in wild eucalypt populations despite the excess of homozygosity in open-pollinated seed (see Potts and Wiltshire 1997). However, in the case of *E. globulus* there is evidence of heterosis in inter-population crosses (Volker *et al.* 2008; Costa e Silva *et al.* 2014), suggesting that some mild build-up of ID within wild populations, is likely due to bi-parental inbreeding (Hardner and Potts 1997; Mimura *et al.* 2009), although the rare survival of selfs cannot be dismissed.

The long-term monitoring of the field trial revealed a dynamic interplay between ID for survival (ID_{surv}) and ID for growth (height and DBH) of survivors (ID_{growth}). This interplay involves ID_{surv} increasing with age but ID_{growth} simultaneously decreasing. This *translation of ID_{self} from growth to survival* appears to be a consequence of size-dependent mortality eliminating the more inviable selfs. Such dynamics have been previously noted in other studies of *E. globulus* (14 years, Costa e Silva *et al.* 2011), *Eucalyptus regnans* (15 years, Hardner and Potts 1997) and *Pinus silvestris* (23 years, Koelewijn *et al.* 1999). However, while ID_{growth} eventually decreased in these studies suggesting declining magnitude of selection against surviving selfs, this was not the case for *E. ovata* in our study and for *Pseudotsuga menziesii* (Stoehr *et al.* 2015). In the latter case, ID_{self} for DBH increased over 26 years, suggesting that selection against selfs was still incomplete. Genome-wide studies of *E. grandis* suggest that it is the more homozygotes of the selfs that are more likely to be eliminated (Hedrick *et al.* 2016). This is consistent with

dominance explaining the inbreeding depression. In *E. globulus*, ID appears to manifest early in the life cycle for growth (DBH) (e.g. by 2 years of age in the present study; 4 years of age in the study by Costa e Silva *et al.* (2010b). Yet, ID_{growth} did not translate to large differences in survival until 10 years of age. Costa e Silva *et al.* (2010b) also reported a similar change whereby significant ID_{surv} was first detected at age 6 years and increased thereafter. In the present study, the greatest increase in ID_{surv} occurred between 10 and 13 years of age in both *E. ovata* and *E. globulus*, which may be due to several factors. Firstly, a threshold in inter-tree competition may have been reached as the stand age increases and trees become larger (Bella 1971; Costa e Silva *et al.* 2011), resulting in greater mortality of the poorer growing selfs. ID involves a dynamic interplay between growth and survival through time, with its magnitude often dependent upon the timing of mortality of poor growing selfs (Koelewijn *et al.* 1999; Costa e Silva *et al.* 2011). Indeed, in the present study, the marked increase in ID_{self} for survival coincided with a decrease in ID_{self} for DBH (Figure 2.1b). This suggests that inter-tree competition may have led to size-dependent mortality, with the surviving selfs being in a less competitive environment and thus growing equivalently to the outcrossed progeny. Secondly, it is possible that the selfs were less buffered against abiotic stress over this time associated with the onset of drought and a period of heat stress (Figure 2.3b). A review by Armbruster and Reed (2005) analysing 34 studies found that in 76% of the cases abiotic stress increased ID. While ID is often assessed in the presence of direct competition between selfs and outcross trees (Costa e Silva *et al.* 2011; Stoehr *et al.* 2015), the present study and that of Costa e Silva *et al.* (2010b) (where selfs were planted separately to outcrosses) clearly show that such competition is not required for the expression of severe ID.

Our results provide strong evidence for ID in the open-pollinated progeny of both species. This is consistent with expectations from a mixed mating system where a fraction of the OP would be due to self-pollination (Goodwillie *et al.* 2005), although a contribution from bi-parental inbreeding cannot be dismissed (Hardner *et al.* 1998; Mimura *et al.* 2009). Following the approach of Charlesworth and Charlesworth (1987) and assuming all inbreeding is due to selfing, a comparison of the reduction in performance of the OPs relative to selfs and outcrosses, yields estimates of outcrossing rates of between 0.56 (13-year survival) and 0.67 (4-year DBH) for *E. globulus*. This compares with similarly derived estimates for *E. globulus* of 0.47 to 0.51 (Costa e Silva *et al.* 2010b) and those from molecular studies of between 0.65 and 0.89 (Mimura *et al.* 2009). In the case of *E. ovata*, we estimated the outcrossing rate at 0.79 (13-year survival) and 0.80 (4-year DBH), however, there are currently no molecular estimates for *E. ovata* for comparison. It is important to note that in both species OP seeds were collected low in the

canopy where controlled pollinations were done, while in the molecular study of Mimura et al. (2009) the seed was collected from the top half of each tree. Previous studies of *E. globulus* show that outcrossing rates increase with increasing height in the canopy (Patterson *et al.* 2004b) and this could explain the difference between some of the estimates. Differences in forest type could also explain variation in estimated outcrossing rates both within and between species. Outcrossing rates can decrease with increasing stand fragmentation in *E. globulus* (Mimura *et al.* 2009), and such differences could explain the greater outcrossing rate in *E. ovata* compared with the *E. globulus* population studied. In addition, performance derived estimates of outcrossing rate assume all trees show equal levels of ID, yet quantitative genetic studies of *E. globulus* have shown that variation in the performance of selfs may result from variation in ID *per se* (Costa e Silva *et al.* 2010a).

In *E. globulus*, the change in ID with time is remarkably similar between self and OP populations. The absence of ID for growth by age 13 is consistent with the purging of smaller inviable selfs from the OP population (Hardner and Potts 1997; Hedrick and Garcia-Dorado 2016). This is supported by the fact that between age 4 and 10 years the outcross population showed no evidence of size-dependent mortality, whereas the self and OP populations did. Such size-dependent mortality in OP progenies has been previously noted in plantation-grown *E. globulus* (Chambers *et al.* 1996; Stackpole *et al.* 2010a) and other eucalypts (Hardner and Potts 1997). *E. ovata* exhibited a different trajectory in ID to *E. globulus*, with significant ID_{self} and ID_{op} for growth maintained to later ages. Further, in the case of the OPs, there was little evidence of ID_{op} for survival except at age 28 years, despite high mortality of selfs and high ID_{self} over this period. In fast growing plantations of *E. globulus,* competition is established early in stand development and increases markedly between 2 and 4 years of age, resulting in faster growing genotypes suppressing their slower growing neighbours (Costa e Silva *et al.* 2017). Such competitive interactions between outcross and selfed offspring in the OPs may have been less marked in the *E. ovata* blocks due to (i) lower growth rates of *E. ovata* compared to *E. globulus*, and (ii) higher later age mortality of *E. ovata* (see below) leading to lower stand densities. Both factors would be expected to lead to less competition and thus less mortality of *E. ovata* selfs in the OPs compared to that experienced by *E. globulus* OPs. Such variation in the extent to which ID is translated from an effect on growth to that on survival is consistent with the site differences observed by Costa e Silva *et al.* (2011) in *E. globulus*, whereby ID_{surv} was markedly greater on the more productive site. While harvesting productivity is positively related to tree size (Hamilton *et al.* 2015a), the extent and timing of the translation of inbreeding depression from growth to survival are unlikely to have a significant economic impact on wood production in eucalypt

plantations. This is because most small selfs would be expected to be dead by harvest age in the case of even short-rotation pulpwood regimes or to have been artificially thinned prior to harvest in the case of longer-rotation solid wood regimes.

While our study indicates that ID is more severe in *E. ovata* compared with *E. globulus*, this does not necessarily represent a species-level difference in genetic load and thus ID. Plant species can differ markedly in levels of ID, particularly due to factors such as breeding system and longevity (Husband and Schemske 1996), and population-level factors such as size and history (Ellstrand and Elam 1993; Charlesworth and Willis 2009). These latter factors, in particular, may see specific species or populations of a species purge their genetic loads of deleterious recessive alleles following prolonged population bottlenecks (Willi *et al.* 2006). Certainly, there are examples of forest tree species and populations which show little ID compared to the norm including eucalypts (Owens *et al.* 1990; Kärkkäinen *et al.* 1996; Bush and Thumma 2013; Bezemer 2018). In the case of *E. globulus*, marked differences in ID_{surv} have been reported between the isolated King Island population and more central populations (36 % versus 74% at age 10 years; Table 2.4) at similar experimental sites, although ID reported for age 4 years DBH are remarkably consistent (21 to 31%; Table 2.4), regardless of site, population and extent of competition with outcrosses. The *E. ovata* trees studied here were native to south-eastern Tasmania, and when compared with native *E. globulus* from the same area and grown at a nearby site to the current trial (Costa e Silva *et al.* 2010a; 2010b) ID_{self} for DBH at age 4 years was greater in *E. ovata* than *E. globulus*, consistent with our result (Table 2.4). The reverse was the case for ID_{self} for survival, although this is likely to reflect differences in timing, as eventually all *E. ovata* selfs in our study died (Figure 2.1a). Nevertheless, such differences in the timing of the ID_{self} from growth to survival are likely to depend on factors such as competition and/or environmental stress (Armbruster and Reed 2005; Fox and Reed 2011), making it difficult to directly relate the level of genetic load *per se* to the level of ID.

While ID appeared to dominate selective filtering over the 4 to 13-year period, the major phase of subsequent mortality appeared to be dominated by differential response of species to climatic stress. The most likely explanation for the dramatic reduction in relative fitness of *E. ovata* compared to *E. globulus* is climate maladaptation of *E. ovata* at this site. Climate records indicated that the site was subjected to a prolonged drought which lasted 15 years (1996 to 2011), with multiple heat days during 2009 and 2010 which were at least 5 °C above the mean yearly maximum temperature of the warmest week observed at this site (25°C*).*

Table 2.4. Inbreeding depression for survival (ID_{surv}) and DBH (ID_{growth}) of the selfed progeny of *E. globulus* and *E. ovata* and comparison with other studies on *E. globulus*. Shown is the trial location in Tasmania, Australia, estimates of ID_{growth} and ID_{surv}, design of the experiment, and the native race of origin of the maternal trees. Experimental designs indicated as 'plot' corresponds to the planting of selfs and outcrosses in separate plots within a replicate thus avoiding competition between them, whereas 'mixed' experimental designs correspond to the planting of self and outcrossed progeny intermixed in the same plot and thus selfs are competing with outcrosses.

	This study		Hardner *et al.* (1996)	Costa e Silva *et al.* (2010b)	Costa e Silva *et al.* (2011)	
Species	*E. ovata*	*E. globulus*	*E. globulus*	*E. globulus*	*E. globulus*	*E. globulus*
Trial location	Ridgley	Ridgley	Geeveston	Ridgley	Ridgley	Southport
ID_{growth} (4 years)	49%	27%	31%	21%	28%	30%
ID_{surv} (10 years)	39%	25%	-	74%	36%	16%
Experiment	plot	plot	mixed	plot	mixed	mixed
Maternal race	*SE Tasmania	*SE Tasmania	SE Tasmania	SE Tasmania	King Island	

*includes some ornamental plantings and native trees from SE Tasma

The decline in fitness coincides with these extreme climate events which are known drivers of tree mortality (Allen *et al.* 2010; Anderegg *et al.* 2013; Mitchell *et al.* 2014), and the synergistic before effects of drought and heat stresses have been reported for native eucalypt forests of Australia (Matusick *et al.* 2013; Mitchell *et al.* 2014). While canopy water stress is known to increase as forests grow (Phillips *et al.* 2003) and tree height increases (e.g. the need to transport water to the top of the canopy; Koch *et al.* 2004), this factor can be dismissed in the present case. Here, *E. globulus* grows faster than *E. ovata* and both their plot and tree-level basal area were greater than *E. ovata* (unpublished data). Indeed, a drought-related fitness decline of *E. ovata* relative to *E. globulus* is consistent with damage reported in native forest in southeastern Tasmania (Kirkpatrick and Marks 1985).

The decline in *E. ovata* fitness was observed regardless of cross-type but was evident earlier in the selfs. The major decline in fitness of *E. ovata* in the selfed population occurred at the beginning of the drought period whereas the major decline in the outcrosses occurred following high temperature stress at the end of the drought. This response is consistent with inbred products being more susceptible to stress (Armbruster and Reed 2005; Fox and Reed 2011) and the greater final ID_{self} in *E. ovata* (100%) than *E. globulus* (64%) for survival. The extent to which poorer establishment success and growth of *E. ovata* compared with *E. globulus* reflects differential climatic adaptation to the site is less clear. Indeed, the performance of *E. ovata* could reflect either differences in (i) maternal environment (López *et al.* 2003), (ii) nursery effects (plant size in nursery affecting survival or growth; Close 2012; Grossnickle 2012), (iii) growth strategy (Davidson and Reid 1980; Otieno *et al.* 2005), or (iv) site-specific adaptation (Davidson and Reid 1985; Davidson and Reid 1989). Nevertheless, the earlier onset of size-dependent mortality of the established *E. ovata* outcrosses compared with *E. globulus* outcrosses would suggest that *E. ovata* is generally less well adapted to the planting site than *E. globulus*. While the planting site is outside the natural geographic range of *E. globulus* but not *E. ovata*, it is relatively well-drained which would favour *E. globulus* over *E. ovata*, which tends to grow on seasonally waterlogged substrates (Williams and Potts 1996). Additionally, we cannot dismiss the possibility that the site preparation method (e.g. rip and mounding) may have been more favourable for *E. globulus* which is a key plantation species amenable to such silvicultural practices.

2.5. Conclusion

The present study shows how under mixed mating, the fitness trajectory of long-lived tree species is shaped by selective filtering associated with the endogenous effects of ID superimposed on maladaptation due to differential responses to exogenous stresses, consistent with our first hypothesis. Also consistent with our second hypothesis, the expression of inbreeding depression changed with age. Selective filtering was initially dominated by ID that resulted in the elimination of most selfs by 13 years, after which environmental stress appeared to be the main cause of differential mortality. The onset and relative importance of these two processes appear to differ between species, as does the manner in which size-dependent mortality shifts ID from growth to survival with age. The interplay and timing of these processes will be species and site-dependent and consistent with our third hypothesis, our data supported the possibility that inbreeding may affect the sensitivity of trees to climatic stress.

Chapter 3 - Genetic correlations among pulpwood and solid-wood selection traits in *Eucalyptus globulus*

3.1. Introduction

Eucalyptus globulus Labill is native to south-eastern Australia, including the islands of Tasmania (Eldridge *et al.* 1993), but is widely planted in temperate regions of the world for pulpwood production (Potts *et al.* 2004; Foelkel 2009). It is Australia's main hardwood plantation species (Rhys and Mijo 2018), where 82.4% of plantations are managed for the production of pulpwood (Downham and Gavran 2017). Therefore, most genetic improvement of the species has focused on pulpwood breeding objectives (Eldridge *et al.* 1993; Cotterill and Macrae 1997; Potts *et al.* 2014). However, in the last few decades, there has been increasing interest in using hardwood plantations for solid-wood products such as sawn timber, veneer and composites (Nolan *et al.* 2005; Hamilton *et al.* 2007; McGavin *et al.* 2014; Derikvand *et al.* 2016). Although there is a growing interest in the improvement of *E. globulus* for solid-wood traits (Potts *et al.* 2014), defining solid-wood breeding objectives and associated breeding objective traits is difficult since this is a relatively new industry and diverse products are being proposed (Raymond 2000; Hamilton *et al.* 2010).

In *E. globulus*, whole tree pulp yield is a breeding objective trait specific to pulp production (Borralho *et al.* 1993). Similarly, in solid-wood, there are some specific breeding objective traits such as stem straightness (Nolan *et al.* 2005). However, improvement of growth and wood basic density are common to both pulpwood and solid-wood breeding (Raymond 2002; Hamilton *et al.* 2007; Hamilton *et al.* 2009a; Hamilton *et al.* 2010; Rezende *et al.* 2014). Not only does basic density determine the amount of dry matter per unit volume of harvested wood (Zobel and Van Buijtenen 1989), but it can also be genetically associated with the yield of pulp from this dry matter (Stackpole *et al.* 2010b). Wood density has also been shown to be genetically correlated with traits which are specific to solid-wood production such as log end-splitting and bowing (*E. grandis* - Santos *et al.* 2004), wood shrinkage (*E. globulus* - Hamilton *et al.* 2010), and wood stiffness (*E. nitens* - Blackburn *et al.* 2010). In the present study, we use base-population trials of *E. globulus* to examine the genetic architecture of key pulpwood and solid-wood selection traits, assessing trait heritability and subrace variation, genotype-by-environment interactions and the genetic correlations among traits.

Our first objective was to understand the extent to which key pulpwood and solid-wood selection traits were associated. Among the various solid-wood selection traits, we focused on

stem straightness (Straightness) and acoustic wave velocity (AWV). The association of Straightness with other selection traits is not well studied in *E. globulus*. Straightness has been shown to affect the stem harvesting time (Hamilton *et al.* 2015a), and recovery of veneer and timber from logs (MacDonald *et al.* 2009; Hamilton *et al.* 2015b). Wood stiffness is one of the most important solid-wood selection traits (Yang and Evans 2003; Vikram *et al.* 2011) and is assessed using the modulus of elasticity (MOE). MOE measures the recoverable deformation when a load is applied to wood (Antony *et al.* 2011). Standing-tree acoustic wave velocity (AWV) is a non-destructive method of indirectly measuring wood stiffness (Farrell *et al.* 2012). Standing-tree AWV is strongly correlated at the genetic and phenotypic levels with AWV of sawlogs (Blackburn *et al.* 2010; Davies *et al.* 2017) as well as the MOE of sawn boards (*E. dunnii* - Dickson *et al.* 2003; *E. nitens* - Blackburn *et al.* 2010) and veneer (*E. nitens* - Blackburn *et al.* 2012). Of specific interest to this study was verifying that AWV was not only positively correlated with basic density but also with pulp yield as reported in various eucalypt species, including *E. globulus* (Blackburn *et al.* 2012; Hamilton *et al.* 2017b).

Our second objective was to consolidate our understanding of the genetic architecture of pulp yield. While the quantitative genetics of growth and wood density are well-studied in *E. globulus* (Raymond 2002; Potts *et al.* 2004), there are only a few studies of pulp yield due to the expense of its assessment (Stackpole *et al.* 2010b). It is only with the application of near-infrared spectroscopy to large numbers of samples obtained from ground stem cores or drill swarf (Meder *et al.* 2010; Downes *et al.* 2011) that it has been possible to obtain reasonably accurate estimates of genetic parameters for this trait. Such studies have shown pulp yield to be under moderate genetic control in *E. globulus*, with narrow-sense heritability (h^2) estimates equal to or higher than 0.40 (Raymond *et al.* 2001; Stackpole *et al.* 2010b). However, there is currently no consistent pattern in the genetic correlations of pulp yield with other traits such as growth and density (Turner *et al.* 1983; Greaves *et al.* 1997; Stackpole *et al.* 2010a). In addition, there has been only one detailed study of the geographic pattern of variation in pulp yield in this species, and this suggested that subraces currently targeted for pulpwood breeding due to their good growth and high density may have low pulp yield (Stackpole *et al.* 2010b). Therefore, the validation of these pulp yield results is required.

3.2. Materials and methods

3.2.1. Field trials and traits assessed

Three *E. globulus* field trials were studied, two in north-west (NW) Tasmania (Salmon River [SR] and Togari [TO]) and one in northern Tasmania (Latrobe [LA]). The Latrobe trial was

the large base population trial from which the previous studies of wood properties, including density and pulp yield, have been published (Stackpole *et al.* 2010a; Stackpole *et al.* 2010b). These published data were used for comparison with the new data collected from the NW trials established with the same open-pollinated families but grown in a higher rainfall zone (Table 3.1). The two NW trials were established on ex-forest sites, and while similar in rainfall and elevation, did differ in soil type. The soil of the Salmon River trial was a yellow-brown mottled clay on Precambarian mudstone whereas the soil at Togari was a red-brown clay on Cambrian inter-layered mudstone, siltstone and sandstone (Hamilton *et al.* 2013; O'Reilly-Wapstra *et al.* 2013). The trials were established using seedlings derived from open-pollinated seed collected from wild trees originating from 13 *E. globulus* subraces extending across the natural range of the species (Figure 3.1 & 3.2). These subraces are defined in Dutkowski and Potts (1999) and represent the genetic group level used in the evaluation of the Australian National Breeding population (Potts *et al.* 2014). Subraces were represented by 9 - 13 families in the north-west trials and 4 - 113 families in the Latrobe trial. Connectivity was high, with 129 families in common to the two NW trials, and 107 families in common to all the three trials. Seedlings were planted at a spacing of 4.0 m between rows (rip-lines) and 2.3 m within rows in the NW trials and 4.0 x 2.5 m at Latrobe. The open-pollinated families in the trials were arranged in an incomplete randomised block design with families represented as single-tree plots in Salmon River and Togari sites, and as two-tree plots in Latrobe site (Table 3.1).

At Salmon River and Togari trials, diameter at breast height (DBH) over bark at 1.3 m above ground level was measured for every tree alive at the age of 9 years 8 months (10 years hereafter) and 9 years 7 months (10 years hereafter), respectively. Stem straightness (Straightness) was assessed using a six-point subjective scale (1 poorest - 6 straightest) at 10 years, for all living trees following Blackburn *et al.* (2013). The class assignment was done so that the frequency distribution approximates a normal distribution as recommended by Cotterill and Dean (1990). The additive genetic correlation between this subjective score and the more time-consuming quantification of stem deviation from straightness is more than -0.9 (Blackburn *et al.* (2013). At age 10 years and 1 month (10 years hereafter), 3-5 trees from each of 9 -10 families per subrace were selected across 8 (Salmon River) to 10 (Togari) replicates for the assessment of standing-tree acoustic wave velocity (AWV), and then cores were taken from the same trees.

Table 3.1. Summary of the establishment, climate and design features of the studied field sites of *Eucalyptus globulus* including number of families and individuals assessed for various traits

	Salmon River (SR)	Togari (TO)	Latrobe (LA)
Site			
Year of establishment	2005	2005	1989
Location	41°01’ S 144°48’ E	40° 56’ S 144° 54’ E	41°16’ S 146°27’E
Altitude (m above sea level)	103	90	116
Climatic variables			
Mean annual rainfall in (mm) [a]	1223	1251	899
Mean annual minimum temperature (°C) [a]	1.4	0.7	-1.3
Mean annual maximum temperature (°C) [a]	26.1	26.1	27.0
Experimental design			
Number of replicates	20	16	5
Number of incomplete blocks per replicate	15	12	24
Sample size			
Subraces	13	13	13 (11)[b]
Family	135	131	489
No. of individuals for DBH	2179	1520	4349
No. of individuals for BD	515	471	1922
No. of individuals for KPY	513	475	1939
No. of individuals for Straightness	2179	1529	NA
No. of individuals for AWV	494	460	NA

[a]Calculated using long-term daily data obtained from the Australian Bureau of Meteorology (http://www.bom.gov.au/jsp/awap/, accessed 1st March 2017). Climate values represent the mean over the 1911-2017 period.
[b]Two subraces (Western Tasmania and Recherche Bay) included in SR and TO were not assessed for wood properties in LA by Stackpole *et al.* (2010b), but were assessed for DBH.
NA - Not Assessed.

AWV was assessed using a Fakopp™ acoustic stress wave timer as detailed in (Blackburn *et al.* 2014). Two sensor spikes were inserted at 0.5 and 1.7 m tree heights in a vertical plane. Sensors were passed through the bark and pierced into the wood for at least 10 mm, at approximately 45° to the stem. The time an acoustic wave takes, to pass through one sensor to another (time-of-flight) was measured by the Fakopp™. AWV was calculated using the time-of-flight and the distance between these sensors. Coring of the NW trials was undertaken at 1.1 m height above ground level using a motorised corer to remove cambium-to-cambium wood cores of 12 mm diameter (Downes *et al.* 1997).

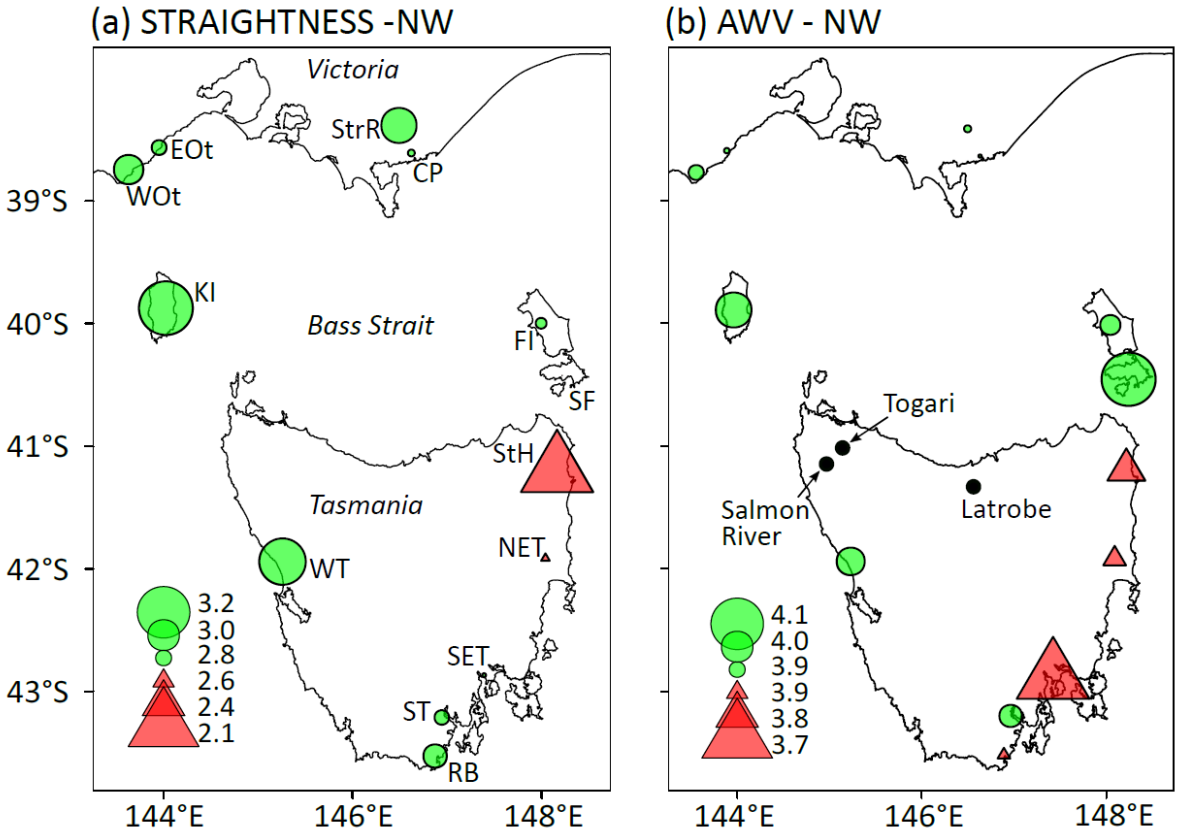

Figure 3.1. The geographic variation in subrace least-square means at the north-west (NW) trials for (a) Straightness (1-6 scale) and (b) AWV (km/s) (see Supp. 3.1). Subrace abbreviations are shown in (a) and are for North-eastern Tasmania [NET], South-eastern Tasmania [SET], Southern Furneaux [SF], Western Tasmania [WT], Southern Tasmania [ST], St Helens [StH], King Island [KI], Western Otways [WOt], Coastal Plain [CP], Recherche Bay [RB], Eastern Otways [EOt], Strzelecki Ranges [StrR] and Flinders Island [FI]. Trial site locations are shown in (b) and are Salmon River and Togari and Latrobe

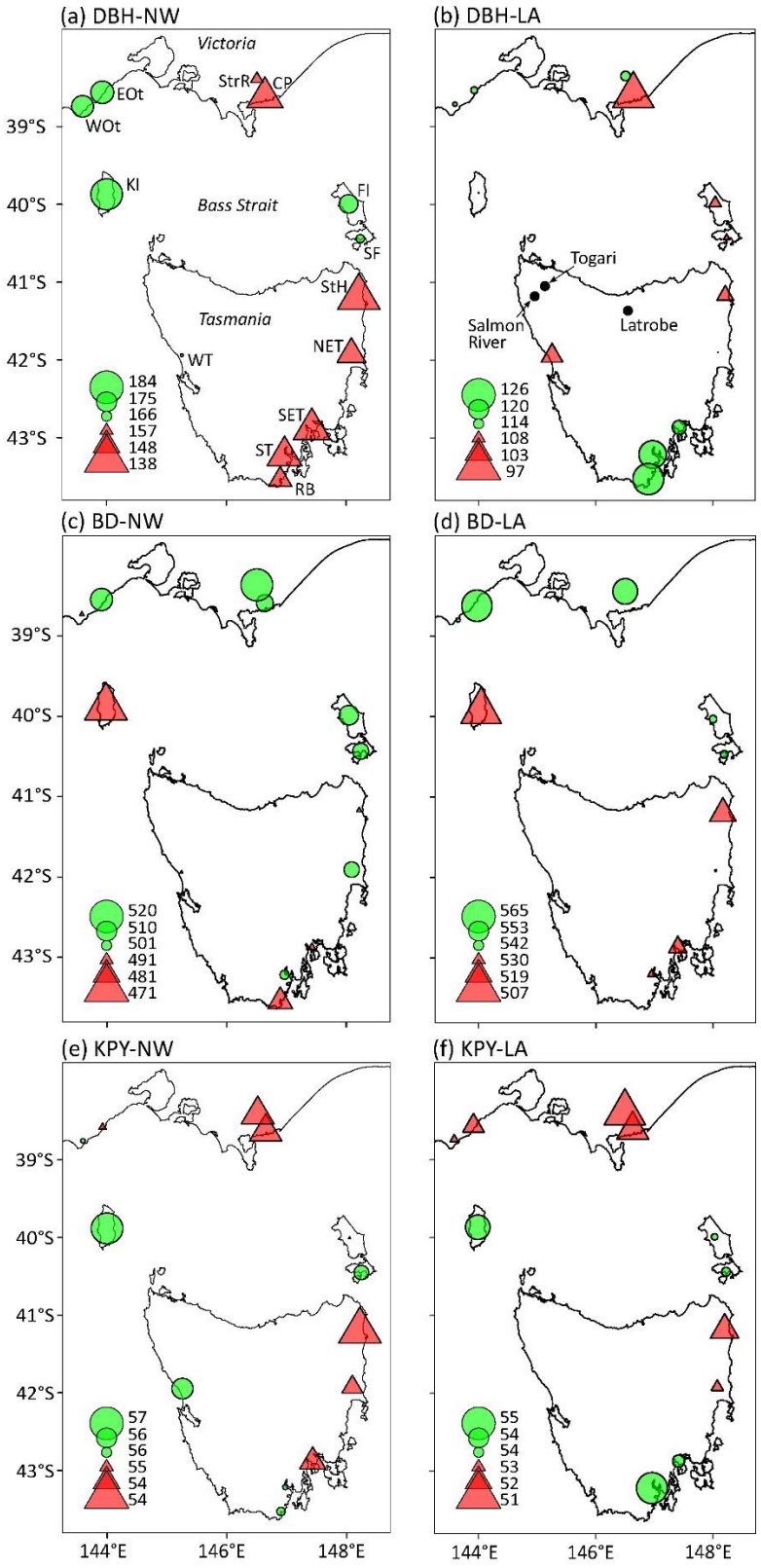

Figure 3.2. The geographic variation in subrace least-square means at the north-west NW and Latrobe (LA) trials for (a & b) DBH (mm); (c & d) BD (kg/m3); (e & f) KPY (%). Subrace abbreviations shown in (a) and trial site locations shown in (b) are as detailed in Figure 3.1

Core alignment was in the east-west horizontal plane and operators endeavoured to include the pith in each core. Following Stackpole *et al.* (2010b), wood basic density (BD) was assessed from cores as per Smith (1954). A second outer wood core, 50 mm in radial length was also taken from a similar position, air-dried and then ground to woodmeal to estimate the pulp yield. A Wiley Mini-mill was used to grind the cores - without the screen for the first few passes to break the core into small pieces, and then with a 20-mesh screen to grind the fragments to woodmeal. For each tree sampled, Kraft pulp yield was estimated using near-infrared spectroscopy (NIRS), based on the spectra obtained from the woodmeal using the global pulp yield model reported by Downes *et al.* (2009, 2011). This global model was developed using NIR spectra from 1,272 wood chip samples, from 40 different eucalypt species from plantations and native forests, which were subjected to laboratory pulping. Plantation grown *E. globulus* was included in the model and subsequent validation of the NIR model in the Latrobe trial yielded R^2 values of 0.82 against whole tree pulp yield estimates based on discs (Stackpole *et al.* 2010b).

At Latrobe, DBH was measured at age 8 years for every tree alive. At age 16 years 6 months (17 years hereafter), one tree from most families was cored from cambium-to-cambium for each of 4 or 5 replicates (provided DBH was greater than 10 cm) and wood basic density (BD) and Kraft pulp yield (KPY) were assessed as detailed in Stackpole *et al.* (2010a) and Stackpole *et al.* (2010b). The sample assessed by NIRS in this study differs from that used by Stackpole *et al.* (2010b) for Latrobe. Stackpole *et al.* (2010b) used half cores for grinding where the cores were sectioned lengthwise (from bark-to-bark), while in this study short 5 cm cores were used.

3.2.2. Statistical Analyses

Univariate analyses of the data were undertaken by fitting the model:

$$y = \mu + replicate + subrace + family(subrace) + residual \qquad \text{Model 1}$$

where, y is the vector of observations, μ is the grand mean, and random effects are replicate, subrace, family within subrace (family(subrace)) terms and the residuals. As families were planted in two-tree plots at Latrobe and both were measured for DBH, a random plot term was added to Model 1 for all analyses involving DBH at Latrobe. The model assumed a normal distribution of residuals for all traits. Although stem straightness was a discrete variable, it was scored to approximate a normal distribution which allowed analysis as a normally distributed trait as traditionally undertaken (Cotterill and Dean 1990; Blackburn *et al.* 2013). However, for a better understanding, we also analysed stem straightness as a multinomial trait at the univariate level. The models were initially fitted for each trial, and the subrace and family(subrace) variance components tested for significance from zero using a one-tailed LRT.

The variance components were used to estimate the narrow-sense heritability (h^2_{op}) of the phenotypic variation within subraces and coefficient of additive genetic variation (CV_a) following (Stackpole *et al.* 2010a; 2010b):

$$h^2_{op} = \frac{\sigma^2_a}{\sigma^2_p}$$

$$\sigma^2_a = \frac{\sigma^2_{f(s)}}{0.4}$$

$$\sigma^2_p = \sigma^2_{f(s)} + \sigma^2_e$$

$$CV_a = \frac{\sqrt{\sigma^2_a}}{\bar{x}}$$

where σ^2_a is the additive genetic variance within subraces and is estimated from the variance between families ($\sigma^2_{f(s)}$) assuming an average coefficient of relatedness (*r*) of 0.4 for open-pollinated progenies corresponding to an average outcrossing rate of 70% (Griffin and Cotterill 1988), a widely used assumption for *E. globulus* (Stackpole *et al.* 2010a; Stackpole *et al.* 2010b; O'Reilly-Wapstra *et al.* 2013); σ^2_p is the phenotypic variance component, σ^2_e is the residual variance and $\bar{x}$ is the trait mean. An adjustment of *r* to account for an average relatedness greater than half-sibs (i.e. *r>0.25*) is usually applied when evaluating open-pollinated eucalypt progeny as eucalypts have a mixed mating system (Potts *et al.* 2004; Tambarussi *et al.* 2018). However, we note this adjustment does neither account for tree-to-tree differences in outcrossing rate (Patterson *et al.* 2005; Mimura *et al.* 2009), nor the average or individual effects of inbreeding depression (Costa e Silva *et al.* 2010a).

The degree of quantitative genetic divergence between subraces was estimated using the quantitative inbreeding coefficient *(Q_{ST})* following Yang *et al.* (1996) and Latta (1998)

$$Q_{ST} = \frac{\sigma^2_s}{\sigma^2_s + 2\,\sigma^2_a}$$

where σ^2_s is the variance between subraces. Following Stackpole *et al.* (2011), a two-tailed LRT was undertaken for each trait to test Q_{ST} against the mean F_{ST} (0.09) derived from eight, putatively neutral, microsatellite markers (Steane *et al.* 2006). The formulation of the LRT followed Dutkowski and Potts (2012). F_{ST} is a measure of the genetic differentiation between populations through random drift or mutation. A significant difference between Q_{ST} and F_{ST} indicates divergent (higher Q_{ST}) or stabilising (lower Q_{ST}) natural selection has impacted directly or indirectly on the trait (Latta 1998; Steane *et al.* 2006). This comparison assumes that mutation rates are equivalent between the molecular markers assayed from which F_{ST} is derived and the

QTL underlying the various quantitative traits (Edelaar and Björklund 2011; Meirmans and Hedrick 2011).

Tests of genotype-by-environment interactions (GxE) for each trait were partitioned into two separate analyses - the homogeneity of variances and inter-site (type-B, Burdon 1977) genetic correlations. Analyses involving the two NW trials fitted a bivariate version of model 1 treating the same trait measured at Salmon River and Togari trials as two different traits (Falconer 1952). The parameterisation of this model involved fixing the residual covariance and replicate correlation to zero and estimating the subrace and family within subrace correlations as well as all variance components (i.e. allowing for heterogeneous variances across sites). The genetic correlations whether type-A (intra-site, inter-trait) or type-B (inter-site, single trait) were estimated following Jordan *et al.* (1999):

$$r_{1,2} = \frac{\sigma_{1,2}}{\sqrt{\sigma_1^2 \, x \, \sigma_2^2}}$$

where, $r_{1,2}$ is the correlation between trait 1 and trait 2, either at subrace or family within subrace level. $\sigma_{1,2}$ is the covariance between the traits, and σ_1^2 and σ_2^2 *a*re the variance components of respective traits. The homogeneity of variances was tested by constraining the variances of Salmon River and Togari trials to be homogeneous separately at the subrace and family levels and testing the difference in likelihoods of the unconstrained model using a two-tailed LRT. At each level, the significance of the type-B correlation was tested against 1 using a one-tailed LRT. Following the threshold defined by Robertson (1959), for traits with the significant type-B genetic correlations below 0.8 were treated as different and likely to reflect biologically relevant GxE. A tri-variate version of model 1 was similarly used to test for GxE between the wetter NW sites and the drier Latrobe site. However, in this case, correlations involving the two NW trials were constrained to be equal, providing a single estimate for the across site correlations at the subrace and family within subrace levels.

For the NW sites, within trial type-A genetic correlations among different traits were estimated using a four-variate version of model 1, where each trait was represented as a separate variable according to the site on which it was measured. In this case all residual and replicate variances across trials were considered independent (i.e. covariances/correlations were constrained to zero). The homogeneity of the type-A correlations at Salmon River and Togari trials at subrace and family within subrace levels were tested using a two-tailed LRT by comparing an unconstrained model to one where the correlations at a given level for both trials were constrained to be equal. These tests were rarely significant and thus pooled correlations are presented and tested against zero using a two-tailed LRT. The pooled inter-trait phenotypic

correlations within trials were Pearsons correlation coefficients, calculated after site standardisation of the phenotypic measurements. Standard errors for these correlations were calculated following Zar (1999). For comparison of the subrace and family within subrace level correlations between the NW trials and the equivalent correlations from Latrobe data, the pooled NW inter-trait correlation values were tested using a two-tailed LRT against (i) those previously published by Stackpole *et al.* (2010a); Stackpole *et al.* (2010b), and (ii) the average of the values from the literature as reported by Stackpole *et al.* (2010b).

The univariate and multivariate analyses and parameter estimation was undertaken using ASReml^{TM} Version 4.1 (Gilmour *et al.* 2015b). The univariate analysis treating stem straightness was undertaken using the !MULTINOMIAL qualifier in ASReml, with the !CUMULATIVE option and a !LOGIT link function. However, in this case, variance components could not be tested with the likelihood ratio test (LRT) (Gilmour *et al.* 2015b). The Proc Corr procedure of SAS^{TM} (version 9.4) was used to calculate phenotypic correlations and partial correlations.

3.3. Results

3.3.1. North-west sites

There was little difference in trait grand means between the two north-west (NW) trials - Salmon River (SR) and Togari (TO) (Table 3.2). Significant subrace and family within subrace level variances were detected at both trials for most traits (Table 3.2). For all traits, the univariate narrow-sense heritability estimates (h^2_{op}), subrace differentiation (Q_{ST}) and coefficient of additive genetic variation (CV_a) from Salmon River and Togari were remarkably similar (Table 3.2). For the Gaussian models, the highest heritability estimates were obtained for stem diameter (DBH) and basic density (BD) and the highest Q_{ST} estimates were obtained for KPY (Table 3.2). Multinomial analyses of Straightness, more than doubled the heritability estimates compared with Gaussian model estimates, but the Q_{ST} estimates were virtually the same (Table 3.2). While all subrace variances were statistically greater than zero (LRT, P<0.01; Table 3.2), KPY was the only trait where the Q_{ST} estimates were significantly greater than the mean (0.09) F_{ST} values for neutral markers at all trials. The CV_a for DBH and Straightness were 3 to 19 times greater than those for wood properties (Table 3.2). Consistent with the similarity in means and genetic parameter estimates, likelihood ratio tests (LRT) indicated that inter-site variance heterogeneity was insignificant (P>0.05) between Salmon River and Togari at both the subrace and family within subrace levels, virtually for all comparisons (Table 3.3).

3.3.2. Genotype-by-environment interaction (GxE)

The inter-site (type-B) genetic correlations at the subrace and family within subrace level ($r_{SR,\ TO}$) among the NW sites were all high (>0.85) and not statistically different from one, except for the subrace correlation for basic density (Table 3.3). Combined with the general homogeneity of variances, these high type-B correlations indicate that there is little GxE and therefore, data from both NW trials can be effectively combined. Accordingly, NW subrace least-square means (Figure 3.1 & 3.2; see Supp. 3.1) and pooled inter-trait correlation coefficients (Table 3.4) were estimated.

3.3.3. Inter-trait correlations

DBH was positively correlated with most traits at the subrace, family within subrace and phenotypic levels, particularly Straightness (Table 3.4). The major exception was BD. However, BD was significantly correlated with KPY, but in opposite directions at the subrace (-0.61) and family within subrace (0.85) levels, resulting in a phenotypic correlation of almost zero. BD was not significantly correlated with Straightness at any level. While it was highly positively correlated with AWV at the family within subrace level (0.78), it was uncorrelated at subrace level. The subraces with the highest AWV (i.e. stiffer wood) were those from the Bass Strait islands (King Island [KI] and Southern Furneaux [SF], Figure 3.1), whereas KI, had the lowest basic density of all subraces studied (Figure 3.2). KPY was significantly positively correlated with Straightness at the subrace level (0.70), but these traits were uncorrelated at the family within subrace level, even after accounting for the joint covariation with DBH (see Supp. 3.2). The western subraces (particularly KI and WT) have straighter stems whereas, apart from Strezlecki Ranges (StrR), the eastern subraces had less straight stems (particularly St Helens [StH]) (Figure 3.1a). In the NW trials, the subraces with the straighter stems also had the highest pulp yield (KI and WT) and the least straight subrace, StH had the lowest pulp yield (Figure 3.2e). KPY was also positively correlated to AWV at the subrace, family within subrace and phenotypic levels, although the family within subrace level correlation was not statistically significant (P=0.103). In particular, the KI subrace stands out as having high KPY (Figure 3.2e) and high AWV (Figure 3.1b). The correlation between AWV and KPY is independent of any covariation with BD or DBH (see Supp. 3.2). There was no major effect on the univariate estimates of h^2_{op} or Q_{ST} when phenotypically correlated traits were included as covariates in the model 1 (see Supp. 3.3).

Table 3.2. Site means and genetic parameters for stem diameter (DBH), stem straightness (Straightness), basic density (BD), acoustic wave velocity (AWV) and Kraft pulp yield (KPY) estimated for two 10-year old field trials of *Eucalyptus globulus* in NW Tasmania (Salmon River - SR, Togari - TO) and Latrobe (LA). The LA estimates were obtained through a re-analysis of the data originally analysed by Stackpole *et al.* (2010a) and Stackpole *et al.* (2010b).

		DBH (mm)	BD (kg/m^3)	KPY (%)	Straightness[3] (1-6 scale)	AWV (km/s)
Mean	SR	161.20 ± 44.62	495.80 ± 35.57	55.51 ± 2.27	2.97 ±1.11	3.94 ± 0.35
	TO	161.30 ± 48.07	503.70 ± 33.55	55.09 ± 2.13	2.77 ± 1.21	3.92 ± 0.30
	LA	112.60 ± 34.45	539.60 ± 36.73	53.24 ± 1.98	-	-
h^2_{op}	SR	0.26 ± 0.05 ***	0.35 ± 0.11 ***	0.04 ± 0.09 [1]	0.17 ± 0.04 ***	0.17 ± 0.11 *
	TO	0.32 ± 0.06 ***	0.34 ± 0.12***	0.10 ± 0.10 [1]	0.21 ± 0.05 ***	0.17 ± 0.11 [1]
	LA	0.19 ± 0.03 ***	0.49 ± 0.06***	0.31 ± 0.05 ***	-	-
Q_{ST}	SR	0.18 ± 0.08 ***	0.21 ± 0.10 ***	0.74 ± 0.53 ***[2]	0.06 ± 0.04 **	0.12 ± 0.11 **
	TO	0.18 ± 0.08 ***	0.19 ± 0.10 ***	0.57 ± 0.28 ***[2]	0.08 ± 0.05 ***	0.23 ± 0.17 ***
	LA	0.08 ± 0.04 ***	0.20 ± 0.08 ***[2]	0.48 ± 0.12 ***[2]	-	-
CV_a	SR	13.40	15.29	3.89	3.07	2.61
(%)	TO	15.92	19.55	3.58	2.69	1.05
	LA	13.22	-	4.34	-	1.73

The table shows grand mean and its standard deviation (mean ± SD), the within subrace narrow-sense heritability estimate with its standard error (h^2_{op}± SE, significant difference of family within subrace variance from zero based on a one-tailed likelihood ratio test is indicated as asterisks), quantitative inbreeding coefficient with its standard error (Q_{ST} ± SE, significant difference of subrace variance from zero based on a one-tailed likelihood ratio test is indicated as asterisks) and coefficient of additive genetic variation (% CV_a). The Q_{ST} values shown were calculated assuming 70% outcrossing. Asterisks indicate the results of the test of significance as ns p>0.05; * p<0.05; ** p<0.01; *** p<0.001. Straightness and AWV were not available for the site Latrobe (-). [1] significantly different from zero at 0.05 level in bivariate analysis but not in the univariate analysis; [2] Q_{ST} is significantly different from the mean F_{ST} (0.09) of Steane *et al.* (2006) at 0.05 level of significance; [3] h^2_{op} and Q_{ST} estimates using the multinomial GLMM model - h^2_{op} SR: 0.50 ± 0.09 and TO: 0.57 ± 0.1; Q_{ST} SR: 0.05 ± 0.04 and TO: 0.08 ± 0.06

Table 3.4. Genetic and phenotypic correlations (± standard error) among DBH, Basic density, KPY, Straightness, and AWV at the subrace (Subrace), family within subrace (Family) and phenotypic (Phenotypic) levels for the two 10-year old *E. globulus* trials in NW Tasmania

		DBH	BD	KPY	Straightness
BD	Subrace	-0.12 ± 0.31 ^{ns}			
	Family	-0.06 ± 0.15 ^{ns}			
	Phenotype	-0.06 ± 0.03 ^{ns}			
KPY	Subrace	0.77 ± 0.02***	-0.61 ± 0.21*		
	Family	0.36 ± 0.17 ^{ns}	0.85 ± 0.33**		
	Phenotype	0.37 ± 0.03 ***	-0.01 ± 0.03 ^{ns}		
Straightness	Subrace	0.57 ± 0.21***	-0.24 ± 0.33 ^{ns}	0.70 ± 0.06***	
	Family	0.64 ± 0.09***	-0.18 ± 0.16 ^{ns}	0.02 ± 0.22 ^{ns}	
	Phenotype	0.35 ± 0.02 ***	-0.03 ± 0.03 ^{ns}	0.26 ± 0.03 ***	
AWV	Subrace	0.74 ± 0.18 **	0.00 ± 0.22 ^{ns}	0.76 ± 0.15***	0.58 ± 0.26 ^{ns}
	Family	0.36 ± 0.18 ^{ns}	0.78 ± 0.19***	0.64 ± 0.26 ^{ns}	0.18 ± 0.21 ^{ns}
	Phenotype	0.23 ± 0.03 ***	0.26 ± 0.03 ***	0.52 ± 0.03 ***	0.15 ± 0.03 ***

Given are the pooled intra-site correlation coefficients of two different trials in NW Tasmania (Salmon River and Togari). The pooled correlations were tested against zero and significance levels based on two-tailed likelihood ratio test are indicated as ns p>0.05; * p<0.05; ** p<0.01; *** p<0.001.

3.3.4. North-west versus Latrobe sites

The previously studied Latrobe trial was on a drier (Table 3.1) and markedly less productive site than the two NW trials, as evidenced by the mean (±sd) DBH comparisons at the only common ages assessed across all trials (4 years: SR [101.5±27.8 mm], TO [98.2±30.7 mm], LA [66.2±19.3 mm] and 10 years: see Table 3.2). There was significant GxE for DBH across these divergent sites (NW *versus* Latrobe). Both the subrace and family within subrace level variances for DBH were significantly different and the across site genetic correlations were significantly less than 1 (Table 3.3). Indeed, the subrace correlation between the NW and Latrobe were effectively zero (LRT from 0, P=1.0). While the faster growing races in the NW were the more local KI and Otway (EOt and WOt) subraces (in terms of geography and climate similarity between trial site and origin of subrace), at Latrobe the faster growing subraces were from southern Tasmania (Recherche Bay [RB], Southern Tasmania [ST] and South-Eastern Tasmania [SET]). Despite the high GxE for growth, the GxE for the wood property traits (KPY and BD) were remarkably low (Table 3.3). Their type-B genetic correlations were 0.77 or greater, and not significantly differed from 1, at the family within subrace level. The geographic pattern of variation in BD was very similar between NW and Latrobe, with the highest density subraces occurring on the mainland (WOt and StrR), and the lowest on KI (Figure 3.2c,d). The type-B correlation observed for KPY (0.77) was slightly lower than that for density, but this could reflect the difference in sampling material (whole-length cores at Latrobe *versus* outer cores in the NW). The pattern of geographic variation in KPY (high in KI subrace and low in the eastern Gippsland subraces [StrR and CP]) was consistent between Latrobe and the NW sites (Fig 3.2e,f). Nevertheless, the ST subrace which had the highest KPY in Latrobe had average KPY in the NW sites. The wood properties of Western Tasmania (WT) and RB subraces were not assessed at Latrobe, but the NW trials reveal that the WT subrace had the second highest KPY of the subraces assessed and RB had average pulp yield comparable to the adjacent ST subrace.

Table 3.3. Tests of homogeneity of variances (P value) and genetic correlations (r ± standard error) across the *E. globulus* sites at the subrace (Subrace) and family within subrace (Family) level, testing for rank order and scale components of genotype-by-environment interaction (GxE) respectively

Trait		Variance homogeneity (SR,TO)	$r_{SR, TO}$	Variance homogeneity (NW,LA)	$r_{NW, LA}$
DBH	Subrace	0.498	0.93 ± 0.07 ^{ns}	0.011	-0.07 ± 0.34 **
	Family	0.303	0.85 ± 0.09 ^{ns}	0.001	0.54 ± 0.26 ***
BD	Subrace	0.480	0.85 ± 0.13 *	0.107	0.91 ± 0.07 *
	Family	0.806	1.00 ± 0.00 ^{ns}	0.180	0.98 ± 0.12 ^{ns}
KPY	Subrace	0.841	1.00 ± 0.00 ^{ns}	0.233	0.77 ± 0.14 ***
	Family	0.806	1.00 ± 0.00 ^{ns}	0.112	0.85 ± 0.35 ^{ns}
Straightness	Subrace	0.007	0.97 ± 0.15 ^{ns}	-	-
	Family	1.000	1.00 ± 0.00 ^{ns}	-	-
AWV	Subrace	0.342	1.00 ± 0.00 ^{ns}	-	-
	Family	0.689	1.00 ± 0.00 ^{ns}	-	-

Comparisons are shown for (i) the two northwest sites of *E. globulus* (Salmon River [SR] *versus* Togari [TO]) and (ii) the northwest sites combined (NW) compared with the Latrobe (LA) site studied by Stackpole *et al.* (2010a); (2010b). The probabilities are given for the tests for the heterogeneity of variances based on the likelihood ratio test comparing the difference between fitting a pooled *versus* independent variance estimates. The genetic correlations and their standard errors are shown as well as the significance of the one-tailed likelihood ratio test from 1 (ns $p>0.05$; * $p<0.05$; ** $p<0.01$; *** $p<0.001$). Straightness and acoustic wave velocity measurements (AWV) were not available for the site Latrobe (-).

3.4. Discussion

3.4.1. Genotype-by-environment interaction (GxE) for growth

In the studies of *E. globulus*, GxE has been detected using quantitative genetics for growth traits in Australia (MacDonald *et al.* 1997; Muneri and Raymond 2000; Costa e Silva *et al.* 2006; Callister *et al.* 2011) and overseas (Potts *et al.* 2004; Salas *et al.* 2014), including at the QTL level between trials across Australia (Freeman *et al.* 2013). While greater than average GxE has been reported among *E. globulus* trials across countries than that of within countries (Potts *et al.* 2004), the present study highlights the importance of local site effects, whereby large GxE can occur when site differences are marked, as was the difference between our wet (NW) and dry (LA) sites. In one of the most detailed studies of *E. globulus* GxE in Australia, water availability was shown to be a major factor driving GxE at the subrace level (Costa e Silva *et al.* 2006). Some subraces (i.e. KI, WOt and EOt) used in our study were more local to the wetter NW sites where they performed well (Figure 3.2a). However, these subraces were only average performers at the Latrobe site (Figure 3.2b), consistent with their relatively poorer growth on dry sites (Costa e Silva *et al.* 2006), greater drought susceptibility (Dutkowski and Potts 2012) and likely local climate adaptation (Leimu and Fischer 2008). The worst performing subraces in the NW sites were those from eastern Tasmania. Of note was the stable, performance of the Strezlecki Range (StrR) subrace in the NW and Latrobe sites in our study (Figure 3.2), consistent with its relatively stable performance in the Australia-wide study of Costa e Silva *et al.* (2006).

3.4.2. Density

One of the key findings from this study was the marked difference between GxE for growth (DBH) and that observed for wood properties. Despite high GxE for growth between the NW and Latrobe sites, that for wood properties were exceptionally small, both at the subrace and family within subrace levels, particularly for basic density. The across site stability of genetic differences in wood properties compared with growth traits is commonly reported in forest tree species (Chen *et al.* 2017; Li *et al.* 2017b). Our subrace level inter-site correlations involving basic density were greater than the threshold (0.8) above which GxE can be considered biologically insignificant (Robertson 1959). Similar results of high inter-site correlations for basic density and pilodyn penetration (an indirect measurement of basic density) have been previously reported at both the subrace and family within subrace levels (MacDonald *et al.* 1997; Muneri and Raymond 2000; López *et al.* 2002; Costa e Silva *et al.* 2009), confirming the extremely low level of GxE for basic density in *E. globulus*. The lower GxE for basic density compared to growth in *E. globulus* is also evident at QTL level. In a study done by Freeman *et al.* (2013), only 24% of basic

density QTLs exhibited GxE, compared to the 38% of growth QTLs, when the same families were compared across wet and dry sites in Australia. The pattern of subrace variation for basic density observed in the present study was very similar to that reported in previous studies of *E. globulus* - the higher basic density of the Strezlecki range subrace and the low basic density of King Island (Stackpole *et al.* 2010b). Similar patterns of geographic variation in density are observed in other sites regardless of the measurement technique used (Pilodyn penetration - Dutkowski and Potts 1999; López *et al.* 2001; discs - Hamilton *et al.* 2010; core - Stackpole *et al.* 2010b) or country (Australia - Dutkowski and Potts 1999; Argentina - López *et al.* 2002). Not only did basic density exhibit high variation among subraces, within subraces it showed the highest narrow-sense heritability (0.35 to 0.49) of all the traits measured in the present study. Many studies reported high heritability of basic density, which averages 0.50 across 11 single-trial estimates from seven studies (Dean *et al.* 1990; Borralho *et al.* 1992; Muneri and Raymond 2000; Raymond *et al.* 2001; Apiolaza *et al.* 2005; Poke *et al.* 2006; Salas *et al.* 2014). This average is double that for DBH, which averages 0.22 across 10 trials from five studies (MacDonald *et al.* 1997; Muneri and Raymond 2000; Stackpole *et al.* 2010a; Callister *et al.* 2011; Salas *et al.* 2014).

The genetic correlation between diameter (DBH) and wood density is well-studied in *E. globulus*, however, results vary considerably in both sign and magnitude. In the present study, subrace, family within subrace, and phenotypic level correlations between basic density and DBH were not significantly different from zero. This finding of a poor association is consistent with the previous observations of *E. globulus* (Downes *et al.* 2006; Stackpole *et al.* 2010a; Salas *et al.* 2014) but differs to the negative association observed by MacDonald *et al.* (1997). Two factors may in part contribute to the variation in the correlation between growth and density. Firstly, the sign of the correlation may be site-dependent. Indeed, MacDonald *et al.* (1997) showed the change of subrace-level type-A correlation from negative at wet sites to positive at dry sites despite the negative within subrace genetic correlation. Secondly, in *E. globulus* the relationship between growth and density changes with age. For example, Stackpole *et al.* (2010a) found a significant negative genetic correlation between basic density and diameter at selection age (4-5 years), however, by the harvest age (16-17 years) the genetic correlation was non-significant and slightly positive. In the present study, while not statistically significant, a small negative trend was noticed at the subrace, family within subrace, and phenotypic levels on wet site (i.e. NW), consistent with Stackpole *et al.* (2010a) for the intermediate age (10 years) of assessment.

3.4.3. Pulp yield

The heritability of pulp yield in the NW trials (0.04-0.10) was the lowest of all traits assessed and only marginally significant. Consistent with previous reports (Raymond *et al.* (2001), low GxE at both the subrace and family within subrace level were observed in pulp yield (high type-B correlations) between the two NW sites and the Latrobe site. The low narrow-sense heritability for pulp yield at the NW trials is at odds to the literature, where considerably higher values have been previously reported (0.42 - Costa e Silva *et al.* 2009; 0.40 - Stackpole *et al.* 2010b; 0.26 - Hamilton *et al.* 2017b). The low heritability for pulp yield could in part be due to the smaller length of core sampled (50 mm cores in this study compared to whole-length cores in other studies) and thus high measurement error, but this does not explain the fact that high subrace differences were detected. The low coefficient of additive genetic variation for pulp yield supports previous studies (Stackpole *et al.* 2010b; Hamilton *et al.* 2017b), suggesting less opportunity for increasing the mean of this trait through selection compared with other traits studied. This argument similarly applies to wood properties in general, exhibiting coefficient of additive genetic variation markedly less than that reported for diameter, in concordance with previous studies (Stackpole *et al.* 2010a; Li *et al.* 2017a).

The subrace differentiation for pulp yield, as assessed using Q_{ST}, was the highest of all traits assessed in both the NW and Latrobe trials (Table 3.2). The Q_{ST} values were significantly greater than the mean F_{ST}, signalling that divergent selection (Leinonen *et al.* 2013) has likely driven the subrace differentiation, consistent with the previous finding by Stackpole *et al.* (2011). This differentiation no doubt reflects the strong genetic correlations of pulp yield with associated wood chemical traits, such as cellulose (>0.9) and lignin (<-0.9) (Stackpole *et al.* 2011), more likely to be under direct selection. The geographic pattern of subrace variation in pulp yield has not been widely studied. The study at Latrobe trial (Stackpole *et al.* 2010b), the largest study to date, showed that the Victorian subraces, currently favoured in breeding programs because of their high density (Potts *et al.* 2014), were among the lowest for pulp yield. They also showed a clinal pattern of decreasing pulp yield among subraces distributed on the east coast of Tasmania, and the subraces with the highest pulp yield were from King Island (KI) and Southern Tasmania (ST). To a large extent, these are confirmed in the wetter NW sites, except that the Southern Tasmania subrace was only average in pulp yield.

The genetic correlation (i.e. family within subrace) of pulp yield with DBH varies greatly with reports ranging from -0.54 to 0.12 (Dean *et al.* 1990; Raymond *et al.* 2001; Apiolaza *et al.* 2005; Costa e Silva *et al.* 2009). In the current study, we observed a marginally significant but positive correlation between growth and pulp yield (0.36; Table 3.4; see Supp. 3.4) in the NW

trials, which was consistent with the significant positive genetic correlation (0.52) observed at the Latrobe trial (Stackpole *et al.* 2010b). At the subrace level also, this correlation was positive and significant in the NW trials, but not significant at Latrobe trial, though it was positive (Stackpole *et al.* 2010b). Given the common set of base-population families, the results suggest that regardless of whether outer-wood or whole-length cores are used, or whether the material (i.e. families within subrace) is tested on wet or dry sites, the association between NIRS predicted Kraft pulp yield and growth is positive at both the subrace and family-within subrace levels. While the magnitude of these correlations is only low to medium, the selection for fast growth should indirectly result in increased pulp yield to some degree.

The positive and significant family within subrace genetic correlation between pulp yield and density was the highest correlation (0.85) observed in the current study. This positive genetic correlation between pulp yield and density was consistent with previous reports (Dean *et al.* 1990; Raymond *et al.* 2001; Apiolaza *et al.* 2005; Costa e Silva *et al.* 2009)(see Supp. 3.4), but differed from the non-significant genetic correlation reported by Stackpole *et al.* (2010b) at Latrobe. In contrast to this family-level genetic correlations, the subrace level correlation between pulp yield and density from the NW trials was not significantly different from that obtained at Latrobe (see Supp. 3.4).

A notable feature of our study was the marked discrepancy in the correlations between pulp yield and density observed at the subrace and family within subrace levels. At the family within subrace level, higher density was significantly associated with higher pulp yield (0.85), whereas at the subrace level higher density was significantly associated with lower pulp yield (-0.61). This trend was also evident in the Stackpole *et al.* (2010b) study and may in part be associated with the significant divergent selection acting on traits associated with pulp yield as noted above. Overall, the correlated response of pulp yield and density to selection for a pulpwood breeding objective will be complex and affected by the relative importance of subrace and family within subrace contributions. Regardless of this complexity, at either genetic level, the expected response to selection on the other key pulpwood selection traits (density and DBH) will likely be independent.

3.4.4. AWV (stiffness)

The current study also found significant genetic variation within *E. globulus* for acoustic wave velocity (AWV), which was independent of growth and basic density. While growth, density and pulp yield are pulpwood selection traits, AWV is associated with wood stiffness which is a solid-wood selection trait. Our heritability estimate (0.17) was lower than that

reported from a Western Australia *E. globulus* trial by Hamilton *et al.* (2017b) (0.26), and from Tasmanian trials of *E. nitens* (0.43) by Blackburn *et al.* (2014). Nevertheless, we found statistically significant subrace variance, as did Hamilton *et al.* (2017b) using a subset of the subraces tested here. In addition, inter-site (type-B) correlations at both the subrace and family within subrace levels were effectively 1.0, indicating no GxE among the NW sites, where the site differences were small. Nevertheless, low GxE was also reported across more divergent sites in *E. nitens* (Blackburn *et al.* 2014). The subrace differences observed were mainly due to the subraces from Bass Strait islands showing high AWV compared with other subraces, particularly those on the east coast of Tasmania. Wood stiffness is believed to enhance the ability of the main stem of the tree to tolerate strong winds without breaking (Moore *et al.* 2018), and it is possible that the high AWV of the island subraces is an adaptation of the main stem to greater wind exposure compared with the other subraces of *E. globulus* (Australian Bureau of Meteorology 2011).

The present study showed a general positive correlation of AWV with DBH, which was highest at the subrace level (0.74), suggesting that selection for fast growth would tend to increase AWV and thus wood stiffness. Similar trends were evident in *E. nitens*, although their significance varied with the site (Blackburn *et al.* 2014). In the same *E. nitens* study, they also found a positive phenotypic correlation between AWV and BD, but this was only significant at the genetic level at two of the three sites studied. These findings accord with the significant correlations we obtained in *E. globulus* at the family within subrace level (0.78) and phenotypic level (0.26), although there was no correlation at the subrace level. A positive relationship between wood density and stiffness (positively correlated to AWV and MOE) is well-established (Evans and Ilic 2001), although at the genetic level exceptions do exist (Li *et al.* 2017a). Indeed, our observed high genetic correlation between AWV and density within subraces suggests a pleiotropic relationship between these two traits, but the absence of a significant subrace level correlation suggests this relationship is uncoupled at the broader geographic scale (Gauli *et al.* 2015). A key aim of this study was to test the strong positive genetic association between AWV and pulp yield in *E. globulus* reported at additive genetic and QTL levels by Hamilton *et al.* (2017b). The present study supports these findings at subrace (0.76), family within subrace (0.64) as well as phenotypic (0.52) levels. Consistent with Hamilton *et al.* (2010), our generally favourable correlations of AWV with DBH, density and pulp yield indicate strong concordance between the pulpwood and solid-wood breeding objectives in this case.

3.4.5. Straightness

The other key solid-wood selection trait studied was stem straightness. The high inter-site type-B genetic correlations (>0.9) for stem straightness at the subrace and family within subrace levels in this study, is consistent with previous studies reporting low GxE for stem straightness in tree species (Li *et al.* 2017b), including eucalypts (Callister *et al.* 2011; Blackburn *et al.* 2013). This was coupled with the significant heritability and subrace variation observed for this trait. The low to moderate heritabilities we estimated for this trait (0.17 & 0.21) were similar to the values obtained in other studies on *E. globulus* (0.28 - Callister *et al.* 2011; 0.19 & 0.33 - Blackburn *et al.* 2013; 0.20 - Hamilton *et al.* 2015a) as well as other tree species (0.15, *E. camaldulensis* - Mahmood *et al.* 2003; 0.28, *E. nitens* - Hamilton and Potts 2008; <0.16, *Acacia mangium* - Hai *et al.* 2015). Our multinomial estimates exceeded these values as well as those previously reported multinomial estimates in *E. globulus* (0.09 - Mora and Serra 2014).

We showed significant but low divergence among the *E. globulus* subraces (Q_{ST}) in stem straightness and similar patterns of geographic variation to that reported for general stem form (of which straightness was one component) by Volker and Orme (1988) and López *et al.* (2001). Of note is the general good form/straightness of King Island (KI) and Western Tasmanian (WT) subraces (Fig. 1a; Volker and Orme 1988), and the south-north clinal decrease in form/straightness along the east coast of Tasmania (Fig. 1a; Volker and Orme 1988; López *et al.* 2001). Volker and Orme (1988) attributed the poor form of St Helens (StH) to its susceptibility to marsupial browsing, which is linked to its low foliar defensive chemistry (O'Reilly-Wapstra *et al.* 2013). This subrace was not included in the studies of Blackburn *et al.* (2013) and Hamilton *et al.* (2015a) which showed non-significant variation in stem straightness between subraces. Browsing has been shown to adversely affect stem straightness in *E. globulus* (Borzak *et al.* 2015) and may contribute to the higher subrace variance and higher Q_{ST} for straightness at Togari (Table 3.2) where marsupial browsing was greater than at Salmon River (O'Reilly-Wapstra *et al.* 2013).

The positive family within subrace level correlation of Straightness with DBH was consistent with the positive additive genetic correlations reported for *E. globulus* by Blackburn *et al.* (2013), but not the non-significant negative correlations reported by Callister *et al.* (2011). Genetic correlations between Straightness and DBH have been reported in the literature ranging from positive (0.53, *E. nitens* - Hamilton and Potts 2008; 0.25 to 0.37, *Acacia mangium* - Hai *et al.* 2015; 0.92, Pinus hybrid - Belaber *et al.* 2018) to negative (-0.42, *E. camaldulensis* - Mahmood *et al.* 2003). This variation in the level and direction of the genetic correlation between these traits across species suggests some uncertainty in the simultaneous improvement for growth

and Straightness traits. For *E. globulus,* most of the genetic correlations are positive as are all phenotypic correlations (0.35 - present study; 0.11, 0.13, 0.20, 0.18 - Blackburn *et al.* 2013). However, as noted by Blackburn *et al.* (2013), with significant positive correlations between Straightness and DBH, we cannot dismiss the possibility that with the subjective measure, assessors tended to give larger stems more favourable straightness scores. Nevertheless, even when such bias is accounted by fitting DBH as a covariate, significant heritability and subrace effects were observed. In addition, similar geographic patterns of subrace variation were obtained in the adjusted and unadjusted analyses arguing for genetic differences in Straightness which are not just a reflection of DBH variation.

The association between Straightness and density in the present study was in a negative direction although non-significant. Similar results were previously reported in some other eucalypt species (Kien *et al.* 2008), but not always (Borralho 1997). However, most of the studies (including this study) consistently report no significant relationship between these two traits. In the present study, the strong association observed between Straightness and pulpyield (0.70) at the subrace level and not at the family within subrace level could be just a correlation by chance due to the indirect association of these traits to other wood properties.

3.5. Conclusions

In conclusion, the present study shows significant genetic variation resides within the *E. globulus* gene pool for all pulpwood and solid-wood selection traits examined. In addition, the significant genetic correlations between traits show generally favourable alignment of pulpwood and solid-wood selection traits. Thus, improvement of the *E. globulus* resource made so far through pulpwood breeding should also be expected to have made gains for solid-wood breeding. Where favourable correlations were not detected, traits were genetically independent as opposed to adversely correlated. Despite large GxE detected for growth between wet and dry sites, the wood properties were remarkably stable. The previously reported geographic pattern of variation between subraces and genetic correlations among pulpwood traits more-or-less confirmed, including the opposing genetic correlations between pulp yield and density at the subrace and family within subrace levels.

3.6. Supplementary material

Supp. 3.1. Estimation of least-square means

For every trait plotted in the Fig. 1 and 2, subrace least-square means were calculated from the subrace main effect in a mixed model that included subrace, trial and their interaction as fixed effects, and replicate within trials, family within subrace and the family by trial interaction terms as random effects in the Model 1. These analyses were undertaken with ASReml following Hamilton *et al.* (2013). The geographic distribution of the subrace means was plotted using the *maptools* (Lewin-Koh 2010) and *sp* packages (Bivand *et al.* 2008) in R version 3.3.1 (R Core Team 2017) in Fig 1 and 2.

Supp. 3.2. Partial correlations and including correlated traits as covariates

The effect of DBH on Straightness-KPY correlations

Straightness and KPY were both significantly positively phenotypically correlated with DBH, but after removing the effect of DBH, the Pearson partial correlation coefficient ($r_{partial}$ = 0.20, P<0.001) between Straightness and KPY was still significant, indicating that there is a positive relationship between these traits that is independent of variation in DBH. This was also confirmed at the subrace level by fitting DBH as a covariate into the multivariate mixed models ($r_{subrace}$ = 0.65±0.07).

The effect of BD and DBH on the AWV-KPY correlations

When BD was fitted as a covariate in the multivariate analyses involving AWV and KPY the high genetic correlations were still maintained ($r_{subrace}$ = 0.84 ± 0.11; r_{family}= 0.61 ± 0.33). In addition, the partial phenotypic correlation coefficient between AWV and KPY was still significant after accounting for BD ($r_{partial}$ = 0.54, P<0.001). Similarly, after accounting for the joint correlation with DBH, the partial phenotypic correlation coefficient between AWV and KPY was still significant (DBH $r_{partial}$ = 0.48, P<0.001) and the genetic correlations were maintained at the subrace level ($r_{subrace}$ = 0.70 ± 0.20) and increased at the family level (r_{family}= 0.98 ± 0.33; LRT from zero P= 0.077).

Supp. 3.3. Results when including covariates in model 1

Many of the traits assessed were significantly correlated at the phenotypic level (see below) which could affect the detection of genetic variation in a focal trait. To test whether this was the case, traits highly correlated with the focal trait were included as a covariate in the linear model (Model 1) fitted for each trial. When BD was fitted as a covariate in the mixed model for AWV, subrace variance persisted as significantly different from zero (LRT - SR and TO: P<0.001), but family variances became non-significant at both sites (LRT – SR: P=0.192, TO: P=0.306). In the same case, Q_{ST} at both sites (AWV SR: Q_{ST} = 0.33±0.30 & TO: Q_{ST} = 0.53±0.52) markedly increased, but only at TO did the value exceeded the mean F_{ST} value. When DBH was included as a covariate for AWV, the subrace effects at SR as well as TO remained significant (SR: Q_{ST} = 0.09±0.08; P<0.05& TO: Q_{ST} = 0.23±0.23; P<0.01). When DBH was fitted as a covariate in the mixed model for Straightness, the subrace as well as family variances at both sites persisted as significantly different from zero in the one-tailed LRT (P<0.01 and P<0.001 respectively- SR: Q_{ST} = 0.07±0.05 & TO: Q_{ST} = 0.07±0.05). The pattern of subrace differences in AWV and Straightness were similar regardless of the inclusion of the covariate in the mixed model analyses (Figure 3.1 *versus* Figure below). Similarly, the inclusion of DBH as a covariate for KPY resulted in the significance of the family term for KPY at TO (P<0.05) but not at SR and had little effect on subraces differences (data not shown).

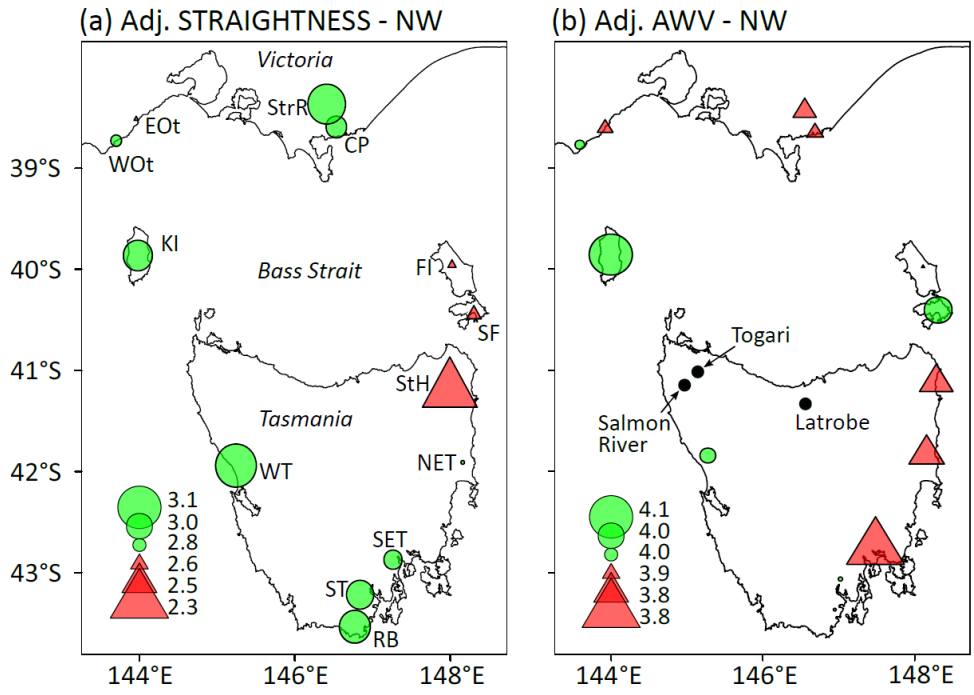

Figure. The geographic variation in subrace least-square means at the NW trials for (a) Adj. Straightness (1-6 scale) and (b) Adj. AWV (km/s) with the inclusion of the covariate DBH in the mixed model analyses

Supp. 3.4. Comparison of NW trials with previously reported values

A key objective of the current study was the comparison of the within-site (type-A) inter-trait genetic correlations between the NW trails and those previously published for the Latrobe trial. Despite the strong GxE for DBH (Table 3.3), the within site inter-trait correlation between DBH and BD at the NW trials were similar to those previously reported for Latrobe (see Table below). At both the subrace and family levels, DBH was effectively uncorrelated with BD (see Table below). In the case of DBH and KPY, the family correlation did not differ significantly between the estimates, but the subrace level correlation was significantly higher in the NW trials (0.77 vs 0.32). The directionality of the correlations between KPY and BD were the same at both sites. While the magnitude of the correlation was not significantly different at the subrace level, the family-level correlation observed at the NW sites was significantly higher than that observed at the Latrobe site (see Table below).

Table: Genetic correlations at the subrace and family within subrace levels and the significance level of the difference of the correlation coefficients obtained in this study (NW) from other reported studies.

		NW	Stackpole	Literature
KPY vs BD	Subrace	-0.65	-0.58 ^{ns}	-
	Family	0.85	0.18*	0.47 ^{ns}
DBH vs BD	Subrace	-0.12	0.05 ^{nc}	-
	Family	-0.06	0.05 ^{ns}	-
DBH vs KPY	Subrace	0.77	0.32 ***	-
	Family	0.36	0.52 ^{ns}	-0.24**

Subrace and family level correlations from NW site were tested against correlations from other studies (Stackpole *et al.* 2010a; Stackpole *et al.* 2010b) and the Literature (average values from other similar old studies as reported by Stackpole *et al.* (2010b)) using a likelihood ratio test and constraining the pooled NW estimates to that reported in other studies. Significance levels were indicated as 'ns' p>0.05; '*' p<0.05; '**' p<0.01; '***' p<0.001, based on two-tailed likelihood ratio test.

^{nc} - not converged in the analysis.

Chapter 4 - Application of resistance drilling to genetic studies of growth, wood basic density and bark thickness in *Eucalyptus globulus*

4.1. Introduction

Growth and wood basic density are key breeding objective traits in eucalypt genetic improvement programmes for pulpwood and solid-wood (Raymond and Apiolaza 2004; Rezende *et al.* 2014). Tree growth is commonly assessed as over-bark diameter at breast height (DBH) using a diameter tape (Husch *et al.* 2002). Wood basic density is either assessed destructively (e.g. discs sampled at intervals along a felled tree) or using non-destructive techniques, which can be direct (cores) or indirect (e.g. Pilodyn pin penetration) (Downes *et al.* 1997; Stackpole *et al.* 2010a). Non-destructive techniques are generally used for the necessarily large-scale measurement of selection traits in tree breeding, as they are quicker than destructive techniques, and the trees remain available for subsequent measurements and selection. The drill resistance profile derived from an IML Power Drill series instrument (RESI) is becoming one of the preferred non-destructive techniques for the assessment of wood density. Although RESI was initially used to measure wood decay (Costello and Quarles 1999; Johnstone *et al.* 2007), it is now being used for the assessment of wood basic density in various tree species (Isik and Li 2003; Silva *et al.* 2017; Fundova *et al.* 2018; Sharapov *et al.* 2019), including eucalypts (Downes *et al.* 2018). Simultaneously, RESI has the additional benefit of being able to measure stem diameter (Isik and Li 2003; Downes *et al.* 2018) and bark thickness (Downes *et al.* 2018).

RESI is a hand-held instrument that measures the radial variation in basic density from the resistance experienced by a metal needle drilled through the stem at a constant rotation rate and forward speed (Downes *et al.* 2018). Compared to other non-destructive techniques for assessing density, the RESI has low cost, high sampling speed, the ability to capture data digitally, and useful processing options include the weighting of the resistance measured to account for the radial variation in stem density (Downes *et al.* 2018). Moreover, evidence to date suggests that it also better predicts density than the Pilodyn, another option for high-speed non-destructive sampling (Downes *et al.* 2018; Fundova *et al.* 2018). In addition, as the RESI trace commences and ends at the outer bark, the marked changes in resistance at the bark/wood boundaries allow bark thickness and under- and over-bark stem diameters (DBH) to be quantified at the same time as the more subtle changes in resistance when drilling through the wood (Downes *et al.* 2018). A study based on *Eucalyptus globulus* and *E. nitens* showed that

at the phenotypic level RESI resistance values well-predict the within site variation in core basic density (R^2= 0.66 to 0.80), as well as DBH measured using the traditional methods (Downes *et al.* 2018). However, to use this tool for breeding purposes, genetic rather than phenotypic correlations are needed, as phenotypic correlations also include environmental covariance among traits (Falconer and Mackay 1996).

Eucalyptus globulus is the main plantation eucalypt grown in pulpwood plantations in temperate regions of the world (Harwood 2011), including Australia (Rhys and Mijo 2018). Field trials have shown significant population and family variation within the species for numerous traits, including growth, basic density and bark thickness (Dutkowski and Potts 1999). While there are numerous estimates of genetic parameters for growth and wood density from open-pollinated families of *E. globulus* (Potts *et al.* 2004; Stackpole *et al.* 2010a), there are few published estimates for bark thickness (Dutkowski and Potts 1999; López *et al.* 2002), despite its potential effect on the accuracy of log volume determined from over-bark DBH (Stayton and Hoffman 1970; Thomas and Bennett 2014) as well as its adaptive significance.

Both bark thickness and wood density are being increasingly recognised as functional traits of adaptative significance in forest trees. Bark thickness has been implicated in susceptibility to insect (*Pinus strobus* - Kriebel 1954; *Eucalyptus globulus* - Jordan *et al.* 2002), mammal (different tree species - Gill 1992), drought (*Eucalyptus globulus* - Dutkowski and Potts 2011) and fire (*Eucalyptus* and *Corymbia* spp - Lawes *et al.* 2011) damage. At the species-level, there is a trend for inner bark thickness to increase in hotter and drier environments in angiosperms, independent of fire, which is hypothesised to reflect its role in water and carbohydrate storage (Rosell 2016). Wood basic density has also been linked to many adaptive characteristics of forest trees, including susceptibility to insect (Lanuza-Garay and Barrios 2018) and drought (Ruiz Diaz Britez *et al.* 2014; Greenwood *et al.* 2017; Nabais *et al.* 2018) damage. In the case of drought, studies of various forest tree species report that increased wood density is associated with xylem properties, such as thicker walls and smaller conductive area, that reduce susceptibility of the xylem to cavitation under water stress (Hacke *et al.* 2001; Santini *et al.* 2016; Venturas *et al.* 2017).

In the present study, we aim to test the reliability of the RESI for genetic studies, and then use this methodology to study the genetic architecture of wood density and bark thickness in *E. globulus*, with a focus on understanding the correlated patterns of genetic variation both within and among subraces of this species.

4.2. Materials and methods

4.2.1. Field trials and traits assessed

Two *E. globulus* field trials, Salmon River [SR] and Togari [TO] in the north-west (NW) Tasmania (Figure 4.1), were used for the assessment of all traits. These trials were established in 2005 on ex-forest sites. The sites have similar past climates but have different soil types (Hamilton *et al.* 2013; O'Reilly-Wapstra *et al.* 2013). The planted seedlings were raised from open-pollinated seeds collected from wild trees representing the 13 *E. globulus* subraces (Dutkowski and Potts 1999; Potts *et al.* 2014). Spacing was 4.0m between and 2.3m within rows. In both trials, families were represented as single-tree plots in a randomised incomplete block design.

At age 10 years 1 month (10 years hereafter), 3-5 trees from each of 10 families in every subrace were drilled with RESI. These trees were spread across the first 8 (SR) or 10 (TO) replicates of the trials, depending on when healthy trees, ≥10 cm DBH were first encountered for each of the chosen families. These trees were those that had been cored and studied by Downes *et al.* (2018) and (Chapter 3). In total 503 trees from SR and 456 trees from TO were used in the assessment. All trees were drilled once at 1.3 m using an IML PD400 instrument (IML Australia) with a needle width of 3.1 mm, feed speed of 200 cm/min and rotation speed of 2500 RPM, attempting to pass through the centre of the tree. For each tree, a trace representing the profile of the resistance to the needle as it pierced the wood was produced. These traces were exported as text files using PD Tools Pro software and Eucalypt ResiProcessor (https://forestquality.shinyapps.io/EucalyptResi Processor/, accessed 6 January 2019) was used to calculate over- and under- bark diameter at breast height and mean cambium-to-cambium resistance (excluding bark) ($Resistance_{RESI}$) following Downes *et al.* (2018). Bark thickness was estimated as half the difference between the over- and under-bark DBH_{RESI} measures.

The RESI measurements of over-bark diameter (DBH_{RESI}) and wood basic density (BD) were compared at the phenotypic-level with analogous traditional measurements by Downes *et al.* (2018). In brief, at Togari and Salmon River, diameter at breast height (DBH) over bark at 1.3 m above ground level was measured using a diameter tape for every tree used for the RESI assessment at the age of 9 years 8 months (10 years hereafter) and 9 years 7 months (10 years hereafter) respectively. The same trees at the same age were also assessed for wood basic density by extracting cambium-to-cambium wood cores of 12 mm diameter taken at 1.1 m height above ground level using a motorised corer (Downes *et al.* 1997). Following Stackpole *et al.* (2010b), wood basic density (BD) was assessed from cores using the water displacement

method described by Smith (1954). The genetic architecture of these traits commonly measured is reported in Chapter 3.

No direct estimates of bark thickness were available for comparison with the RESI measurements from the two NW trials, but direct measurements were available from a large base population trial at Latrobe (LA; Figure 4.1). This trial was established using 561 open-pollinated families, collected from trees thorough out the geographical distribution of *E. globulus*. The trial comprised 5 replicates with 24 incomplete randomised blocks. Each block contained 30 plots with 2 trees of a family in every plot. This trial had a spacing of 4.0 m between rows and 2.5 m within rows. Here, bark thickness was measured from the bark window made while undertaking a Pilodyn assessment of the 5.5 year-old (hereafter referred to as six years) trail for wood density (Dutkowski and Potts 1999). In this case, the bark depth was measured by resting the Pilodyn on the stem and manually pushing the Pildyn pin until it hit the wood exposed by the bark window. The Pilodyn wood penetration and bark thickness data from this trial were used in previous studies by MacDonald *et al.* (1997) and Dutkowski and Potts (1999), respectively. The LA trial had 107 families and 11 subraces common to the NW trials, thus our bark thickness comparison was based on subrace and family-level correlations

4.2.2. Statistical Analyses

For each trial, univariate analyses of the data were undertaken in ASReml (Gilmour *et al.* 2015a; see also - Isik *et al.* 2017) by fitting the model:

$$y = \mu + replicate + subrace + family(subrace) + residual \qquad \text{Model 1}$$

where y is the vector of observations, μ is the grand mean, and random effects (in italics) are *replicate, subrace, family within subrace (family(subrace))* terms and the *residual*. As bark thickness is positively related to tree size, a size-adjusted estimate of bark thickness was analysed by fitting DBH in Model 1 as a covariate, and thus all analyses refer to adjusted bark thickness (Adj.BT_{RESI}). Estimated variance components were used to calculate the narrow-sense heritability (h^2_{op}) of the variance within subrace following Griffin and Cotterill (1988):

$$h^2_{op} = \frac{\sigma^2_a}{\sigma^2_p}$$

$$\sigma^2_a = \frac{\sigma^2_{f(s)}}{0.4}$$

$$\sigma^2_p = \sigma^2_{f(s)} + \sigma^2_e$$

where σ^2_a is the additive genetic variance within subraces that is estimated using the variance between families ($\sigma^2_{f(s)}$).

We assumed an average coefficient of relatedness (*r*) of 0.4. based on Griffin and Cotterill (1988) considering average outcrossing rate of 70% for open-pollinated progenies, which is a widely used assumption for this species (Stackpole *et al.* 2010a; Stackpole *et al.* 2010b; O'Reilly-Wapstra *et al.* 2013). σ_p^2 is the phenotypic variance component and σ_e^2 is the residual variance. Following Yang *et al.* (1996) and Latta (1998), the quantitative inbreeding coefficient (Q_{ST}) was estimated to understand the degree of quantitative genetic divergence between subraces:

$$Q_{ST} = \frac{\sigma_s^2}{\sigma_s^2 + 2\,\sigma_a^2}$$

where σ_s^2 is the variance between subraces.

Following Dutkowski and Potts (2012), a two-tailed likelihood ratio test (LRT) was undertaken for each trait to test Q_{ST} against the mean F_{ST} as well as the maximum F_{ST} derived from eight putatively neutral microsatellite markers (0.09, Steane *et al.* 2006), of two studies (0.158, Astorga *et al.* 2004; Steane *et al.* 2006). F_{ST} indicates the genetic differentiation between populations through random drift or mutation. While these microsatellite studies sampled different *E. globulus* trees to the present study, they were from multiple populations (200 trees from 43 provenances - Astorga *et al.* 2004; 340 trees from 10 races - Steane *et al.* 2006) from across a similar geographic range. Under appropriate assumptions (Edelaar and Björklund 2011; Meirmans and Hedrick 2011), a significant difference between Q_{ST} and F_{ST} signals that divergent ($Q_{ST} > F_{ST}$) or stabilising ($Q_{ST} < F_{ST}$) natural selection across the species range has impacted directly or indirectly on the trait (Latta 1998; Steane *et al.* 2006; Leinonen *et al.* 2013).

The correlations ($r_{1,2}$) whether type-A (intra-site, inter-trait) or type-B (inter-site, same trait) were estimated following Jordan *et al.* (1999):

$$r_{1,2} = \frac{\sigma_{1,2}}{\sqrt{\sigma_1^2 \, x \, \sigma_2^2}}$$

where, $r_{1,2}$ is the correlation between trait 1 and trait 2, $\sigma_{1,2}$ is the covariance between the traits, and σ_1^2 and σ_2^2 are the respective traits variance components. These correlations were obtained with ASReml by extending Model 1 to the multivariate level (see Isik *et al.* 2017 for examples). We fitted an unstructured (US) residual covariance matrix and a correlation matrix with heterogeneous variances (CORGH) for each fitted random term.

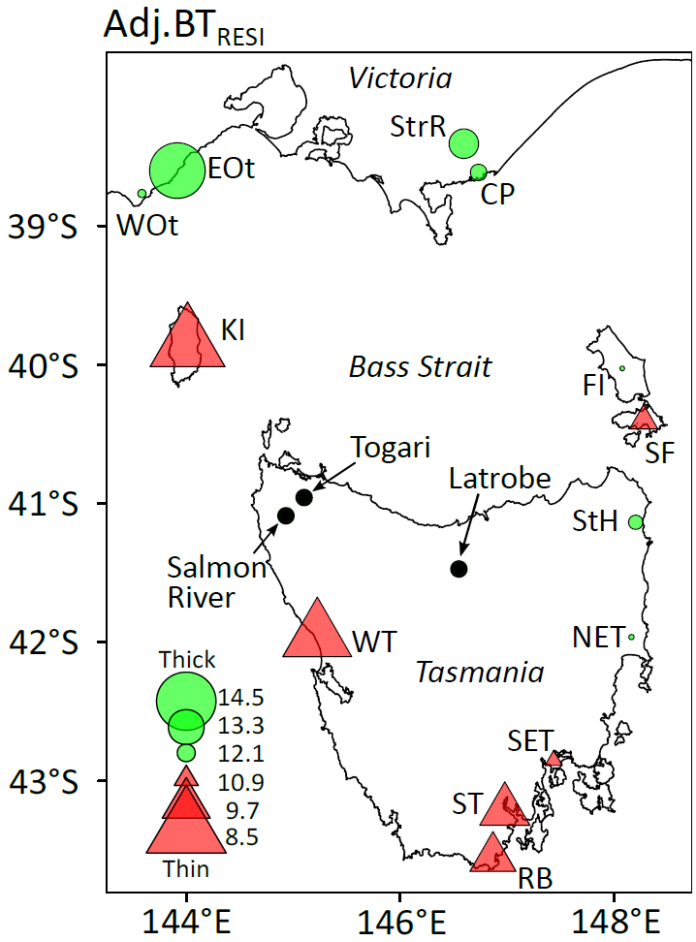

Figure 4.1 The geographic variation in subrace least-square means for adjusted bark thickness (Adj. BT_{RESI}; mm), pooled across the two *Eucalyptus globulus* trials at Salmon River and Togari. Subrace abbreviations are shown and are for North-eastern Tasmania [NET], South-eastern Tasmania [SET], Southern Furneaux [SF], Western Tasmania [WT], Southern Tasmania [ST], St Helens [StH], King Island [KI], Western Otways [WOt], Coastal Plain [CP], Recherche Bay [RB], Eastern Otways [EOt], Strzelecki Ranges [StrR] and Flinders Island [FI]. Trial site locations at Salmon River, Togari and Latrobe are also shown by the black circle symbols. Large green circles correspond to subraces with thick bark, grading into large red triangles that correspond to subraces with thin bark.

The genetic stability (genotype by environment interaction [GxE]) of traits across the two north-west trials (SR and TO) were tested using type-B genetic correlations following Burdon (1977). Type-B correlations were estimated using bivariate analyses where measurements of the same trait from different trials were treated as separate variables. In this case, residual and replicate covariances across trials were assumed independent (i.e. covariances/correlations were constrained to zero) and, provided significant variance was evident for both traits, genetic correlations were estimated at the family and subrace levels. Following Stackpole *et al.* (2011) a one-tailed LRT was used to test the deviation of these correlations from one, each test constraining the correlation at either the subrace or family level.

Type-A genetic correlations between RESI and traditional measurements were similarly estimated for DBH and wood basic density for the NW trials but using a four-variate version of Model 1. In this case, across-site residual and replicate variances across trials were considered independent (i.e. covariances/correlations were constrained to zero), and covariances/correlations only estimated where the traits were measured from the same trees in the same trial. The pooled intra-site correlations among traits were estimated by separately constraining the subrace and family correlations for Togari and Salmon River to be equal. These type-A correlations were tested against zero using a two-tailed LRT. To test the measurements of bark thickness, a tri-variate version of Model 1 (including DBH as a covariate) was used with observations from three different sites (SR, TO and LA). However, in this case, the correlations of the RESI estimates of bark thickness from the two north-west (SR and TO) trials with the Pilodyn estimates from the LA trial were constrained to be equal, providing a single estimate of the correlation between the different measurement techniques at both the subrace and family within subrace levels. Correlations between traditional and RESI measurements of the same trait were tested against one and zero using one- and two-tailed LRTs, respectively.

Tests for associations between subrace trait means and targeted subrace home-site climate variables were undertaken using a Pearson product moment correlation using the 'cor.test' function of the *stats* package in R (R Core Team 2018). Subrace least-square means were estimated with ASReml by treating subrace as a fixed effect in Model 1. The climate at the provenance origin was characterised using two bioclimatic variables (TSPAN and TVAR) estimated as their mean during the 1976-2005 period with ANUClim v6.1 (Xu and Hutchinson 2013), which reflects biologically important facets of home-site temperature.

4.3. Results and discussion

There was little difference in the grand means estimated for Salmon River (SR) and Togari (TO), reflecting their proximity and similar high-rainfall climates (Hamilton *et al.* 2013; Chapter 3). All traits assessed were found to be heritable, exhibiting significant family and subrace variance (Table 4.1). The type-B genetic correlations between these two NW trials for every trait were not significantly different from one at subrace as well as family level, consistent with the studies of other traits from these trials, including growth (Chapter 3) and disease susceptibility (TG05 vs SR05 - Hamilton *et al.* 2013). This absence of significant GxE is noteworthy as DBH is one of the traits most sensitive to GxE in *E. globulus* (Costa e Silva *et al.* 2006; Callister *et al.* 2011; Salas *et al.* 2014). Accordingly, the data from the NW trials (Salmon River and Togari) were pooled for studying the inter-trait correlations.

One of the key objectives of this study was to understand the genetic relationship between the traditional and RESI methods in the assessment of growth, wood density and bark thickness. All three traits assessed using RESI were highly genetically correlated with their traditional measurements at both the subrace and family levels. The correlation between DBH measured using the traditional method and RESI was not significantly different from 1 at the family (r_{fam} = 0.99 ± 0.01) and subrace (r_{sub} = 1.0 [at the boundary of the parameter space]; Figure 4.2a) levels. This highly significant genetic correlation in the present study supports the use of RESI for *E. globulus* breeding and genetic studies. However, there may be an issue if there is a bias in the selection of samples assayed with RESI. Our results presented (Table 4.1 and Figure 4.2) are based only on the subset of trees assessed for RESI. In the present case, there was a size limit to the trees assessed as only trees above 10 cm DBH were initially cored and thus, trees with DBH below 10 cm were not drilled with RESI. This selection design resulted in the over-estimation of DBH for one poor-performing subrace (St Helens, results not shown).

The $Resistance_{RESI}$ measures were also highly genetically correlated with the traditional method of estimating basic density from cores, as the LRTs indicated the two were not statistically different from one at the family (r_{fam} = 0.95 ± 0.04) or subrace (r_{sub} = 0.99 ± 0.01; Figure 4.2b) levels. This finding was similar to loblolly pine (*Pinus taeda*) where a strong genetic correlation between RESI and core basic density (>0.90) was reported (Isik and Li 2003). Such high correlations for *E. globulus* were expected based on the previously reported phenotypic R^2 (>0.66) values (Downes *et al.* 2018). In the case of bark thickness, no traditional measurements were available from the trees assessed with RESI. However, the same families had been assessed for bark thickness using the bark window made during Pilodyn assessment of a trial on a dry

Tasmanian site (Latrobe) (pilodyn data reported in Stackpole *et al.* 2010a). This trial exhibits high GxE compared to the two wetter NW trials for growth but not for wood properties (Chapter 3). In the present study, the genetic correlations of RESI measured bark thickness ($Adj.BT_{RESI}$) with the Pilodyn measured bark thickness (from Latrobe - Figure 4.1) were very high at the subrace (0.96 ± 0.03) and family (1.0; at the boundary of the parameter space) levels, providing further validation for the RESI measurements. This high value is particularly noteworthy as our comparison of bark thickness measured across sites confounds different measurement techniques and growth GxE, although low GxE is expected for bark thickness (*E. urophylla* - Wei and Borralho 1997; *E. globulus* - López *et al.* 2002). In addition to these high correlations, estimates of the heritability and subrace differentiation (Q_{ST}) using the traditional and RESI methods for DBH and wood basic density (core BD *versus* $Resistance_{RESI}$) were also comparable (Table 4.1), as were the genetic correlations among traits (DBH_{RESI}-$Resistance_{RESI}$ *versus* DBH-BD and $Resistance_{RESI}$-$Adj.BT_{RESI}$ versus BD-$Adj.BT_{RESI}$; Table 4.2). Thus, we conclude that the RESI technique can confidently replace the traditional assessment methods in breeding and genetic studies for the traits assessed.

Bark thickness had the highest Q_{ST} (0.64) of our studied traits (Table 4.1). These Q_{ST} estimates surpass the previously reported Q_{ST} estimate for bark thickness in *E. globulus* (0.228 - Steane *et al.* 2006) and are among the highest values reported for any trait in this species (Figure 4.3). Our Q_{ST} estimates for bark thickness were significantly greater than the mean and maximum F_{ST} for *E. globulus* (Table 4.1), signalling that divergent selection across the species range (Leinonen *et al.* 2013) has led to subrace differentiation in this trait (Figure 4.1). The *E. globulus* subraces broadly exhibited a latitudinal cline in bark thickness (negative degrees south; Pearson's correlation coefficient $r = 0.54$, $P = 0.211$), with bark thickness tending to be higher in mainland than Tasmanian subraces and increasing northward within the more-or-less continuous distribution on the east coast of Tasmania (Figure 4.1). This geographic pattern of variation is similar to that reported in other studies at the provenance level (Dutkowski and Potts 1999; López *et al.* 2001).

Table 4.1 Site-level means and genetic parameters estimated from two 10-year old field trials of *Eucalyptus globulus* in NW Tasmania (Salmon River [SR], Togari [TO]) for over-bark stem diameter at breast height (DBH_{RESI} and DBH), wood basic density ($Resistance_{RESI}$ and BD) and DBH adjusted bark thickness ($Adj.BT_{RESI}$)

		DBH_{RESI} (mm)	[c]DBH (mm)	$Resistance_{RESI}$	[c]BD (kg/m^3)	$Adj.BT_{RESI}$ (mm)
Mean	SR	173.4 ± 42.1	169.3 ± 39.5	40.5 ± 4.2	495.8 ± 35.6	11.42 ± 3.5
	TO	170.8 ± 41.1	169.1 ± 39.4	40.6 ± 4.2	503.7 ± 33.6	10.83 ± 3.3
h^2_{op}	SR	0.38 ± 0.12***	0.43 ± 0.12 ***	0.45 ± 0.12***	0.35 ± 0.11***	0.30 ± 0.12**
	TO	0.21 ± 0.12*	0.20 ± 0.12 ***	0.36 ± 0.12***	0.34 ± 0.12***	0.26 ± 0.12**
Q_{ST}	SR	0.12 ± 0.07***	0.11 ± 0.06 ***	0.18 ± 0.09***	0.21 ± 0.10***	[b]0.63 ± 0.14 ***
	TO	[a]0.30 ± 0.16***	0.28 ± 0.16 ***	0.21 ± 0.11***	0.19 ± 0.10***	[b]0.64 ± 0.15 ***

The table shows grand mean and its standard deviation (mean ± SD), the narrow-sense heritability estimate with its standard error (h^2_{op}± SE, significant difference of family variance from zero based on a one-tailed likelihood ratio test is indicated as asterisks) and quantitative inbreeding coefficient with its standard error (Q_{ST} ± SE, significant difference of subrace variance from zero based on a one-tailed likelihood ratio test is indicated as asterisks). h^2_{op} *and* Q_{ST} values shown were calculated assuming 70% outcrossing. Asterisks indicate the results of the test of significance as ns p≥0.05; * p<0.05; ** p<0.01; *** p<0.001.

[a] Q_{ST} is significantly different from the mean F_{ST} (0.09) of Steane *et al.* (2006) at the 0.05 level of significance.

[b] Q_{ST} is significantly different from the maximum F_{ST} of any locus (0.158) from the studies of Astorga *et al.* (2004) and Steane *et al.* (2006) at the 0.05 level of significance.

[c] (Chapter 3)

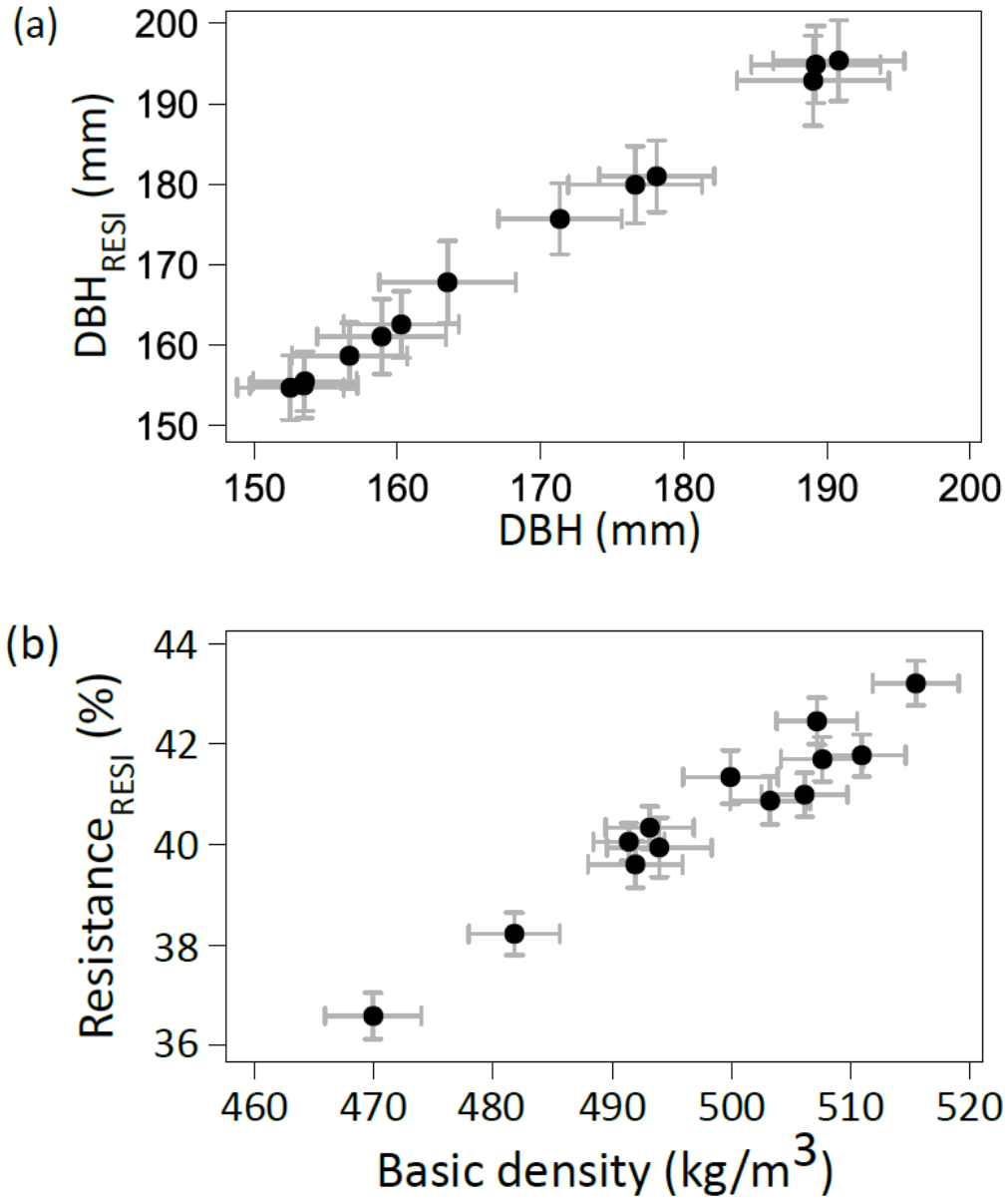

Figure 4.2 Subrace least-square means with standard errors for (a) DBH and (b) Basic density in *Eucalyptus globulus*, showing the association between RESI measured values and traditional methods. The pooled intra-site subrace correlations (r_{sub}) were tested against one and found to be at the boundary of the parameter space for DBH (r_{sub} = 1.00) and was non-significant for Basic density (r_{sub} = 0.99 ± 0.01)

In terms of climate-trait associations, we target specific traits to test based on the reported trends in Dutkowski and Potts (1999). These authors report a positive locality-level association between bark thickness and the home-site temperature annual range (TSPAN r= 0.56, P<0.01 after Bonferroni adjustment), which was consistent with the positive association detected at the subrace level in the present study (TSPAN *r* = 0.77, P = 0.043). Dutkowski and Potts (2012) previously reported that the temperature seasonality (TCVAR) at the subrace origin was positively correlated to the tolerance of the subraces to drought damage. They also reported a negative correlation of drought damage with bark thickness and wood density (Dutkowski and Potts 2011), which is consistent with the correlations with temperature seasonality observed in the present study (TCVAR with BT *r* = 0.82, P = 0.023 and wood basic density r = 0.85, P=0.016).

With subrace variation in both bark thickness and wood density significantly correlated with the same climatic variable, we tested for the genetic association between these two traits at the subrace and within subrace levels. Bark thickness ($Adj.BT_{RESI}$) was significantly positively correlated to the wood density at the subrace level ($Resistance_{RESI}$ 0.61; BD 0.75 - Table 4.2). When traits are influenced by divergent selection, such parallel patterns of variation may result from selection acting on one of a pair of pleiotropically related, genetically correlated traits (i.e. correlated response to selection - Falconer and Mackay 1996) or be independent responses to the same or independent, but spatially correlated, selection gradient(s) (selective covariance - Armbruster and Schwaegerle 1996). The magnitude of the genetic correlation between these two traits, when measured within genetic groups (e.g. subrace) compared to that between genetic groups, provides information that helps to differentiate these two hypotheses involving divergent selection (Armbruster and Schwaegerle 1996; Gauli *et al.* 2015). An insignificant within-group genetic correlation would be consistent with selection acting independently on cospecialized traits and thus selective covariance Whereas, a significant within-group positive genetic correlation would signal the traits are co-dependent (pleiotropy has constrained evolution) or interdependent and correlational selection has shaped variation in the functionally related trait (Peiman and Robinson 2017). In the present case, the family level (within-group) genetic correlation between bark thickness and either $Resistance_{RESI}$ or BD was non-significant (Table 4.2), suggesting trait co-specialisation and that selective covariance accounts for the subrace-level correlations between wood density and bark thickness (Peiman and Robinson 2017). However, while weaker and not statistically significant, genetic correlations were in the same direction (0.33 and 0.26) as that observed at the subrace level (0.61 and 0.75), the

possibility that these two traits are in some way weakly pleiotropically related or functionally interdependent cannot be dismissed.

Adjusted bark thickness was not significantly genetically correlated with DBH_{RESI} at either the subrace or family levels in the present study (Table 4.2), although in some other species a positive genetic correlation has been reported (*Eucalyptus urophylla* - Wei and Borralho 1997; *E. urophylla* × *E. grandis* hybrids - Retief and Stanger 2009). The within subrace variation in bark thickness had a moderate narrow-sense heritability (0.26 – 0.29; Table 4.1). Our phenotypic adjustment for tree size using DBH_{RESI} as a covariate meant our measure of bark thickness was also uncorrelated with DBH at the genetic level (family or subrace, Table 4.2), consistent with previous reports by López *et al.* (2002). While there were significant subrace and family variations in bark thickness, this had no practical effect on the genetic rankings for stem diameter as the subrace and family level correlations between under- and over-bark DBH measures were effectively one (1.0; at the boundary of the parameter space). Wood density and DBH were not significantly correlated at either family or subrace level (DBH_{RESI}-$Resistance_{RESI}$ or DBH-BD; Table 4.2), affirming the previously reported low genetic correlations between DBH and wood basic density in *E. globulus* (Stackpole *et al.* 2010a; Salas *et al.* 2014).

Table 4.2 Genetic correlations (± standard error) among DBH_{RESI}, $Resistance_{RESI}$, $Adj.BT_{RESI}$, DBH and Basic density (BD) at the subrace (Subrace) and family within subrace (Family) levels of *Eucalyptus globulus*.

	Level	**Type-A genetic correlation**
DBH_{RESI} vs $Resistance_{RESI}$	Subrace	-0.21 ± 0.33^{ns}
	Family	0.11 ± 0.17^{ns}
[a]DBH-BD	Subrace	-0.12 ± 0.31^{ns}
	Family	-0.06 ± 0.15^{ns}
DBH_{RESI} - $Adj.BT_{RESI}$	Subrace	0.11 ± 0.31^{ns}
	Family	-0.09 ± 0.22^{ns}
$Resistance_{RESI}$ - $Adj.BT_{RESI}$	Subrace	0.61 ± 0.21*
	Family	0.33 ± 0.18^{ns}
BD - $Adj.BT_{RESI}$	Subrace	0.75 ± 0.16**
	Family	0.26 ± 0.18^{ns}

Given are the pooled intra-site correlation coefficients of two trials in NW Tasmania (Salmon River and Togari) which were tested against zero and significance levels based on two-tailed likelihood ratio test are indicated as ^{ns} $p \geq 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. [a] (Chapter 3)

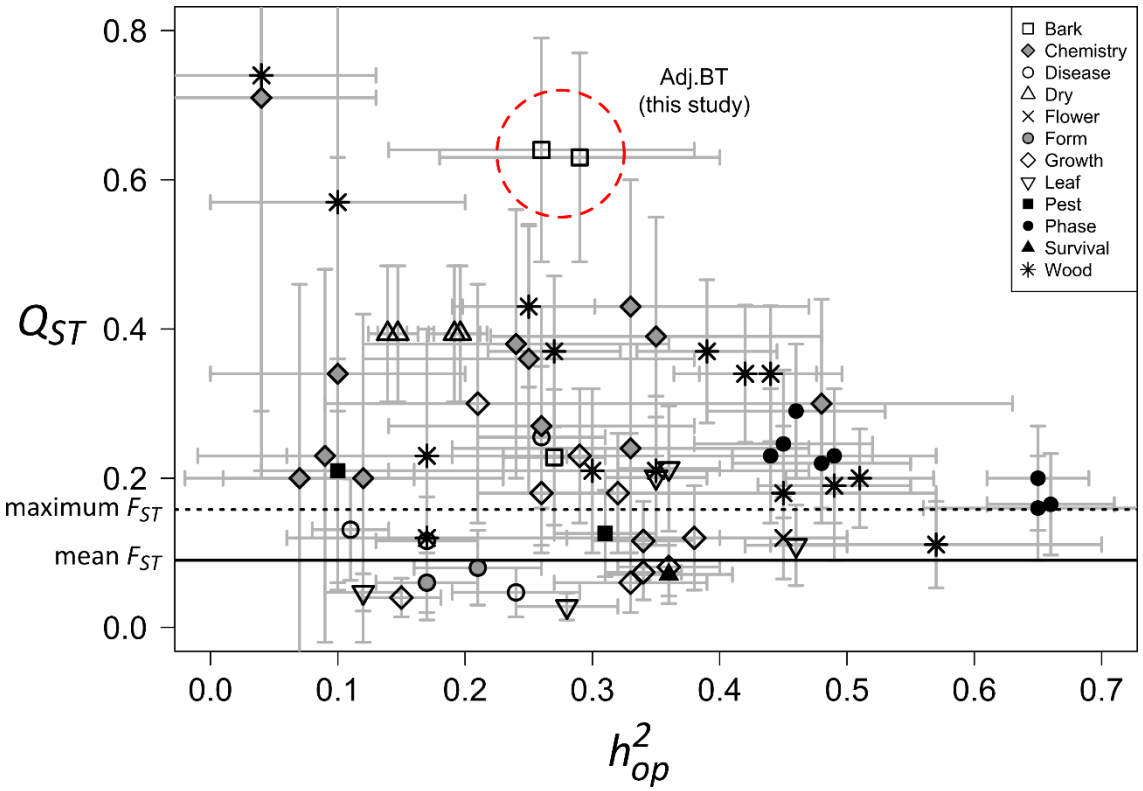

Figure 4.3 Q_{ST} and h^2_{op} estimates with standard errors for traits reported in the present and other studies of *Eucalyptus globulus*. The current estimates for the RESI derived adjusted bark thickness values (Adj.BT) from the two NW trials studied are circled. The figure follows that presented in Dutkowski and Potts (2012) with updated data from Hamilton *et al.* (2013), O'Reilly-Wapstra *et al.* (2013), Chapter 3 and the present study. The solid horizontal line indicates the mean F_{ST} of Steane *et al.* (2006) and the dotted line indicates the maximum F_{ST} of any microsatellite locus (see Table 4.1 footnote). The traits plotted include bark thickness (Bark), *Teratosphaeria* leaf disease (Disease), drought damage (Dry), flowering precocity (Flower), stem height and diameter (Growth), stem straightness (Form), juvenile leaf size and shape traits (Leaf), sawfly damage (Pest), the onset of vegetative phase change (Phase), survival (Survival), wood density and chemistry (Wood).

4.4. Conclusion

There was no noteworthy difference in genetic parameter estimates for DBH, wood density and bark thickness measured using RESI compared to those estimated using traditional measurements. This finding combined with the high genetic correlations reveals that the non-destructive RESI measures of DBH, wood density and bark thickness of *Eucalyptus globulus* are as reliable as analogous traditional measurements for genetic studies The simultaneous assessment of these three traits using RESI change the cost of trial assessment, but would allow more wood density measurements to enhance genetic gain from breeding. Although in the present study the RESI was used in a progeny trial established for breeding purposes, the results highlight the potential of this tool for ecological studies of wood density and bark thickness, as many ecological responses of trees, especially their adaptability to drought, are associated with these traits.

Chapter 5. Heterosis and genetic architecture of growth and wood properties in an inter-race diallel of *Eucalyptus globulus*

5.1. Introduction

Forest trees are foundation species which dominate a large component of the world's terrestrial ecosystems (Whitham *et al.* 2006; FAO 2010). However, due to their large size, long-life cycle and complex genetic structure, understanding of the genetic architecture of traits of ecological and economic importance in forest trees is challenging (Petit and Hampe 2006; White *et al.* 2007). Forest tree species frequently exhibit marked genetic variation across their geographic range (Harfouche *et al.* 1995a; Boshier and Billingham 1999; White *et al.* 2007; Kremer *et al.* 2014) and understanding the manner in which within and between population differences are inherited is important from multiple perspectives. Within populations, the levels of additive genetic variation, heritability and genetic correlation among traits determine the possibilities for, and constraints to, evolutionary changes (Armbruster *et al.* 2014; Kremer *et al.* 2014; Peiman and Robinson 2017). From an evolutionary perspective, an understanding of the patterns of population differentiation provides insights into the relative roles of drift and selection in shaping gene pools (Steane *et al.* 2006; Eckert *et al.* 2008; Kremer *et al.* 2014). A knowledge of the genetic basis of population differentiation and the consequences of their crossing is becoming increasingly relevant to forest conservation and restoration, as population mixtures are being used to avoid inbreeding (Frankham 2015; Hamilton *et al.* 2017a) and increase resilience to climate change (Aspinwall *et al.* 2015; Prober *et al.* 2015; Aitken and Bemmels 2016). Such issues are also relevant to tree breeding where the genetic gain is dependent on exploiting genetic differences both between and within populations (Dhir and Mohn 1976; Eldridge *et al.* 1993; White *et al.* 2007).

Inter-population crossing, involving different provenances, races or subspecies is often used in tree breeding to avoid inbreeding depression and capture heterotic effects (Ying 1978; Schmidtling and Nelson 1996; Joseph *et al.* 2000; Johnston 2001). In forest trees, such crossing has generally revealed positive heterosis for growth (Ying 1978; Harfouche *et al.* 1995b; Harfouche *et al.* 2000; Joseph *et al.* 2000; Volker *et al.* 2008; Costa e Silva *et al.* 2014). However, in other plants more varied results have been reported, and inter-provenance crossing in *Arabidopsis thaliana* (Oakley *et al.* 2015) and *Primula vulgaris* (Barmentlo *et al.* 2018) can even result in the reduction in fitness of the progeny, termed outbreeding depression (Waser and Price 1989; Tallmon *et al.* 2004; Oakley *et al.* 2015; Barmentlo *et al.* 2018). Although the

underlying mechanism is not very clear, suggested causes for outbreeding depression are mal-adaptation (a dilution of adaptive parental genes due to hybridization), chromosomal rearrangements as well as the breakdown of the gene complexes linked to adaptation and thus disrupted epistatic interactions (Edmands 2007; Barmentlo *et al.* 2018). Moreover, some evidence in plants suggests that there is an optimal degree of genetic divergence between parents for the expression of heterosis, which represents a balance between inbreeding and outbreeding depression (Waser and Price 1989; Grindeland 2008; Ayre *et al.* 2019). When there is spatial genetic structure within native populations (Jones *et al.* 2007), such divergence is reflected in crossing success being proximity-dependent (Hardner *et al.* 1998). Selfing or biparental mating among relatives can lead to inbreeding depression (Uyenoyama 1986; Baskin and Baskin 2015) which has been identified and widely studied in forest trees (Charlesworth and Charlesworth 1987; White *et al.* 2007; Baskin and Baskin 2015). In contrast, there are few studies of outbreeding depression at either the inter-specific level (Potts *et al.* 1992; López *et al.* 2000b; Costa e Silva *et al.* 2012; Larcombe *et al.* 2014) or intra-specific level (Harfouche *et al.* 1995b; Hardner *et al.* 1998; Stacy 2001; Goto *et al.* 2011).

Another important factor in determining offspring performance is the directionality of crossing, in terms of whether a parent is used as a male or a female. Any asymmetry in performance will impact tree breeding operations as well as the directionality of gene flow in nature. Reciprocal effects may be due to maternal (general reciprocal) and non-maternal (specific reciprocal) effects (Cockerham and Weir 1977; Lynch and Walsh 1998; Wu and Matheson 2001), hereafter referred to as maternal and non-maternal reciprocal effects respectively (López *et al.* 2003). These effects arise either from environmental or genetic causes (Roach and Wulff 1987; Rossiter 1996; Zas *et al.* 2013). Maternal effects occur when the genetic or environmental characteristics of a mother influence the phenotype of its progeny, beyond the direct inheritance of nuclear alleles (Roach and Wulff 1987; Lynch and Walsh 1998). There is considerable evidence for maternal effects in plants, including forest trees, for seed traits, including dormancy, germination, as well as early-age performance (Roach and Wulff 1987; Lindgren and Wei 1994; López *et al.* 2003; Rix *et al.* 2012; Vivas *et al.* 2017; Vivas *et al.* 2019). Environmentally induced maternal effects may be caused by factors affecting maternal seed provisioning as well as an embryonic epigenetic 'memory' (Holeski *et al.* 2012; Bräutigam *et al.* 2013; Zas *et al.* 2013; He and Li 2018). The most obvious maternal genetic effect is that due to the uniparental inheritance of organellular DNA, which in most angiosperms is inherited maternally (Hagemann 2004). However, while maternal and non-maternal reciprocal effects may impact offspring performance, there is limited information on their importance compared

with the traditional additive and dominance sources of genetic variation, especially in forest trees. Moreover, while asymmetry in inter-specific hybrid success and performance has been shown in forest trees (Potts and Dungey 2004; Hamzeh *et al.* 2007; Zhu *et al.* 2017), only a few studies have addressed such issues in intra-specific crossing (Lindgren and Wei 1994; Harfouche and Kremer 2000).

Most forest tree species undergoing domestication are only a few generations removed from the wild, and progeny testing is usually undertaken using open-pollinated seed (Harfouche *et al.* 2012; Ingvarsson and Dahlberg 2019). Therefore, most of the knowledge of trait genetic architecture in forest trees, including patterns of provenance variation and quantitative genetic parameters such as heritability, comes from the study of open-pollinated progenies (see Carson 2019 for examples). Without pedigree recovery using molecular markers (Bush *et al.* 2011; Klápště *et al.* 2014; El-Dien *et al.* 2016), open-pollinated progeny trials do not allow the estimation of non-additive genetic effects and therefore forest tree breeding is mainly focused on exploiting additive genetic variance (White *et al.* 2007). However, even then the accuracy of additive variance estimates and breeding value predictions may be compromised due to unknown male parentage and levels of inbreeding, particularly selfing (Charlesworth and Charlesworth 1995; Lynch and Walsh 1998; Walsh 2005). Large full-sib family crossing schemes are needed for the partitioning of genetic variations into additive and non-additive components. In tree species, additive genetic effects generally appear to be more important than non-additive effects for traits such as growth (*Pinus taeda* L. - Isik *et al.* 2003; Baltunis *et al.* 2007; *Pinus pinaster* - Lepoittevin *et al.* 2011; *Callitropsis nootkatensis* - Russell *et al.* 2015), wood density (*Pinus pinaster* Ait. - Pot *et al.* 2002; *Eucalyptus globulus* - Costa e Silva *et al.* 2004; *Picea abies* - Hannrup *et al.* 2004), wood chemistry (*Pinus pinaster* Ait. - Pot *et al.* 2002), and insect resistance (*Picea abies* - Mottet *et al.* 2015). However, there are studies suggesting substantial non-additive genetic control of some traits, particularly those associated with growth (López *et al.* 2003; Costa e Silva *et al.* 2004; Waldmann *et al.* 2008; Berlin *et al.* 2019). Non-additive genetic effects include various components such as dominance and epistatic effects, as well as maternal and non-maternal reciprocal effects (Falconer and Mackay 1996; Lynch and Walsh 1998), but most crossing designs in forest trees only allow separation of the dominance (4 x specific combining ability) and additive genetic components (White *et al.* 2007).

Within eucalypts species, the quantitative genetic architecture of traits has not been well-studied using full-sib family crossing designs, with the exception of a few studies (Van Wyk 1977- *E. grandis*; Hardner and Tibbits 1998- *E. nitens*; see below for *E. globulus*). Published studies on the effect of inter-population crossing have only been reported for *Eucalyptus*

globulus. *Eucalyptus globulus* (Tasmanian blue gum), endemic to south-eastern Australia and islands of Tasmania (Nicolle 2006), is part of a complex of four intergrading taxa (*E. maidenii*, *E. bicostata*, *E. pseudoglobulus* and *E. globulus*; Jones *et al.* 2012). *E. globulus* (including its integrade populations) is widely grown in temperate regions of the world and is the subject of domestication programmes in at least 10 countries, mainly for pulpwood production (Potts *et al.* 2004). The species is highly variable for traits relevant to breeding with large differences occurring between geographic races (Dutkowski and Potts 1999; Steane *et al.* 2006). Its large flower and advances in pollination techniques (Potts *et al.* 2008) has made control crossing and full-pedigree control the norm in many breeding programmes (Potts *et al.* 2014). While the species can be vegetatively propagated and there is clonal deployment and testing in countries such as Chile, Portugal, Spain and Uruguay (Costa e Silva *et al.* 2004; Potts *et al.* 2008; Araújo *et al.* 2012), deployment by seed is more common, particularly in Australia (Potts *et al.* 2008). With the development of mass supplementary pollination techniques for the species (Patterson *et al.* 2004a), seed deployment not only includes seed from open-pollinated seed orchards but also full-sib families from or single-pollen crossing or half-sib families from polymix pollen (Potts *et al.* 2008). The extent to which selection traits are under non-additive genetic control and affected by maternal and non-maternal reciprocal effects is thus a key issue for both breeding and deployment of this species. In the case of breeding, this will affect the genetic evaluation models and accuracy (Falconer and Mackay 1996; Hodge *et al.* 1996; Hallander and Waldmann 2009; Denis and Bouvet 2013; Bouvet *et al.* 2016). From a deployment perspective, this will determine the extent to which additional genetic gain can be captured by deploying targeted full-sib families (Jansson and Li 2004; Wu and Matheson 2004). In the case of full-sib family production, a key issue is whether the directionality of crossing matters as this will affect the economics of seed production (Collins and Callister 2010) as well as the accuracy of genetic predictions of performance (Wu and Matheson 2001; Potts *et al.* 2004).

With most first generation crossing in the Australian National *E. globulus* Breeding Programme focused on inter-race hybrids (Potts *et al.* 2014), the magnitude of non-additive genetic effects such as inter-race heterosis, and the extent to which it varies between races is a key issue. The few studies in *E. globulus* of the relative importance of non-additive effects compared to additive have focused on specific combining ability (SCA)/dominance effects. These studies suggest that non-additive effects are more important for growth than for wood property traits, i.e. wood density and pulp yield (Hodge *et al.* 1996; Potts *et al.* 2004; Volker *et al.* 2008; Callister *et al.* 2011; Araújo *et al.* 2012; Callister *et al.* 2013; Hamilton *et al.* 2017b; Mora *et al.* 2019). Using the increased power of clonal replication of progeny, Costa e Silva *et al.*

(2004) was able to separate the additive, dominance and epistatic genetic effects on growth and wood density and found only the additive component was significant. However, few studies have separated the effects of inter- and intra-population crossing (Vaillancourt *et al.* 1995; Hodge *et al.* 1996; Volker *et al.* 2008). In most cases where inter-population (or race) crossing has been studied, mid-parent heterosis is reported (Vaillancourt *et al.* 1995; Hodge *et al.* 1996; Volker *et al.* 2008; Costa e Silva *et al.* 2014) which, as previously noted, may be confounded with many estimates of the levels of dominance variance within the species (Li *et al.* 2007; Callister *et al.* 2011). *E. globulus* is one of the few forest tree species where the importance of maternal and non-maternal reciprocal effects have been studied (Lopez *et al.* 2003; Rix *et al.* 2012; Costa e Silva *et al.* 2013a). As with most angiosperms, the chloroplast and mitochondria of *E. globulus* are maternally inherited (McKinnon *et al.* 2001; Vaillancourt *et al.* 2004), and there is some evidence to suggest that genetic variation in plastid genes may affect traits of adaptive significance (Kahrood *et al.* 2019). Maternal effects have been shown on the germination response of *E. globulus* seed subject to high temperature stress (Rix *et al.* 2012). López *et al.* (2003) found significant maternal and non-maternal reciprocal effects for early germination and nursery growth, but these effects on growth rapidly diminished with age after field planting, while additive genetic effects increased. While not statistically tested, Costa e Silva *et al.* (2013b) reported the maternal and non-maternal reciprocal variances were minor compared with additive variances for both growth and disease resistance.

A complete diallel allows the estimation of additive and dominance variances, as well as maternal and non-maternal reciprocal effects, assuming no epistasis (Cockerham and Weir 1977; Wu and Matheson 2001; López *et al.* 2003; Muñoz *et al.* 2014). However, implementing large scale diallel crossing designs in forest trees faces practical limitations and therefore, accurate estimation of these non-additive genetic variances is challenging. For example, to estimate the dominance variance with equal accuracy to that of the additive variance, about 20 times more data is required (Misztal 1997). Diallel mating systems involve a large number of crosses on a relatively small number of parents to obtain good estimates of non-additive genetic effects. Many studies of non-additive genetic effects in intraspecific crosses of eucalypts have been based on less than 10 parents or have sparse parental crossing (Hodge *et al.* 1996; López *et al.* 2003; Li *et al.* 2007; Costa e Silva *et al.* 2014), and it is only in recent years that larger trials have become available (Van den Berg *et al.* 2017), and at the intra-specific level, these mainly involve *E. globulus* (Costa e Silva *et al.* 2004; Araújo *et al.* 2012; Callister *et al.* 2013; Costa e Silva *et al.* 2017). The present study is based on the largest of these field trials which involves a diallel crossing scheme among parents from the three races of *E. globulus*, most important to the

National Breeding Programme. This trial has been previously used for the study of indirect genetic effects for growth and disease damage (Costa e Silva *et al.* 2013a; Costa e Silva *et al.* 2017). The focus here is on inter-race heterosis and the genetic architecture of growth, wood density, pulp yield and bark thickness, with the specific aims of determining:

(i) the magnitude and direction of the average inter-race heterosis, and whether this varies with trait, age, race and directionality of the cross. It is assumed that the growth traits will show positive mid-parent heterosis similar to previous studies in this and other forest tree species, but hypothesise that (a) increasing competition among trees will result in increasing magnitude of heterosis with age, and (b) heterosis will be less in crosses among the more divergent races; and

(ii) the importance of dominance, maternal and non-maternal reciprocal effects within races relative to the additive genetic variation. It is hypothesised that dominance will be only important for growth traits and within race estimates of dominance variance will be inflated when not accounting for inter-race heterosis. It is also hypothesised that maternal and non-maternal reciprocal effects, particularly for later age growth and wood properties, will be insignificant.

5.2. Materials and methods

5.2.1. Genetic material and crossing design

This study used a progeny trial established using full-sib families from a diallel mating of *E. globulus* parents. The trial has been previously studied by Costa e Silva (Costa e Silva *et al.* 2013a), and the design is summarised as follows (also depicted in Figure 5.1). For the mating programme, forty-eight *E. globulus* parent trees were selected which were first generation selections from Tree Breeding Australia (TBA; formerly Southern Tree Breeding Association) National *E. globulus* breeding programme (see Potts *et al.* 2014 for programme overview). These parents were from base population progeny trials in Australia. They originate from open-pollinated seed lots collected from native trees in three races – Furneaux (F), Strzelecki Ranges (S) and Western Otways (W). These races effectively correspond to three of the 13 *E. globulus* races described by Dutkowski and Potts (1999), with the exception that one of the females in the Western Otways group was from the adjacent and closely related Eastern Otways race (Steane *et al.* 2006) which is part of the continuous distribution of *E. globulus* in the Otways region (Figure 5.1). Two of the races were from mainland Australia (Western Otways and Strzelecki Ranges) and one from the Bass Strait Islands (Figure 5.1). Molecular markers indicate

that Western Otways and Strzelecki Ranges races are more related to each other than to the Furneaux race (Steane *et al.* 2006). Approximately 77% of the first generation (G1) selections in the National *E. globulus* breeding programme come from these three races (Potts *et al.* 2014). The inter-race full diallel studied comprised successful crosses from a 30x30 crossing scheme which excluded selfs but included many reciprocals, and involved 10 parents from each of the three races, each of which was descended from a different base-population grandparent (Suitor *et al.* 2009a). In total, these parents produced 433 families and included 137 male-female combinations with full reciprocals. These families were supplemented with additional crosses from the same race combinations derived from the same generation of the breeding programme. Thus, in total the inter-race diallel trial included 515 families produced from 51 parents - (19 from Furneaux, 15 from Western Otways and 17 from Strzelecki Ranges). Of the 51 parents, 48 were represented as females and 38 as males (Figure 5.1).

5.2.2. Field trial and trait assessment

The trial was established in August 2007 at Manjimup, Western Australia (34°13’34’’S, 116°8’37’’E), outside the native range of *E. globulus*. It was established on an ex-pasture, high productivity site, with a mean annual rainfall of 987 mm and mean annual maximum temperature of 20.5 °C (Bureau of Meteorology 2020). The area was rip-mounded and strip-sprayed with herbicide (Glyphosate and Simazine) prior to planting. Plants were fertilised at planting (135 kg/ha MAP and MOP®), and in the first (250 kg/ha of Agras®) and fourth (300 kg/ha of Urea) years after planting. The trial comprised 15 contiguous replicates, each consisting of 35 rows and 18 columns within rows, with a spacing of 5.0 m between rows and 2.125 m between columns (i.e. within rows). In general, each family was represented once per replicate (single-tree plots) and allocated randomly to a single-tree plot within each replicate using a row-column design (Williams *et al.* 2002). Surplus family positions in the design were filled with families from breeding crosses, as were diallel family positions which could not be filled due to insufficient plantable seedlings. These additional breeding families were excluded from the current analysis. In total, 7827 seedlings were planted from the inter-race diallel.

Female parent \ Male parent	Furneaux (F) n=15	Strzelecki Ranges (S) n=12	Western Otways (W) n=11
Furneaux (F) n=18	**FF** 95 (1547)	**FS** 47 (723)	**FW** 31 (471)
Strzelecki Ranges (S) n=16	**SF** 87 (1323)	**SS** 62 (865)	**SW** 55 (804)
Western Otways (W) n=14	**WF** 62 (979)	**WS** 45 (672)	**WW** 31 (443)

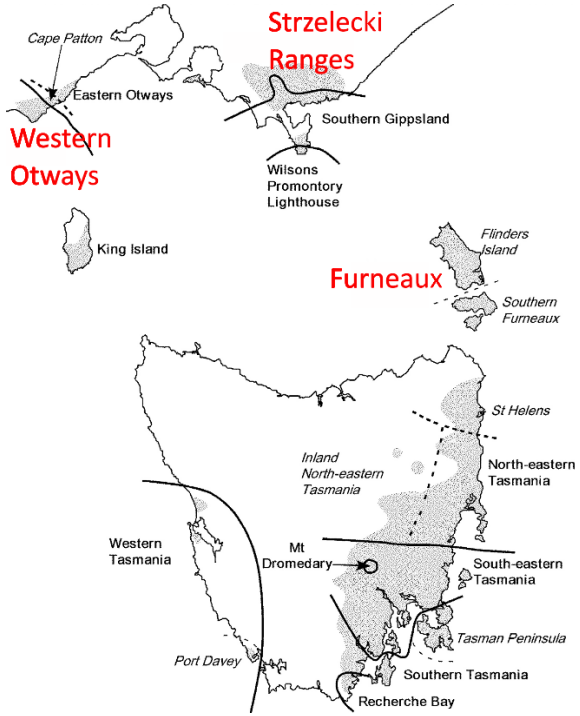

Figure 5.1 Summary of the intra- and inter-race diallel of *Eucalyptus globulus* and the location of the three races involved – Furneaux (F), Strzelecki Ranges (S), Western Otways (W). The number of parents used as male and female (n) in each of the three races is shown, and within each cell of the mating design, the number of full-sib families and individuals (in parenthesis) planted is indicated beneath the abbreviation (in bold) used for each unique race combination. The shaded diagonal cells represent the intra-race crosses (FF, SS, WW) and the remaining cells represent the inter-race crosses (FS & SF, WS & SW, WF & FW). The map shows the distribution of the 13 races of *E. globulus* as defined by Dutkowski and Potts (1999). The three races used in the inter-race diallel crossing programme are indicated in red letters (modified from Potts *et al.* 2004)

All 15 replicates in the trial were assessed for diameter at breast height (1.3m; DBH) using a diameter tape at the age of 2 years 4 months (2 years hereafter - DBH_2), 4 years 1 month (4 years hereafter - DBH_4), 6 years 3 months (6 years hereafter - DBH_6), and 8 years 1 month (8 years hereafter - DBH_8. Survival percentage ($Survival_8$) at the age of 8 years was also estimated as the presence or absence of an individual with the DBH measurements from that age. At 9 years 2 months (9 years hereafter), three replicates were assessed for wood basic density (BD_9), diameter (DBH_9), and bark thickness (BT_9) were derived from bark-to-bark traces obtained from IML Resi PD400 power drill (RESI) as explained in Chapter 4. Traces produced by RESI were transferred to PD Tools Pro software (https://forestquality.shinyapps.io/EucalyptResi Processor/, accessed 6 January 2019) and exported as text files for analysis. RESI resistance values were used to calculate the diameter and bark thickness using a customised script written in R (Downes *et al.* 2018; Chapter 4). Basic density was estimated using linear regression, relating variance in the mean RESI values to that of core basic density (Downes *et al.* 2018). Core basic density values were obtained from 12 mm diameter bark to bark cores taken from 200 trees at the site immediately after RESI sampling. These 200 trees were chosen to represent the full range of observed RESI resistance values and were used to define the relationship between mean RESI values for a tree and its core basic density (BD). Core BD values were well correlated with the RESI values ($r=0.83$, $P<0.001$) and at a level comparable with calibration results reported for other *E. globulus* trials (Downes *et al.* 2018; Chapter 4). At the same time, swarf samples were taken from the same trees as those assessed with RESI to estimate Kraft pulp yield (KPY_9) using near-infrared spectroscopy (NIRS). Swarf samples were taken from the outerwood (not including bark) at breast height by drilling to a depth of 45–50 mm using a 12 mm diameter Auger drill bit. Samples were immediately transferred into a paper envelope and air-dried. A Wiley Mini-mill with a 20-mesh screen was used to grind the swarf to woodmeal. Spectra were obtained from the woodmeal using a Bruker MPA FT-NIR instrument and KPY predicted using the multi-site and multi-species pulp yield model reported by Downes *et al.* (2009 & 2011). This model was developed based on the NIR spectra obtained from 1272 wood chip samples, from 40 different eucalypt species from plantations and native forest. Meder (2015) reported a coefficient of determination, (R^2) of 0.88 for NIR-predicted KPY obtained from outerwood swarf samples at breast height to the whole-tree KPY for eucalypts. Similar high R^2 (0.82; n = 20) was also reported between NIR-predicted and whole-tree pulp yield for *E. globulus* by Stackpole *et al.* (2010b).

5.2.3. Data analysis

Due to the high survival levels in the trial, genetic comparisons of survival were restricted to that of trees at the age of 8 years. Differences in survival were compared using homogeneity chi-square test statistics from the difference between the observed and expected number of trees alive and dead following Zar (1999) and implemented using the MS office Excel CHISQ.TEST function.

For the remaining traits, genetic analyses were undertaken by fitting linear mixed models with ASReml™ (Version 4.1), which estimates variance components using restricted maximum likelihood (REML) (Gilmour *et al.* 2015b). These models included a numerator relationship matrix derived from a three-generation pedigree file (native grandparents - G0, selected parents - G1 and progeny - G2) with the male of the G1 parents unknown as they were open-pollinated progeny collected from the G0 trees in native stands. Linear mixed models were persued to estimate the fixed race effects, inter-race heterosis, random genetic effects including maternal and non-maternal reciprocal effects, and the additive as well as dominance variance within inter- and intra-race crosses (detailed below). The eight degrees of freedom associated with the fixed difference amongst the nine combinations of the three races (Figure 5.1) were partitioned in various ways, all of which produced effectively the same results for the random terms in the model. The main fixed effects partition involved fitting terms for cross-type (CT: intra- versus inter-race crosses; df = 1), the difference between the three intra-race crosses ($Race_{intra}$: SS vs FF vs WW, df = 2), the difference between the three inter-race crosses ignoring reciprocals ($Race_{inter}$: SF/FS vs SW/WS vs FW/WF, df = 2), and the difference between the inter-race reciprocals ($Recip_{inter}$**:** SF vs FS, SW vs WS and FW vs WF; df = 3).

5.2.4. Maternal and non-maternal reciprocal effects

The first analysis fitted a full model aimed at determining the significance of non-maternal reciprocal and maternal effects:

$$y = \mu + \mathbf{Replicate} + \mathbf{CT} + \mathbf{Race_{intra}} + \mathbf{Race_{inter}} + \mathbf{Recip_{inter}}, + \mathit{Row} + \mathit{Column} + \mathit{Additive} + \mathit{SCA} + \mathit{Maternal} + \mathit{Reciprocal} + \varepsilon \quad \text{Model 1}$$

where y is a vector of observations on a trait, μ is the grand mean, Replicate is the fixed difference between trial replicates (for DBH: df = 14, for BD, BT and KPY: df = 2) and other fixed effects are as defined above. Random terms (*italics*) were *Row* (planting row), *Position* (position within planting row), *Additive* (individual-tree additive genetic effects), *SCA* (specific combining effect), as well as the *Maternal* (maternal) and *Reciprocal* (non-maternal reciprocal) effects, and ε was the vector of random residuals. The *SCA* effect was the full-sib family effect estimated

treating reciprocals as the same (AxB = BxA), whereas the *Reciprocal* effects (remaining unknown effects due to reciprocal crosses) were estimated using a factor coded such that reciprocal families are treated as different (AxB ≠ BxA). The *Reciprocal* term was only fitted for the subset of families for which a reciprocal cross was present. All random genetic effects refer to variation within races and their combinations. As bark thickness is positively related to tree size (Wei and Borralho 1997; Retief and Stanger 2009), a size-adjusted estimate of bark thickness was analysed by fitting DBH_9 as a covariate in the model, and thus all analyses refer to adjusted bark thickness ($_{Adj.}BT_9$). The testing of the significance of the variance components were undertaken using one-tailed likelihood ratio tests (LRT; Gilmour *et al.* 2015b; Isik *et al.* 2017). In the case of the *Reciprocal* term, this was done by fixing it to zero and comparing this constrained model against the unconstrained Model 1. Using the variance components estimated using Model 1, the proportion of the non-maternal reciprocal variance to the total phenotypic variance were calculated:

$$\sigma^2_{pr} = \sigma^2_{recip} + \sigma^2_{ma} + \sigma^2_{a} + \sigma^2_{SCA} + \sigma^2_{e}$$

$$recip^2 = \frac{\sigma^2_{recip}}{\sigma^2_{pr}}$$

where, σ^2_{pr} is the total phenotypic variance; σ^2_{recip} is the non-maternal reciprocal variance obtained from the *Reciprocal* term, σ^2_{ma} is the maternal variance obtained from the *Maternal* term, σ^2_{a} is the additive genetic variance obtained from the *Additive* term; σ^2_{SCA} is the *SCA* variance and σ^2_{e} is the residual variance. $recip^2$ is the proportion of the non-maternal reciprocal variance to the total phenotypic variance.

Non-maternal reciprocal effects (*Reciprocal*) were generally not significantly greater than zero (see results) and this term was thus removed from Model 1 to form a reduced model to estimate and test the significance of the random maternal effect. This test was also undertaken by fixing the maternal term to zero and comparing against the unconstrained reduced model. Using the variance components estimated using this reduced model, the proportion of the maternal variance (ma^2) was calculated:

$$\sigma^2_{pm} = \sigma^2_{ma} + \sigma^2_{a} + \sigma^2_{SCA} + \sigma^2_{e}$$

$$ma^2 = \frac{\sigma^2_{ma}}{\sigma^2_{pm}}$$

where, σ^2_{pm} is the phenotypic variance and the remaining terms are as described above but obtained from this reduced model. In most cases, the *Maternal* term was also not significantly

different from zero (see results) and therefore, both *Maternal* and *Reciprocal* terms were removed from further models.

5.2.5. Dominance and additive effects

The following model was used to estimate and test the fixed effects and all the remaining components of variance:

$y = \mu + \mathbf{Replicate} + \mathbf{CT} + \mathbf{Race}_{intra} + \mathbf{Race}_{inter} + \mathbf{Recip}_{inter}, + Row + Column + Additive + SCA + \varepsilon$ Model 2

where fixed and random terms are as described for Model 1.

Using the variance components estimated using Model 2, the following genetic parameters were calculated:

$$\sigma_p^2 = \sigma_a^2 + \sigma_{SCA}^2 + \sigma_e^2$$

$$h^2 = \frac{\sigma_a^2}{\sigma_p^2}$$

$$\sigma_d^2 = \sigma_{SCA}^2 \times 4$$

$$D^2 = \frac{\sigma_d^2}{\sigma_p^2}$$

where, σ_p^2 is the phenotypic variance within races, pooled across the intra- and inter-race cross-types removing replicate, row and position variation, h^2 is the narrow sense heritability, D^2 is the proportion of the dominance variance to the phenotypic variance and the remaining terms are as described above but obtained from Model 2, and estimated assuming no epistasis (Costa e Silva *et al.* 2004; Araújo *et al.* 2012). Variances, covariances and their standard errors were calculated based on restricted maximum likelihood (Gilmour *et al.* 2015b). The one-tailed LRT of the significance of σ_{SCA}^2 from zero were undertaken by comparing Model 2 with a constrained model where σ_{SCA}^2 was fixed to zero. The significance of σ_a^2 was tested fixing σ_a^2 and σ_{SCA}^2 to zero and comparing the likelihood of this model with that from the previously constrained model where σ_{SCA}^2 was fixed to zero. The least-square means (LSM) for each fixed effects were estimated using Model 2. The significance of the fixed effects were tested using the Wald F statistics following on the method proposed by Kenward and Roger (Gilmour *et al.* 2015b).

The percentage heterosis of the inter-race crosses with respect to intra-race crosses was estimated as follows:

$$heterosis = \frac{LSM_{inter} - LSM_{intra}}{LSM_{intra}} \times 100$$

where *heterosis* is the percentage mid-parent heterosis, LSM_{inter} and LSM_{intra} is the least-square means of inter- and intra-race crosses respectively. In addition, the LSM of the six various cross combinations at the race level (FF, SS, WW, FS, WF and WS) were estimated using a modified version of Model 2 with only the terms Replicate and Race (with no conditioning) as fixed effects. The pairwise difference between each of these cross combinations was tested using a standard error of the pairwise differences (SED) and the percentage heterosis estimated for each race combination as described above but with the LSM_{intra} term replaced with the average of the LSM of the specific intra-race combinations.

To test whether the interaction between races influences the estimates of the SCA variance and dominance ratio, a model with an alternative parameterisation of the fixed effects was fitted:

$$y = \mu + \mathbf{Replicate} + \mathbf{Race}_{female} + \mathbf{Race}_{male} + \mathbf{Race}_{female} \times \mathbf{Race}_{male} + Row + Column + Additive + SCA + \varepsilon \quad \text{Model 3}$$

where the fixed effects of the female race ($\mathbf{Race}_{female}$ df = 2), male race ($\mathbf{Race}_{male}$, df = 2), and their interaction ($\mathbf{Race}_{female} \times \mathbf{Race}_{male}$, df = 4) were included. The estimates of σ^2_{SCA} and D^2 from this model (which were effectively the same as those estimated from Model 2) were compared to the model where the $\mathbf{Race}_{female} \times \mathbf{Race}_{male}$ interaction term was removed. The later model is comparable to the treatment of race effects in a genetic group model where only additive genetic effects are considered. To test whether the σ^2_{SCA} estimates were significantly different between these two models, a two-tailed likelihood ratio test was undertaken by constraining the σ^2_{SCA} estimate in Model 3 to be equal to that obtained from the model with no fixed interaction term.

5.2.6. Genetic correlations

Age-age genetic correlations for DBH and inter-trait genetic correlations among the traits assessed at age 9 years were determined using a bivariate version of the Model 2 and allowing for heterogeneous variances by using CORGH variance structures. For age-age correlations, DBH measured at two different growth periods were fitted as different traits.

These correlations were estimated following Jordan *et al.* (1999):

$$r_{1,2} = \frac{\sigma_{1,2}}{\sqrt{\sigma_1^2 \, x \, \sigma_2^2}}$$

where, $r_{1,2}$ is the correlation between age 1 and age 2 or trait 1 and trait 2; $\sigma_{1,2}$ is the covariance between ages or traits; σ_1^2 and σ_2^2 *a*re the variance components of respective ages or traits. The genetic correlations were estimated at the *Additive* and *SCA* levels but only presented where the variance components of both traits at the level being tested were significantly greater than zero. The significance of the age-age correlations was tested from one using a one-tailed LRT; whereas the inter-trait correlations were tested from zero using a two-tailed LRT. In each case, the correlations were only constrained at the specific level being tested (*Additive* or *SCA*). ASREML uses an approximate likelihood technique based on first order Taylor series approximation (Gilmour *et al.* 2015a)

5.3. Results

5.3.1. Between race effects

While survival was high in the trial (overall $Survival_8$ = 91%), the large sample size (n = 7827) gave the power to statistically detect a marginally significant difference between cross-types (CT: intra- versus inter-race) despite only a small difference in survival (χ_1^2 = 5.11; P=0.024). Survival was slightly higher in the intra-race crosses (92.2%) than inter-race crosses (90.7%), reflecting inter-race heterosis of -1.63% (Table 5.1). When decomposed into pairwise contrasts this negative heterosis was due to slightly reduced survival of the Strzelecki Ranges x Western Otways crosses (WS) compared with the respective intra-race crosses (Figure 5.2; χ_2^2 = 12.22; P=0.002). No significant differences in survival were detected between the reciprocal crosses of this combination of races (WS vs SW; χ_1^2 = 0.13; P=0.716), and there was no specific parent or family combination which could explain the negative heterosis suggesting it is a general phenomenon of this specific race combination. No significant differences were detected in survival for the other race combinations (Figure 5.2).

For the quantitative traits, significant differences between the intra- and inter-race crosses were detected for all traits except wood density (BD_9) and bark thickness ($_{Adj}BT_9$) (Table 5.2). This heterosis was consistently positive and most evident in DBH where it increased with age from 2.2% at age 2 years to 6.5% by age 8 years (Table 5.1). Pairwise comparison of the race combinations revealed that this heterosis was more than mid-parent heterosis and, while not statistically significant, the inter-race crosses out-performed the better intra-race cross in all

pairwise combinations in DBH_8 (Figure 5.2), consistent with better-parent heterosis for growth. The significant (P<0.05) positive heterosis of 0.86% observed for pulp yield (KPY_9 in Table 5.1), mainly reflected the significantly lower pulp yield of the Strzelecki Ranges race being on average inherited slightly above the mid-parent value in crosses with the other two races (Figure 5.2). In contrast, the significantly thicker bark observed in the Strzelecki Ranges race was on average inherited in an additive manner in crosses with the thinner-barked Furneaux and Western Otways races (Figure 5.2). Pulp yield and bark thickness were the only traits for which significant differences were detected among the intra- (FF, SS, WW) and inter-race (FS, WS, WF) crosses (Table 5.2-$Race_{intra}$ and $Race_{inter}$ terms respectively). The Strzelecki Ranges intra-race crosses had lower pulp yield, thicker bark and higher wood density than the intra-race crosses of Furneaux and Western Otways, these differences were only significant for pulp yield and bark thickness (Table 5.2 and Figure 5.2). In no case were the differences between the inter-race reciprocal crosses statistically significant (Table 5.2).

5.3.2. Within-race genetic parameters

Within-race variance ratios pooled across the intra- and inter-race cross-types are presented in Table 5.2. Non-maternal reciprocal effects ($recip^2$) were insignificant in all traits assessed except bark thickness (P<0.05), but even then, it was very small compared to the additive and dominance effects. Similarly, maternal effects were generally insignificant, the exception being the earliest DBH assessment (DBH_2) and pulp yield (KPY_9). However, while statistically significant these effects explained ≤ 3% of the phenotypic variance within races (after removing the effects by replicate, row and positional effects), which is minor when compared to the combined additive and dominance genetic effects which accounted for 67% and 42% phenotypic variance in DBH_2 and KPY_9, respectively (Table 5.2). Based on these results, both maternal and non-maternal reciprocal effects were considered negligible and these terms were removed from models used for subsequent analyses. Using the reduced model (Model 2), additive variances (σ_a^2) were shown to be highly significant (P<0.001) for all traits and were the major source of phenotypic variation (Table 5.2). Narrow-sense heritabilities (h^2) were high ranging from 0.40 to 0.60 across traits. The highest h^2 estimate was for basic density (BD_9), but even the DBH estimates were high (0.41 to 0.56). SCA variance was highly significantly (P<0.001) different from zero for DBH at all ages (data not shown). The dominance ratios D^2 for DBH ranged from 0.10 to 0.19, with the dominance variance 22 to 34% the magnitude of the additive variance (Table 5.2). The SCA variance was insignificant for both wood properties (KPY_9 and BD_9) but was significant for bark thickness ($_{Adj.}BT_9$) where the dominance variance was 29% of the magnitude of the additive variance (Table 5.2).

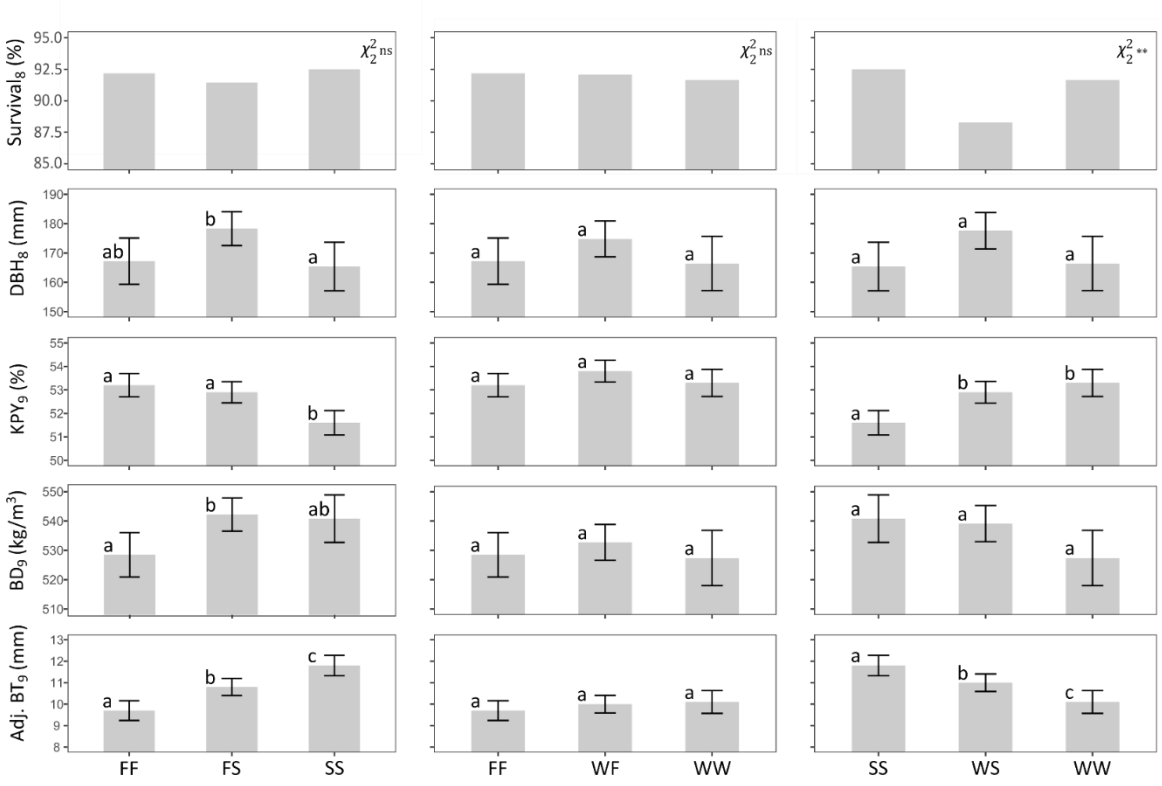

Figure 5.2 Percentage survival and trait least-square means (Diameter [DBH], Kraft pulp yield [KPY], wood basic density [BD] and adjusted bark thickness [$_{Adj.}$BT], where the age of measurement is shown as a subscript). for each cross combination tested in an *E. globulus* diallel. For each pairwise cross combination, the mean of the intra-race crosses is plotted with the mean of their respective inter-race cross. For the four quantitative traits, the standard errors of the means are shown and within each pairwise combination, common letters indicate means which are not significantly different based on pairwise comparisons exceeding the standard error of each difference (SED). For Survival$_8$, the difference among the three crosses indicated within each plot was tested for significance using a homogeneity χ^2 test and the significance is indicated as ns p>0.05 and ** p<0.01.

Table 5.1 Percentage survival and least-square means (± standard errors) of the quantitative traits for two cross-types and the percentage inter-race heterosis for traits measured

Trait	Cross-type	Mean	% heterosis
$Survival_8$	Intra-race	92.2%	-1.63^{*}
	Inter-race	90.7%	
DBH_2	Intra-race	94.8 ± 2.4	2.20^{**}
	Inter-race	96.9 ± 2.4	
DBH_4	Intra-race	130.2 ± 2.9	3.20^{***}
	Inter-race	134.4 ± 2.9	
DBH_6	Intra-race	152.4 ± 4.2	4.93^{***}
	Inter-race	159.9 ± 4.2	
DBH_8	Intra-race	166.4 ± 4.9	6.51^{***}
	Inter-race	177.2 ± 4.8	
KPY_9	Intra-race	52.6 ± 0.3	0.86^{*}
	Inter-race	53.1 ± 0.3	
BD_9	Intra-race	532.2 ± 4.9	1.18^{ns}
	Inter-race	538.5 ± 4.8	
$_{Adj}BT_9$	Intra-race	10.5 ± 0.3	0.30^{ns}
	Inter-race	10.5 ± 0.3	

Traits include survival ($Survival_8$), Diameter at breast height (DBH_2-DBH_8), Kraft pulp yield (KPY_9), wood basic density (BD_9) and adjusted bark thickness ($_{Adj}BT_9$), where the age of measurement is shown as a subscript. For $Survival_8$, the difference in percentage survival between cross-type was tested using a homogeneity chi-square test. For quantitative traits, the difference between the two cross-types was tested using the Wald F statistics. Significance levels are indicated as ^{ns} $p \geq 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 5.2 The variance ratios (± standard errors) and Wald F test for fixed effects and their significance for growth and wood property traits

Source of variation	DBH_2	DBH_4	DBH_6	DBH_8	KPY_9	BD_9	$_{Adj.}BT_9$
Random effects							
$recip^2$	0.00 ± 0.01 ^{ns}	0.01 ± 0.01 ^{ns}	0.01 ± 0.01 ^{ns}	0.01 ± 0.01 ^{ns}	0.01 ± 0.03 ^{ns}	0.00 ± 0.00 ^{ns}	0.07 ± 0.04 *
ma^2	0.01 ± 0.00 *	0.00 ± 0.00 ^{ns}	0.00 ± 0.00 ^{ns}	0.00 ± 0.00 ^{ns}	0.03 ± 0.02 *	0.02 ± 0.02 ^{ns}	0.02 ± 0.01 ^{ns}
h^2	0.56 ± 0.02 ***	0.41 ± 0.03 ***	0.52 ± 0.03 ***	0.51 ± 0.03 ***	0.40 ± 0.09 ***	0.60 ± 0.11 ***	0.56 ± 0.11 ***
D^2	0.19 ± 0.03 ***	0.10 ± 0.02 ***	0.12 ± 0.02 ***	0.12 ± 0.02 ***	0.02 ± 0.08 ^{ns}	0.10 ± 0.08 ^{ns}	0.16 ± 0.08 **
σ_D^2/σ_a^2	0.34 ± 0.05	0.25 ± 0.06	0.22 ± 0.05	0.25 ± 0.05	0.05 ± 0.20	0.16 ± 0.13	0.29 ± 0.16
Fixed effects							
CT (intra- *vs* inter-race)	10.0 **	25.4 ***	48.1 ***	69.1 ***	4.15 *	1.83 ^{ns}	0.12 ^{ns}
$Race_{intra}$ (Within intra-race)	0.07 ^{ns}	0.02 ^{ns}	0.01 ^{ns}	0.02 ^{ns}	6.46 **	0.99 ^{ns}	9.27 ***
$Race_{inter}$ (Within inter-race)	0.04 ^{ns}	0.44 ^{ns}	0.36 ^{ns}	0.18 ^{ns}	8.57 **	1.09 ^{ns}	7.21 **
$Recip_{inter}$ (Inter-race reciprocals)	1.87 ^{ns}	0.77 ^{ns}	0.94 ^{ns}	0.51 ^{ns}	1.79 ^{ns}	1.50 ^{ns}	1.37 ^{ns}

Table shows the ratio of the reciprocal variance to the total phenotypic variance (***recip²***), ratio of the maternal variance to the total phenotypic variance (***ma²***), narrow-sense heritability estimate (***h²***), the ratio of dominance variance to total phenotypic variance (***D²***), the ratio of dominance variance to additive variance (***σ_D^2/σ_a^2***) and Wald *F* test for the significance of the fixed effect terms fitted in the univariate model for different traits (Diameter [DBH] at different ages, Kraft pulp yield [KPY], wood basic density [BD] and adjusted bark thickness [$_{Adj.}$BT], where the age of measurement is shown as a subscript). ***recip²***, was based on the model with all random terms included (Model 1) and ***ma²*** was estimated from the model with the non- or marginally-significant reciprocal term dropped. Remaining estimates were based on the model with reciprocal and maternal terms dropped (Model 2). The diallel comprised crosses between parents from three races – Strzelecki Ranges (S), Western Otways (W) and the Furneaux Islands (F). **CT** tests the significance of intra-race crosses (FF, SS, WW) *vs* inter-race crosses (FS, WS, WF). **$Race_{intra}$** tests between various intra-race crosses (FF vs SS vs WW). $Race_{inter}$ tests between various inter-race crosses (FS vs WS vs WF). $Recip_{inter}$ includes the test of all inter-race reciprocals (FS *vs* SF; WS *vs* SW; WF *vs* FW). The significance levels for the random terms (i.e. σ^2_{recip}, σ^2_{ma}, σ^2_a *or* σ^2_{SCA}) refers to whether these variance components are significantly greater than zero based on a one-tailed likelihood ratio test. Significances are indicated as ^{ns} p≥0.05; * p<0.05; ** p<0.01; *** p<0.001.

The extent to which inter-race heterosis has the potential to inflate estimates of SCA and the dominance ratio was explored by estimating these parameters in models with (Model 3) and without the fixed interaction between female and male races (Table 5.3). Consistent with the increasing heterosis with age (Table 5.1), the SCA variance and D^2 estimates for DBH were increasingly inflated with the model excluding the fixed race interaction term, but this inflation was only significant at the α = 0.10 level (P=0.054) by the age 8 years when there was a 42% increase in the D^2 estimate over that obtained in the full model (Table 5.3). There was no significant difference between the two models in the SCA variance estimates for the wood properties or bark thickness (Table 5.3), consistent with the low inter-race heterosis observed for these traits (Table 5.1).

Age-age additive and SCA genetic correlations for DBH were greater than 0.90 but were highly significantly different from one except for the age of 6 to 8 year (Table 5.4). As expected, there was a slight reduction in the magnitude of correlation as the difference between ages increased. However, even over the maximum age span from two to eight years the additive and SCA correlations only dropped marginally compared with those among DBH measurements taken two-years apart. Inter-trait genetic correlations showed that DBH was significantly positively correlated with adjusted bark thickness at the additive ($r_{additive}$: 0.48 ± 0.17; P≤0.05) and SCA (r_{SCA}: 1.0 at boundary; P<0.001) levels (Table 5.5), and there was no significant difference between these additive and SCA estimates (two-tailed LRT; P=0.056). While there was a trend for positive additive genetic correlations of pulp yield with DBH and basic density, these were not significant (Table 5.5). As SCA variances for the two wood property traits were not significantly different from zero (Table 5.2), genetic correlations at this level were not calculated.

Table 5.3 Comparison of the dominance ratio estimated from Model 3 to the estimates from Model 3 with no interaction term for traits measured in *E. globulus* diallel.

Trait	D^2	
	Model 5	Model 5 with no interaction term
DBH_2	0.19 ± 0.03	0.19 ± 0.03 ^{ns}
DBH_4	0.10 ± 0.02	0.12 ± 0.02 ^{ns}
DBH_6	0.12 ± 0.02	0.15 ± 0.02 ^{ns}
DBH_8	0.12 ± 0.02	0.17 ± 0.03 ^{a}
KPY_9	0.02 ± 0.08	0.02 ± 0.08 ^{ns}
BD_9	0.10 ± 0.08	0.09 ± 0.07 ^{ns}
$_{Adj.}BT_9$	0.16 ± 0.08	0.16 ± 0.08 ^{ns}

Traits included Diameter [DBH] at different ages, Kraft pulp yield [KPY], wood basic density [BD] and adjusted bark thickness [$_{Adj.}$BT], where the age of measurement is shown as a subscript. To test the significance of the interaction term based on a two-tailed likelihood ratio test, the SCA variance in Model 3 was fixed as the variance estimated from the same model excluding the interaction term, and significance levels are indicated as ^{ns} p≥0.05 and ^{a} p=0.054.

Table 5.4 Age-to-age genetic correlations (± standard error) for stem diameter at breast height (DBH where the age of measurement is shown as a subscript) in an *E. globulus* diallel

		DBH_2	DBH_4	DBH_6
DBH_4	Additive	0.99 ± 0.00 ***		
	SCA	0.97 ± 0.01 ***		
DBH_6	Additive	0.95 ± 0.01 ***	0.97 ± 0.01 ***	
	SCA	0.92 ± 0.02 ***	0.98 ± 0.01 ***	
DBH_8	Additive	0.93 ± 0.02 ***	0.96 ± 0.01 ***	1 ± 0 ^{b}
	SCA	0.90 ± 0.02 ***	0.95 ± 0.01 ***	1 ± 0 ^{b}

The table shows the genetic correlations at the additive and SCA levels among the DBH measurements at different ages (age is shown as a subscript). The significance of whether correlation estimates are less than one, based on a one-tailed likelihood ratio test, is indicated as ^{ns} p≥0.05; * p<0.05; ** p<0.01; *** p<0.001.

^{b}Correlation value is at the boundary of the parameter space.

Table 5.5 Inter-trait additive genetic correlations (± standard error) among diameter (DBH_9), basic density (BD_9), Kraft pulp yield (KPY_9) and bark thickness ($_{Adj.}BT_9$) at the age 9 years in *E. globulus* diallel.

	DBH_9	KPY_9	BD_9
KPY_9	0.35 ± 0.19 ^{ns}		
BD_9	-0.16 ± 0.21 ^{ns}	0.35 ± 0.17 ^{ns}	
$_{Adj.}BT_9$	0.48 ± 0.17 *	0.05 ± 0.2 ^{ns}	0.14 ± 0.19 ^{ns}

Here the stem diameter (DBH_9) was measured from the resistance trace from power drill (RESI) at age 9 years for the same trees as wood property traits were assessed. As the SCA variance estimates were not significantly different from zero for KPY_9 and BD_9, genetic correlations at SCA level were not estimated for the combinations including those traits. DBH_9 - $_{Adj.}BT_9$ SCA level correlation was estimated as 1 ± 0, which was at the boundary of the parameter space. The significant difference from zero based on a two-tailed likelihood ratio test (LRT) is indicated as ^{ns} $p \geq 0.05$; * $p < 0.05$

5.4. Discussion

5.4.1. Inter-race heterosis

A key finding of this study was the positive mid-parent heterosis for growth (DBH) resulting from inter-race crossing, which was consistent regardless of the race combination tested. Such mid-parent heterosis for survival or growth has been reported for other race combinations in *E. globulus* (King Island x Southern Tasmania - Vaillancourt *et al.* 1995; Volker *et al.* 2008; Northern-eastern Tasmania x Southern Tasmania - Costa e Silva *et al.* 2014), which suggests that this is a common phenomenon. The magnitude of the inter-race heterosis for growth has been shown to be site and age-dependent in *E. globulus* (Costa e Silva *et al.* 2014). Consistent with our initial hypothesis and other studies (Volker *et al.* 2008; Costa e Silva *et al.* 2014), heterosis increased in magnitude and significance with age in the present study. Indirect genetic effects reflecting heritable competition have been shown to increase with age in the studied trial (Costa e Silva *et al.* 2017), and as cross-types are randomly intermixed as single-tree plots within the trial, such increasing competition is the most likely explanation for the increasing positive inter-race heterosis for growth with age. By 8 years of age, mid-parent heterosis for DBH had reached 6.5%, which was greater than the 5% at age 10 years reported by Volker *et al.* (2008) for crosses involving the King Island and Southern Tasmanian races. Further, while not statistically significant, each race combination separately exhibited better-parent heterosis in the present study, a phenomenon not reported in the study by Volker *et al.* (2008), where on average the inter-race hybrids did not exceed the DBH of the better performing race (King Island).

Positive heterosis for growth in inter-provenance hybrids is commonly reported in forest tree species (*Pinus taeda* L.- Schmidtling and Nelson 1996; *Pinus pinaster* - Harfouche and Kremer 2000; *Pinus caribaea* var. hondurensis - Joseph *et al.* 2000; *Populus balsamifera* L. - Hu and Thomas 2019). The level and direction of heterosis would be expected to depend upon multiple factors including differences in provenance adaptation, as well as susceptibility to inbreeding and outbreeding depression (Edmands 2007). In the present case, there were no significant differences in DBH or survival among the three races when assessed as intra-race crosses suggesting that, at least in terms of the breeding selections, they are equally adapted to the trial site in Western Australia. Thus, as suggested by Potts *et al.* (2000), the most likely explanation for the better-parent heterosis regardless of race combination is the release from inbreeding. In the present case, the consistent better-parent heterosis observed for growth in all inter-race combinations suggests that selections of the three races are equally subject to

inbreeding depression. Inbreeding depression for growth and survival has been reported for multiple populations of *E. globulus.* Based on comparisons of the 2-year stem volume of inter-race crosses of *E. globulus* from two provenances (King Island and Southern Tasmania), Hodge *et al.* (1996) suggests that on average the intra-race crosses exhibited 4% inbreeding depression. Severe inbreeding depression has been observed following selfing (Costa e Silva *et al.* 2010b; Costa e Silva *et al.* 2011; Chapter 2), with the effects on performance declining linearly with the degree of relatedness between parents (Costa e Silva *et al.* 2011). This inbreeding depression is thought to be a consequence of the genetic load of rare recessive, or partially recessive, deleterious alleles that exists within forest tree populations (Charlesworth and Willis 2009; Hedrick *et al.* 2016), including *E. globulus* (Costa e Silva *et al.* 2010b). When trees in close proximity (< 50 m) within native forests are crossed, their progeny also exhibit inbreeding depression (Hardner *et al.* 1998). This finding is consistent with a family group structure within native forests extending 1-2 canopy heights, hypothesised to arise mainly from limited seed dispersal (Eldridge *et al.* 1993; Skabo *et al.* 1998; Jones *et al.* 2007). Parents used in the present study were selected from open-pollinated progenies from different wild trees (Potts *et al.* 2014), sampled at a spacing sufficient to transgress this family group structuring (Gardiner 1989). Thus, the positive heterosis observed may well be a consequence of mild inbreeding depression in the intra-race crosses arising from low levels of relatedness among the founding wild trees (i.e. grandparents) sampled from the same race. In *E. globulus* there is a gradual decline in genetic similarity with distance once the family group structure is transgressed (Skabo *et al.* 1998; Yeoh *et al.* 2012), with significant genetic similarity extending beyond 40 km likely due to long-distance pollen movement (Yeoh *et al.* 2012). However, this gene flow appears to be insufficient to prevent the build-up of low levels of inbreeding within races.

For the studied races, there was no evidence to support the second hypothesis that heterosis for growth would be less evident in crosses among the more divergent races. Such decline could occur if outbreeding depression increased with increasing genetic divergence between hybridising populations (Thornhill 1993; Edmands 2007). In the present case, molecular studies suggest that the two mainland Australian races (Strzelecki Ranges and Western Otways) are more closely related than they are to the Furneaux race (Steane *et al.* 2006; Jones *et al.* 2012; Yeoh *et al.* 2012), yet there is no evidence for a decline in heterosis in the growth of crosses involving the Furneaux race as would be expected with increasing influence of outbreeding depression. This is consistent with the general observation that for hybridisation within species the risk of outbreeding depression is not as great as the risk of inbreeding depression (Edmands 2007). However, in contrast to the improved growth of inter-race hybrids,

there was minor but significant, negative heterosis (outbreeding depression) for survival at the age of 8 years, a trend evident as early as age 2 years (results not shown). A similar discrepancy between growth and survival was reported by Magnussen and Yeatman (1988) in *Pinus banksiana*, where the survival of inter-provenance hybrids was intermediate but the growth trait (height) showed significant positive heterosis. In the present study, this negative heterosis for survival was solely due to one inter-race cross combination - Western Otways x Strzelecki Ranges (WS) - in which the survivors exhibited positive heterosis for growth. These two races were the most genetically similar and thus, the mild outbreeding depression observed for survival is not related to general genetic divergence nor are the factors involved having a negative impact on survivors which exhibit positive heterosis for growth. Outbreeding depression has been reported at the inter-specific level for first (López *et al.* 2000a) and second generation (Costa e Silva *et al.* 2012) hybrids of *Eucalyptus* and this is the first indication of its manifestation at the inter-population level within a species. However, this effect on survival of one race combination is minor compared with the positive heterosis observed for growth in all the inter-race combinations tested (present study; Volker *et al.* 2008; Costa e Silva *et al.* 2014). Nevertheless, it should be noted that while the genetic divergence between the Furneaux and mainland subraces is comparatively high, as were the crosses studied by Volker *et al.* (2008), there are slightly more divergent race combinations in *E. globulus* which have not been tested (e.g. SE Tasmania x King Island; Steane et al 2006). Whether that greater level of population divergence would result in outbreeding depression countering the positive effects of heterosis is unclear. More importantly, outbreeding depression may not be evident in the first generation of inter-population hybridisation but mainly expressed subsequently following recombination of co-adapted gene complexes in subsequent generations (Edmands 1999). Indeed, Edmands (2007) notes that in the context of conservation biology where inter-population hybridisation is often considered for genetic rescue, that while there is much more empirical evidence for inbreeding depression than outbreeding depression, the risks associated with outbreeding, particularly in the second generation, maybe on par with the risks of inbreeding.

Unlike growth, wood property traits (pulp yield and wood basic density) showed a very low level of positive inter-race heterosis for the trees sampled. A study by Volker *et al.* (2008) also reported the absence of significant heterosis of basic density for intra-specific crosses as in the present study, although such studies are rare. However, inter-specific hybridisation of forest tree species showed widely varying basic wood density for hybrids (Dungey 2001; Bison *et al.* 2006). Although small, pulp yield exhibited slightly significant heterosis in the present study for inter-population crosses. Similar hybrid vigour for pulp yield was earlier reported in inter-

specific crosses (de Assis 2000) leading to hybridisation being suggested as a strategy for increasing pulp yield. The absence or low level of inter-race heterosis for wood properties were reflected in the negligible dominance effects for these traits due to their strong additive genetic control as reported in the present study.

5.4.2. Maternal and non-maternal reciprocal effects

Another important finding of the present study is the absence of significant reciprocal effects at race level and their virtual absence within-races, as hypothesised. For growth, the estimated maternal effect was significant at age 2 years, but this effect was very small (explaining only 1% of the total phenotypic variation) and, as hypothesised rapidly diminished with age. While significant maternal effects were not detected for height growth of *E. globulus* in the study of López *et al.* (2003), the significant non-maternal reciprocal effect was reported on early age growth (age 7 months). They showed that early age field growth was positively affected by seed mass and nursery block, but these effects and the non-maternal reciprocal effect were insignificant at later ages when race and within race additive effects became increasingly significant. While seed and germination characteristics were shown to affect nursery performance, the transitional nature of these, and the non-maternal reciprocal effect on growth is consistent with the present study. A reciprocal effect at the race level was detected for stem diameter in the smaller-scale study of Tasmanian races of *E. globulus* by Costa e Silva *et al.* (2014). However, this effect was only significant at one of the two sites studied and was of minor significance compared to the general heterotic effect of inter-race crossing.

Maternal and non-maternal reciprocal effects are non-Mendelian in nature and could reduce the precision of the estimated genetic effects (Roach and Wulff 1987). However, as selection of *E. globulus* for chip export or pulp production is based on early age (4-8 years of age) stem diameter, wood density and pulp yield (depending on the breeding objective; Potts *et al.* 2014), the present study suggests the maternal and non-maternal reciprocal effects are insignificant (diameter and wood density) or very minor (pulp yield), and will have little impact on breeding and deployment considerations. This is particularly relevant given the option for deploying full-sib families of *E. globulus* through mass supplementary pollinations (Potts *et al.* 2008), suggesting that the choice of the maternal parent can be made based on accessibility and reproductive characteristics, and does not have to copy the direction of the cross from previous trials. Similar conclusions on the practical importance of reciprocal effects were also reported for *Pinus radiata*, a major softwood plantation tree species deployed in Australia, where weak reciprocal effects were identified (Wu and Matheson 2001) and there is also the option for deployment of full-sib families from seed (Baltunis *et al.* 2007).

5.4.3. Relative importance of within-race dominance variation

In addition to inter-race heterosis, the relative importance of additive and dominance variation within races is a key consideration in determining the benefits of deploying full-sib families as opposed to deploying polymix or open-pollinated families. If dominance is not important then there is a little additional benefit in the deployment of full-sib-families apart from the avoidance of the deleterious effects arising from, for example, self-pollination in the case of open-pollinated progeny (Potts *et al.* 2014; Chpater 2). Consistent with theoretical expectations and observations in other systems (Hill *et al.* 2008), most of the genetic variation within races for the traits studied was additive. However, as originally hypothesised, estimated dominance variation was highly significant for growth trait but not for wood property traits (pulp yield and wood density). For growth, dominance accounted for 19% of the within race phenotypic variation in diameter at age 2 years but stabilised between 10 and 12% at later ages. While the dominance ratio is slightly higher in the intra-race crosses compared to the inter-race crosses (e.g. DBH_8 D^2 is 0.11 ± 0.04 compared with 0.09 ± 0.03, respectively; results not shown) as evident in the early study by Hodge *et al.* (1996), these increases in dominance variance were not statistically significant. Reports of the relative importance of dominance variation for growth traits vary markedly in *E. globulus*, partly dependent upon the populations and growth trait studied, as well as the accuracy of parameter estimation due to small sample size. While no significant dominance variation for DBH was reported by Costa e Silva *et al.* (2004), most studies report significant dominance variation explaining up to 19% of the phenotypic variation at a single site (12% - Li *et al.* 2007; 0-100% - Callister *et al.* 2011; 2-10% - Callister *et al.* 2013; 18% - Hamilton *et al.* 2015a; 19% - Hamilton *et al.* 2017b).

The ratio of dominance variance to total phenotypic variance (D^2) estimates for later age DBH from the studied trial accord well with the maximum D^2 estimate reported for *E. globulus*. However, in terms of the importance of dominance variation relative to the additive genetic variation (σ_D^2/σ_a^2) the estimates are low in the present study. The dominance variation for later age (> 2 years) DBH was 22 to 25% of the magnitude of the additive variation, whereas other smaller-scale studies reported values as high as 167% (100% - Li *et al.* 2007; 0-167% - Callister *et al.* 2011; 80% - Araújo *et al.* 2012; 13-131% - Callister *et al.* 2013; 113% - Hamilton *et al.* 2015a; 126% - Hamilton *et al.* 2017b) and even 400% in the small trial of 62 full-sib families reported by Mora *et al.* (2019). Inaccuracies due to small full-sib family representation in many trials may explain much of the variation in the dominance to additive ratio. However, it is noteworthy that the narrow-sense heritability and thus contribution of additive genetic variation to the phenotypic variation in DBH in the present study were among the highest

reported for full-sib family trials of *E. globulus*. Single-site estimates of the narrow-sense heritabilities for DBH in the above mentioned studies do not exceed 0.16 (e.g. 0.08-0.10 by Costa e Silva *et al.* 2004; 0.12 by Li *et al.* 2007; 0.10 - Araújo *et al.* 2012; 0.08–0.12 by Callister *et al.* 2013; 0.16 - Hamilton *et al.* 2015a; 0.07 - Mora *et al.* 2019), whereas the values estimated for DBH in the present study ranged between 0.41-0.56, depending upon age. This high heritability for DBH could be due to multiple factors including a uniform trial site, large numbers of full-sib families per parent, coupled with the accentuation of additive genetic differences through competition as indirect genetic effects on DBH have been demonstrated at this trial (Costa e Silva *et al.* 2013a; Costa e Silva *et al.* 2017). Nevertheless, the significant additive and dominance effects revealed early in stand development (by age 2 years) are stable with age as evidenced by the extremely high age to age correlations for DBH evident at both genetic levels.

While the genetic architecture for DBH was somewhat different from that previously reported for *E. globulus*, the genetic architecture of wood property traits was in accordance with previously published reports. Variation in both pulp yield and wood density were under significant and strong additive genetic control with high narrow-sense heritabilities (h^2; 0.40 to 0.60, respectively) and no significant dominance variation. Wood density generally exhibits higher heritability than growth traits (Li *et al.* 2007; Stackpole *et al.* 2010a; Chapter 3 & 4) and the few studies using full-sib family trials of *E. globulus*, have not revealed significant dominance variation (Costa e Silva *et al.* 2004; Li *et al.* 2007). There are few studies of the genetic architecture of pulp yield in *E. globulus*, and more recent studies based on the same NIR methodology and open-pollinated progenies have reported h^2 estimates ranging from 0.04 to 0.40 (Stackpole *et al.* 2010b; Chapter 3). Only two studies of pulp yield were found, which were based on full-sib family trials, allowing dominance to be estimated (Costa e Silva *et al.* 2009; Hamilton *et al.* 2017b). Both of these studies revealed highly significant additive genetic variation and non-significant dominance variation, as found in the present study. The heritability estimates varied (0.42±0.14 - Costa e Silva *et al.* 2009; 0.26±0.07 - Hamilton *et al.* 2017b), and the current estimate (0.40±0.09) is more consistent with the higher of these heritability estimates reported, and accords with the higher of the estimates from open-pollinated progeny trials (0.40±0.06 - Stackpole *et al.* 2010b). No previous studies on the significance of non-additive effects for bark thickness were found. This trait is clearly under significant additive genetic control (present study; Chapter 4), but the dominance variation is significant and 29% of the additive genetic variance in magnitude. Given bark thickness is the only trait that is genetically correlated with DBH, it is possible that the dominance variation in bark thickness is

a reflection of the residual correlated variation in DBH which has remained even after fitting DBH as a covariate in the statistical model at the phenotypic level.

5.5. Conclusion

Significant levels of genetic variance were evident in this selected population of *E. globulus* for all traits studied – three of which are key selection traits for pulpwood breeding (DBH, wood density and pulp yield). This genetic variation was manifested at different scales (i.e. between race or with race levels) and involved additive and, depending on trait, dominance effects. With the sample of parents studied, all traits exhibited significant additive genetic variation within races, but growth, in particular, was also influenced by significant non-additive genetic effects. The high levels of additive genetic variance and low genetic correlations among selection traits, combined with the inter-race heterosis for growth in the first generation bode well for combining germplasm from multiple races in the breeding population. However, the possibility that outbreeding depression may be expressed following recombination in subsequent generations and the levels of heterosis needs to be monitored in the subsequent generations of breeding as inter-race crossing extends beyond the first generation. Nevertheless, as hypothesised, and similar to other forest tree species, first generation inter-race hybrids of *E. globulus* showed positive heterosis for growth, suggesting that intra-race crosses exhibit low levels of inbreeding depression. This finding of positive heterosis, coupled with the significant dominance variation for growth within races, suggests additional genetic gain in growth can be captured in deploying clones or full-sib families from inter-race crossing. The absence or minor significance of maternal and non-maternal reciprocal effects estimated for the three pulpwood selection traits shows that the directionality of crossing has little effect on performance. This finding supports the deployment of full-sib families of *E. globulus* using mass supplementary pollination easier, simplifies crossing for breeding purposes, and simplifies genetic evaluation models.

Chapter 6 - General discussion

6.1. Introduction

The present study has used both open-pollinated (OP) and control-pollinated progeny trials to provide novel insights into the genetic architecture of growth, wood property traits and bark thickness in *Eucalyptus globulus*. Using a progeny field trial from one of the largest diallel crossing schemes undertaken in *E. globulus*, I report inter-race heterosis, additive and non-additive genetic effects. In addition, the study on the effects of inbreeding is the longest studies to date in this species (and *E. ovata*), and one of the very few long-term studies of inbreeding depression in forest trees. Moreover, the generally positive association between pulpwood and solid wood selection traits reported here is an important finding given the increasing interest in using parts of the pulpwood estate for solid wood products from *E. globulus*, a species which had been mainly studied for the genetic improvement for pulpwood, but not for solid wood. The key findings and their implications are discussed below.

6.2. Genetics of growth traits

As demonstrated in other studies, the growth (DBH) of *E. globulus* is severely affected by inbreeding depression (ID; Chapter 2). Utilising one of the longest (28 years) studies on the effects of selfing in eucalypts, it is shown that there is significant early age ID for growth with the sampled trees studied. Subsequent mortality estimates were size-dependent and, with the death of smaller selfs, the interplay between ID for growth and survival resulted in the translation of ID from growth to survival, as previously noted in other studies (*E. regnans* - Hardner and Potts 1997; *Pinus silvestris* - Koelewijn *et al.* 1999; *E. globulus* - Costa e Silva *et al.* 2011). The present study was only recently exceeded in age by the 29 year study of *E. regnans* by Griffin *et al.* (2019). That study also reported the selective elimination of most selfs from the population with age, as shown for *E. globulus* and *E. ovata* in Chapter 2. These species show severe inbreeding depression, which is comparable to other forest tree species (75% in Scots pine [23 years] - Koelewijn *et al.* 1999; 80% in Douglas fir [26 years] - Stoehr *et al.* 2015), and the purging of most selfs by the age of 10-15 years. However, as eucalypts have a mixed mating system which produces both selfed and outcrossed seed under open-pollination, the rare survival of selfs cannot be neglected (Chapter 2), especially in the absence of competition with more vigorous outcross progeny, and may even contribute to inter-population heterosis (Chapter 5). However, in some species, populations exhibiting little inbreeding depression have

also been noted (*Eucalyptus cladocalyx* - Bush and Thumma 2013; *Eucalyptus caesia* - Bezemer *et al.* 2019), which is hypothesised to be a consequence of the purging of deleterious alleles in small and isolated populations.

Comparable to previous reports, the present study also identified racial differences in *E. globulus* in growth rate as assessed using stem diameter. Previous studies based on open-pollinated base population progeny trials reported Furneaux as the slowest growing race among the three races used in the diallel study - Furneaux, Strzelecki Ranges and Western Otways (Dutkowski and Potts 1999; Raymond *et al.* 2001; Stackpole *et al.* 2010b). However, a different rank order for these races has been found in other studies (Raymond *et al.* 2001; NW in Chapter 3). The discrepancies in race ranks among these studies/trials could be due to differences in the trial environments (genotype x environmental interaction; GxE) or the age of assessment (Stackpole *et al.* 2010a). The GxE interaction for growth shown by this study (Chapter 3) emphasises the importance of local environmental effects on the relative growth of families and provenances/races of *E. globulus*, potentially reflecting differences in local climate adaptation (Leimu and Fischer 2008). However, these reports were from open-pollinated progeny trials and differences between families and races could reflect differences in levels of inbreeding (Hodge *et al.* 1996). Therefore, the variation showed between these races in their OP progenies might be due to differences in selfing rate between races, which can negatively affect growth rate (e.g. Chapter 2). In the diallel study where races were compared as intra-race cross-pollinated progenies, there was no significant difference between races for growth at any age of assessment (Chapter 5), which could in part reflect the impact of artificial selection on the parents, homogenising the race differences. Alternatively, differential rank order of the races could reflect a GxE interaction for growth.

The controlled intra-race crosses in the diallel trial showed differences in their estimated additive genetic effects compared to the OP progeny trials, in this study as well as the literature. OP progeny trials, including the present study (Chapter 3), have produced lower narrow-sense heritability estimates than that found in our full-sib diallel progeny trial (Chapter 5). Regardless, of the cause of these differences (e.g. sampling effects, GxE), the diallel study shows that there is considerable additive genetic variance for growth among the first-generation selections in the Australian National *E. globulus* Breeding Population, which can be exploited. Although the genetic variation in other traits is mostly additive, the significant dominance effects for growth emphasise the importance of controlled crossing. Exclusion of dominance effects from genetic models may affect the accuracy of genetic parameter and breeding value estimates. Such non-additive effects are evident in both between- and within-

race crosses, with between race crosses resulting in significant positive heterosis. Moreover, signals of heterosis in inter-race crossing (Chapter 5) is consistent with other studies on this species (King Island x Southern Tasmania - Vaillancourt *et al.* 1995; Volker *et al.* 2008; Northern-eastern Tasmania x Southern Tasmania - Costa e Silva *et al.* 2014) as well as other forest tree species (*Pinus banksiana* Lamb. - Magnussen and Yeatman 1988; *Pinus taeda* L.- Schmidtling and Nelson 1996; *Pinus pinaster* - Harfouche and Kremer 2000; *Pinus caribaea* var. *hondurensis* - Joseph *et al.* 2000; *Populus balsamifera* L. - Hu and Thomas 2019), and suggests that inter-race hybrid vigour could be utilised for capturing additional gain in deployment programmes of *E. globulus*.

6.3. Genetics of bark thickness

Larger trees tend to have thicker bark (Pinard and Huffman 1997; Lawes *et al.* 2013; Poorter *et al.* 2014). Bark thickness in *E. globulus* has been observed to vary with tree age, heights within tree and site productivity (Quilhó *et al.* 2000; Quilhó and Pereira 2001; Hamilton *et al.* 2007). However, reported genetic correlations between these traits show varying results from negative [-0.42] (Wei and Borralho 1997; Retief and Stanger 2009; Chapter 4) to positive [0.48] correlation (Wei and Borralho 1997; Chapter 5). This may be due to the variation in the age of measurement as well as the environmental interaction and its genetic effects on growth, which may be confounded with variation in the assessment of bark thickness as an absolute or relative measure used in different studies.

Understanding the genetic control of bark thickness *per se*, independent of stem size, is thus important. While some studies have accounted for stem diameter by expressing back thickness as a proportion of stem diameter (Wei and Borralho 1997; Retief and Stanger 2009), in the present case the association with diameter was removed at the phenotypic level by using size-adjusted estimates of bark thickness, by fitting diameter (DBH) as a covariate in the linear model used to estimate genetic variances.

Most of the above-mentioned studies of bark thickness, including the present one (Chapter 4 & 5), reported moderate to high heritability for relative/adjusted bark thickness, regardless of the type of crossing or population studied, emphasising the potential for selection on this trait. Moreover, the variation in relative bark thickness between various races of this species is clear (Chapter 4). For the three races used in Chapter 5 (Furneaux, Strzelecki Ranges and Western Otways), the rank order of the bark thickness was identical to that of other studies, although they were tested at multiple locations and as both open- and cross-pollinated progenies (Dutkowski and Potts 1999; Chapter 4 & 5). A slight difference in the rank order of

these races was reported between trials by Hamilton *et al.* (2007), but all evidence to date suggests that there is little GxE interaction for this trait (Wei and Borralho 1997; Rosell *et al.* 2014; Chapter 4). Therefore, the above-mentioned stable rank order confirms that among these three races, the Strzelecki Ranges race possesses the thickest bark. The potential adaptive implications of variation in bark thickness, including its links with stress tolerance (e.g. drought susceptibility and protection of the stem from fire; Rosell 2016) make this trait of potential interest in tree improvement programmes aimed at better adapting the plantation estate to future climates.

6.4. Genetics of wood property traits

Unlike growth, wood property traits such as basic density and pulp yield showed a uniform ranking of the races (Furneaux, Strzelecki Ranges and Western Otways), irrespective of trial site, assessment age or cross-type (Dutkowski and Potts 1999; Stackpole *et al.* 2010b; Chapter 3 & 4). Of the *E. globulus* races dominating the National *E. globulus* Breeding programme, Strzelecki Ranges appeared to be the race with the densest wood, but the lowest pulp yield. Both open-pollinated base population trials and the diallel trial based on first generation selections produced similar conclusions, although the differences in wood density were not statistically significant in the diallel trial when the intra-race crosses were compared (Chapter 5). These results support the absence of significant GxE for these traits (Chapter 3). In addition, the absence of significant dominance effects (Chapter 5) for these wood property traits for the sampled trees, supports the use of additive models in tree improvement programmes. No studies were found, either at the inter- or intra-specific levels, which assessed maternal effects for wood basic density and pulp yield. The present study showed the absence of maternal effect on basic density, but a statistically significant maternal effect was observed on pulp yield. However, this effect contributed only 3% to the phenotypic variance and was very small compared with the additive genetic effects (40%). Although this effect on pulp yield is unlikely to be of practical importance, further exploration is warranted to confirm this conclusion.

The estimated additive genetic correlation of basic density with diameter was not significantly different from zero, in both the open-pollinated and the controlled pollinated trials (Chapter 3 & 5). These non-significant correlations were consistent with previous reports for this species (Downes *et al.* 2006; Stackpole *et al.* 2010a) suggesting little genetic association between basic density and growth in *E. globulus*. Unlike basic density, pulp yield exhibited a positive additive genetic correlation with the diameter in both the open-pollinated and diallel

trials studied. Previous reports of this correlation are variable, ranging from negative [-0.16] to positive [0.12] (Dean *et al.* 1990; Raymond *et al.* 2001; Apiolaza *et al.* 2005; Costa e Silva *et al.* 2009). Such variation in the relationship of these wood property traits with diameter may well reflect the significant GxE reported for growth traits. Indeed, the association between basic density and pulp yield is complex. At the additive-level, both traits exhibit a significant positive association (in both open- and cross-pollinated studies; Chapter 3 & 5), however, they are significantly negatively associated at the subrace level (Chapter 3). Such discrepancy has been observed previously (Stackpole *et al.* 2010b), and emphasises the need to separate and understand the different hierarchies of genetic variation within the species. Indeed, such comparisons are fundamental to understanding the extent to which the patterns of population divergence may be constrained by the patterns if genetic variation and covariation within populations (Costa e Silva *et al.* 2020).

6.5. Implications of this study

While most of the results of this thesis are based on samples of base population parents and seed lots or, in the case of Chapter 5, selections from these seed lots, coupled with the caveat that several of the studies are based on single site experiments, the findings of this thesis do have multiple implications for the breeding and deployment of *E. globulus* for production forestry. The current Australian National *E. globulus* breeding programme primarily focused on inter-race crossing following the first wave of selections from the base population trials. Mid-parent heterosis for growth was expected as seen in other forest trees (Chapter 5) and the earliest study of *E. globulus* involving crossing among the King Island and Southern Tasmania races (Vaillancourt *et al.* 1995; Volker *et al.* 2008). However, this hypothesis had not been tested for the main races where most of the first-generation selections were derived (Furneaux, Strzelecki Ranges and Western Otways). The present study not only supports this expectation of mid-parent heterosis but shows such heterosis is evident regardless of race combination and even extends to better race (parent) heterosis. Thus, in genetic evaluation models, race effects could effectively be considered additive for wood property traits, but in the case of growth, the genetic interaction between races needs to be considered. In addition, with the option of deploying full-sib families of *E. globulus* through mass-supplementary pollination (MSP), there is the opportunity of capturing such inter-race heterosis along with the significant dominance effects for deployment. Additional gains from MSP should be possible through eliminating selfing which has a marked deleterious effect on tree growth and survival (Chapter 2). The present study also showed that maternal and non-maternal reciprocal effects are effectively

negligible (Chapter 5). This means that it doesn't matter from a quantitative genetic perspective whether a selected genotype is used as a male or a female in breeding or deployment crossing, and other factors such as accessibility or reproductive attributes can be given priority.

In the case of using *E. globulus* plantations as a source of raw material for solid-wood products, the present study suggests that the genetic improvements made through decades of pulpwood breeding (Eldridge *et al.* 1993; Potts *et al.* 2014) should also be expected to have made gains for solid-wood uses, as the present study showed a favourable alignment of selection traits for pulpwood and solid wood (Chapter 3). Thus, breeding for a pulpwood objective is expected to have improved the current plantations in use for solid-wood products. Similar conclusions were reported in *E. nitens,* suggesting the possibility of making pulpwood breeding goals compatible with those for solid wood products (Kube and Raymond 2001; Blackburn *et al.* 2012), although exceptions exist (Hamilton *et al.* 2009b). However, with growing interest in solid wood products from plantations of *E. globulus*, increasing emphasis on specific solid wood traits (e.g. form and stiffness) may be warranted. Indeed, enhanced gains with this objective in mind may be possible by revisiting opportunities for selection from base population trials to make the plantations more suitable for diverse end products. For example, the King Island race is poorly represented in the current breeding population (Potts *et al.* 2014). However, this race is consistently reported to have high pulp yield (Stackpole *et al.* 2010b; Chapter 3), the straightest stems and relatively higher wood stiffness (Chapter 3), but greater drought susceptibility (Dutkowski and Potts 2012) and poorer growth on drier sites (Costa e Silva *et al.* 2006) which suggests suitability for only the wetter component of the current plantation estate.

Reference

ABARES (2016a) Australia's plantation log supply 2015-2059. Australian Bureau of Agricultural and Resource Economics and Sciences, Canberra.

ABARES (2016b) Australian plantation statistics 2016. Australian bureau of agricultural and resource economics and sciences, Canberra, Australia.

ABARES (2018) Australian forest and wood products statistics: September and December quarters 2017. Australian Bureau of Agriculture and Resource Economics and Sciences, Australia.

Abram NJ, McGregor HV, Tierney JE, Evans MN, McKay NP, Kaufman DS, PAGES 2k Consortium (2016) Early onset of industrial-era warming across the oceans and continents. *Nature* **536**, 411-418.

Acquaah G (2009) 'Principles of plant genetics and breeding.' (John Wiley & Sons: Blackwell, Oxford, UK).

Aitken SN, Bemmels JB (2016) Time to get moving: assisted gene flow of forest trees. *Evolutionary Applications* **9**, 271-290.

Allen CD, Macalady AK*, et al.* (2010) A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecology and Management* **259**, 660-684.

Anderegg WR, Kane JM, Anderegg LD (2013) Consequences of widespread tree mortality triggered by drought and temperature stress. *Nature Climate Change* **3**, 30-36.

Antony F, Jordan L, Schimleck LR, Clark III A, Souter RA, Daniels RF (2011) Regional variation in wood modulus of elasticity (stiffness) and modulus of rupture (strength) of planted loblolly pine in the United States. *Canadian Journal of Forest Research* **41**, 1522-1533.

Apiolaza L, Raymond C, Yeo B (2005) Genetic variation of physical and chemical wood properties of *Eucalyptus globulus*. *Silvae Genetica* **54**, 160-165.

Araújo JA, Borralho NMG, Dehon G (2012) The importance and type of non-additive genetic effects for growth in *Eucalyptus globulus*. *Tree Genetics & Genomes* **8**, 327-337.

Armbruster P, Reed DH (2005) Inbreeding depression in benign and stressful environments. *Heredity* **95**, 235-242.

Armbruster W, Schwaegerle K (1996) Causes of covariation of phenotypic traits among populations. *Journal of Evolutionary Biology* **9**, 261-276.

Armbruster WS, Pélabon C, Bolstad GH, Hansen TF (2014) Integrated phenotypes: understanding trait covariation in plants and animals. *Philosophical Transactions of the Royal Society B* **369**, 20130245.

Aspinwall MJ, Loik ME, Resco de Dios V, Tjoelker MG, Payton PR, Tissue DT (2015) Utilizing intraspecific variation in phenotypic plasticity to bolster agricultural and forest productivity under climate change. *Plant, Cell & Environment* **38**, 1752-1764.

Astorga R, Soria F, Basurco F, Toval G (2004) Diversity analysis and genetic structure of *Eucalyptus globulus* Labill. In '*Eucalyptus* in a Changing World. ' Ed. NMG Borralho) pp. 351-363. (RAIZ , Instituto de Investigacao da Floresta e Papel: Aveiro, Portugal)

Australian Bureau of Meteorology (2011) Australian climate averages: Average wind velocity. In. ' (Bureau of Meteorology, Commonwealth of Australia)

Ayre BM, Roberts DG, Phillips RD, Hopper SD, Krauss SL (2019) Near-neighbour optimal outcrossing in the bird-pollinated *Anigozanthos manglesii*. *Annals of Botany* **124**, 423-436.

Baltunis BS, Huber DA, White TL, Goldfarb B, Stelzer HE (2007) Genetic analysis of early field growth of loblolly pine clones and seedlings from the same full-sib families. *Canadian Journal of Forest Research* **37**, 195-205.

Barmentlo SH, Meirmans PG, Luijten SH, Triest L, Oostermeijer JGB (2018) Outbreeding depression and breeding system evolution in small, remnant populations of *Primula vulgaris*: consequences for genetic rescue. *Conservation Genetics* **19**, 545-554.

Baskin JM, Baskin CC (2015) Inbreeding depression and the cost of inbreeding on seed germination. *Seed Science Research* **25**, 355-385.

Bates D, Maechler M, Bolker B, Walker S (2014) lme4: Linear mixed-effects models using Eigen and S4. *R package version 1.0-5.* *http://CRAN.R-project.org/package=lme4*

Batish DR, Singh HP, Kohli RK, Kaur S (2008) *Eucalyptus* essential oil as a natural pesticide. *Forest Ecology and Management* **256**, 2166-2174.

Beadle C, Medhurst J, Forrester D (2011) Linking silvicultural management to solid-wood values. In 'Developing a eucalypt resource: learning from Australia and elsewhere '. Ed. J Walker) pp. 77-92: Wood Technology Research Centre, University of Canterbury, Christchurch)

Beadle C, Volker P*, et al.* (2008) Solid-wood production from temperate eucalypt plantations: a Tasmanian case study. *Southern Forests: a Journal of Forest Science* **70**, 45-57.

Belaber EC, Gauchat ME, Reis HD, Borralho NM, Cappa EP (2018) Genetic parameters for growth, stem straightness, and branch quality for *Pinus elliottii* var. *elliottii* × *Pinus caribaea* var. *hondurensis* F_1 hybrid in Argentina. *Forest Science* **64**, 595–608.

Bella I (1971) A new competition model for individual trees. *Forest Science* **17**, 364-372.

Berlin M, Jansson G, Högberg K-A, Helmersson A (2019) Analysis of non-additive genetic effects in Norway spruce. *Tree Genetics & Genomes* **15**, 42.

Bertrand R, Riofrío-Dillon G, Lenoir J, Drapier J, De Ruffray P, Gégout JC, Loreau M (2016) Ecological constraints increase the climatic debt in forests. *Nature Communications*, 7:12643.

Bezemer N (2018) Wild seedlings of a tree endemic on granite outcrops show no evidence of inbreeding depression. *Australian Journal of Botany* **66**, 39-47.

Bezemer N, Krauss SL, Roberts DG (2019) No evidence for early inbreeding depression in planted seedlings of *Eucalyptus caesia*, an anciently fragmented tree endemic on granite outcrops. *Plant Ecology* **220**, 1101-1108.

Bison O, Aguiar AM, Rezende G, Ramalho MAP (2004) Inbreeding depression in *Eucalyptus* clones. *Crop Breeding and Applied Biotechnology* **4**, 459-464.

Bison O, Ramalho MAP, Rezende G, Aguiar AM, de Resende MDV (2006) Comparison between open pollinated progenies and hybrids performance in *Eucalyptus grandis* and *Eucalyptus urophylla*. *Silvae Genetica* **55**, 192-196.

Bivand RS, Pebesma EJ, Gomez-Rubio V, Pebesma EJ (2008) 'Applied spatial data analysis with R.' (Springer: New York).

Blackburn D, Farrell R, Hamilton M, Volker P, Harwood C, Williams D, Potts B (2012) Genetic improvement for pulpwood and peeled veneer in *Eucalyptus nitens*. *Canadian Journal of Forest Research* **42**, 1724-1732.

Blackburn D, Hamilton M, Harwood C, Innes T, Potts B, Williams D (2010) Stiffness and checking of *Eucalyptus nitens* sawn boards: genetic variation and potential for genetic improvement. *Tree Genetics & Genomes* **6**, 757-765.

Blackburn D, Hamilton M, Williams D, Harwood C, Potts B (2014) Acoustic wave velocity as a selection trait in *Eucalyptus nitens*. *Forests* **5**, 744-762.

Blackburn DP, Hamilton MG, Harwood CE, Baker TG, Potts BM (2013) Assessing genetic variation to improve stem straightness in *Eucalyptus globulus*. *Annals of forest science* **70**, 461-470.

Blada I (1999) Diallel crossing in *Pinus cembra*. *Silvae Genetica* **48**, 179-187.

Booth TH (2017) Going nowhere fast: a review of seed dispersal in eucalypts. *Australian Journal of Botany* **65**, 401-410.

Borralho N (1997) Genetic parameters for growth and wood density traits in *Eucalyptus nitens* in New Zealand. *New Zealand Journal of Forestry Science* **27**, 237-244.

Borralho N, Cotterill P, Kanowski P (1992) Genetic parameters and gains expected from selection for dry weight in *Eucalyptus globulus* ssp. *globulus* in Portugal. *Forest science* **38**, 80-94.

Borralho N, Potts B (1996) Accounting for native stand characteristics in genetic evaluations of open-pollinated progeny from a *Eucalyptus globulus* base population. *New Forests* **11**, 53-64.

Borralho NMG, Cotterill PP, Kanowski PJ (1993) Breeding objectives for pulp production of *Eucalyptus globulus* under different industrial cost structures. *Canadian Journal of Forest Research* **23**, 648-656.

Borzak CL, O'Reilly‐Wapstra JM, Potts BM (2015) Direct and indirect effects of marsupial browsing on a foundation tree species. *Oikos* **124**, 515-524.

Boshier D, Billingham M (1999) Genetic variation and adaptation in tree populations. In 'Ecological Consequences of Habitat Heterogeneity. ' (Eds M Hutchings, E John and A Stewart) pp. 267-289: University of Sussex,)

Bouvet JM, Makouanzi G, Cros D, Vigneron P (2016) Modeling additive and non-additive effects in a hybrid population using genome-wide genotyping: prediction accuracy implications. *Heredity* **116**, 146-157.

Bräutigam K, Vining KJ*, et al.* (2013) Epigenetic regulation of adaptive responses of forest tree species to the environment. *Ecology and evolution* **3**, 399-415.

Brooker MIH (2000) A new classification of the genus *Eucalyptus* (Myrtaceae). *Australian Systematic Botany* **13**, 79-148.

Burdon R (1977) Genetic correlation as a concept for studying genotype-environment interaction in forest tree breeding. *Silvae Genet* **26**, 168-175.

Bureau of Meteorology (2020) Climate statistics for Australian locations: Summary statistics Manjimup. In. ' (Commonwealth of Australia Australia)

Bush D, Kain D, Matheson C, Kanowski P (2011) Marker-based adjustment of the additive relationship matrix for estimation of genetic parameters—an example using *Eucalyptus cladocalyx*. *Tree genetics & genomes* **7**, 23-35.

Bush D, Thumma B (2013) Characterising a *Eucalyptus cladocalyx* breeding population using SNP markers. *Tree Genetics & Genomes* **9**, 741-752.

Byrne M (2008) Phylogeny, diversity and evolution of eucalypts. In 'Plant genome: biodiversity and evolution. Vol. 1. Part *E. Phanerogams*–Angiosperm'.(Eds AK Sharma, A Sharma) pp. 303–346. In. ' (Science Publishers: Berlin, Germany)

Callister AN, Collins SL (2008) Genetic parameter estimates in a clonally replicated progeny test of teak (*Tectona grandis* Linn. f.). *Tree Genetics & Genomes* **4**, 237.

Callister AN, England N, Collins S (2011) Genetic analysis of *Eucalyptus globulus* diameter, straightness, branch size, and forking in Western Australia. *Canadian Journal of Forest Research* **41**, 1333-1343.

Callister AN, England N, Collins S (2013) Predicted genetic gain and realised gain in stand volume of *Eucalyptus globulus*. *Tree Genetics & Genomes* **9**, 361-375.

Cappa EP, El-Kassaby YA, Muñoz F, Garcia MN, Villalba PV, Klápště J, Marcucci Poltri SN (2017) Improving accuracy of breeding values by incorporating genomic information in spatial-competition mixed models. *Molecular Breeding* **37**, 125.

Carson M (2019) The future of forest tree improvement in New Zealand. In 'Tree Breeding and Genetics in New Zealand. pp. 181-197. (Springer).

Chambers P, Potts B, Borralho N (1996) Genetic analysis of survival in *Eucalyptus globulus* ssp. *globulus*. *Silvae Genetica* **45**, 107-112.

Charlesworth D, Charlesworth B (1987) Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics* **18**, 237-268.

Charlesworth D, Charlesworth B (1995) Quantitative genetics in plants: The effect of the breeding system on genetic variability. *Evolution* **49**, 911-920.

Charlesworth D, Willis JH (2009) The genetics of inbreeding depression. *Nature Reviews Genetics* **10**, 783-796.

Chen Z-Q, Karlsson B, Wu HX (2017) Patterns of additive genotype-by-environment interaction in tree height of Norway spruce in southern and central Sweden. *Tree Genetics & Genomes* **13**, 25.

Close DC (2012) A review of ecophysiologically-based seedling specifications for temperate Australian eucalypt plantations. *New Forests* **43**, 739-753.

Cockerham CC, Weir BS (1977) Quadratic analyses of reciprocal crosses. *Biometrics* **33**, 187-203.

Collins S, Callister A (2010) Genetic and environmental influences on capsule retention following controlled pollination of *Eucalyptus globules* flowers. *Australian forestry* **73**, 198-203.

Cook BI, Smerdon JE, Seager R, Coats S (2014) Global warming and 21st century drying. *Climate Dynamics* **43**, 2607-2627.

Coppen JJ (2002) 'Eucalyptus: the genus Eucalyptus.' (Taylor & Francis: London).

Costa e Silva J, Borralho NMG, Araujo JA, Vaillancourt RE, Potts BM (2009) Genetic parameters for growth, wood density and pulp yield in *Eucalyptus globulus*. *Tree Genetics & Genomes* **5**, 291-305.

Costa e Silva J, Borralho NMG, Potts BM (2004) Additive and non-additive genetic parameters from clonally replicated and seedling progenies of *Eucalyptus globulus*. *Theoretical and Applied Genetics* **108**, 1113-1119.

Costa e Silva J, Hardner C, Potts BM (2010a) Genetic variation and parental performance under inbreeding for growth in *Eucalyptus globulus*. *Annals of Forest Science* **67**, 606p1-606p8.

Costa e Silva J, Hardner C, Tilyard P, Pires AM, Potts BM (2010b) Effects of inbreeding on population mean performance and observational variances in *Eucalyptus globulus*. *Annals of Forest Science* **67**, 605p1-605p9.

Costa e Silva J, Hardner C, Tilyard P, Potts BM (2011) The effects of age and environment on the expression of inbreeding depression in *Eucalyptus globulus*. *Heredity* **107**, 50-60.

Costa e Silva J, Potts BM, Bijma P, Kerr RJ, Pilbeam DJ (2013a) Genetic control of interactions among individuals: contrasting outcomes of indirect genetic effects arising from neighbour disease infection and competition in a forest tree. *New Phytologist* **197**, 631-641.

Costa e Silva J, Potts BM, Dutkowski GW (2006) Genotype by environment interaction for growth of *Eucalyptus globulus* in Australia. *Tree Genetics & Genomes* **2**, 61-75.

Costa e Silva J, Potts BM, Gilmour AR, Kerr RJ (2017) Genetic-based interactions among tree neighbors: identification of the most influential neighbors, and estimation of correlations among direct and indirect genetic effects for leaf disease and growth in *Eucalyptus globulus*. *Heredity* **119**, 125-135.

Costa e Silva J, Potts BM, Harrison PA (2020) Population divergence along a genetic line of least resistance in the tree species *Eucalyptus globulus*. *Genes* **11**, 1095.

Costa e Silva J, Potts BM, Lopez GA (2014) Heterosis may result in selection favouring the products of long-distance pollen dispersal in *Eucalyptus*. *PloS one* **9**, e93811.

Costa e Silva J, Potts BM, Tilyard P (2012) Epistasis causes outbreeding depression in eucalypt hybrids. *Tree genetics & genomes* **8**, 249-265.

Costa e Silva J, Potts BM, Tilyard P (2013b) Stability of genetic effects across clonal and seedling populations of Eucalyptus globulus with common parentage. *Forest Ecology and Management* **291**, 427-435.

Costello LR, Quarles SL (1999) Detection of wood decay in blue gum and elm: an evaluation of the Resistograph and the portable drill. *Journal of Arboriculture* **25**, 311-318.

Cotterill P, Macrae S (1997) Improving *Eucalyptus* pulp and paper quality using genetic selection and good organization. *Tappi Journal* **80**, 82-89.

Cotterill PP, Dean CA (1990) 'Successful tree breeding with index selection.' (CSIRO, Division of Forestry and Forest Products: Australia).

Crawley MJ (2012) 'The R book.' (John Wiley & Sons: UK).

Davidson N, Reid J (1980) Comparison of the early growth characteristics of the *Eucalyptus* subgenera *Monocalyptus* and *Symphyomyrtus*. *Australian Journal of Botany* **28**, 453-461.

Davidson N, Reid J (1985) Frost as a factor influencing the growth and distribution of subalpine eucalypts. *Australian Journal of Botany* **33**, 657-667.

Davidson N, Reid J (1989) Response of eucalypt species to drought. *Austral Ecology* **14**, 139-156.

Davies NT, Apiolaza LA, Sharma M (2017) Heritability of growth strain in *Eucalyptus bosistoana*: a Bayesian approach with left-censored data. *New Zealand Journal of Forestry Science* **47**, 5.

de Assis TF (2000) Production and use of *Eucalyptus* hybrids for industrial purposes. In 'Hybrid breeding and genetics of forest trees. Proceedings of QFRI/CRC-SPF Symposium. ' pp. 63-74. (Queensland Forestry Research Institute, Department of Primary Industries: Noosa, Australia)

Dean G, French J, Tibbits W (1990) Variation in pulp and papermaking characteristics in a field trial of *Eucalyptus globulus*. In '44th Appita Annual General Conference. ' (Australian Pulp and Paper Industry Technical Association: Rotorua, New Zealand)

Denis M, Bouvet J-M (2013) Efficiency of genomic selection with models including dominance effect in the context of *Eucalyptus* breeding. *Tree Genetics & Genomes* **9**, 37-51.

Derikvand M, Nolan G, Jiao H, Kotlarewski N (2016) What to do with structurally low-grade wood from Australia's plantation *Eucalyptus*; building application? *BioResources* **12**, 4-7.

Dhir N, Mohn C (1976) A comparative study of crosses between and within two geographically diverse sources of eastern cottonwood. *Canadian Journal of Forest Research* **6**, 400-405.

Dickson RL, Raymond CA, Joe W, Wilkinson CA (2003) Segregation of *Eucalyptus dunnii* logs using acoustics. *Forest Ecology and Management* **179**, 243-251.

Dong L, Xie Y, Sun X (2019) Full-diallel-based analysis of genetic parameters for growth traits in Japanese larch (Larix kaempferi). *New Forests*.

Downes G, Meder R, Bond H, Ebdon N, Hicks C, Harwood C (2011) Measurement of cellulose content, Kraft pulp yield and basic density in eucalypt woodmeal using multisite and multispecies near infra-red spectroscopic calibrations. *Southern Forests* **73**, 181-186.

Downes G, Worledge D, Schimleck L, Harwood C, French J, Beadle C (2006) The effect of growth rate and irrigation on the basic density and kraft pulp yield of *Eucalyptus globulus* and *E. nitens*. *New Zealand journal of forestry* **51**, 13.

Downes GM, Hudson I, Raymond CA, Dean GH, Michell AJ, Schimleck LR, Evans R, Muneri A (1997) 'Sampling plantation eucalypts for wood and fibre properties.' (CSIRO: Victoria, Australia).

Downes GM, Lausberg M, Potts B, Pilbeam D, Bird M, Bradshaw B (2018) Application of the IML Resistograph to the infield assessment of basic density in plantation eucalypts. *Australian Forestry* **81**, 177-185.

Downes GM, Meder R, Hicks C, Ebdon N (2009) Developing and evaluating a multisite and multispecies NIR calibration for the prediction of Kraft pulp yield in eucalypts. *Southern Forests* **71**, 155-164.

Downham R, Gavran M (2017) Australian plantation statistics 2017 update. Australian Bureau of Agricultural and Resources Economics and Sciences, Canberra.

Downham R, Gavran M (2019) Australian plantation statistics 2019 update. Canberra, Australia.

Droogers P, Allen RG (2002) Estimating reference evapotranspiration under inaccurate data conditions. *Irrigation and Drainage Systems* **16**, 33-45.

Dungey H, Potts B, Carnegie A, Ades P (1997) Mycosphaerella leaf disease: genetic variation in damage to *Eucalyptus nitens*, *Eucalyptus globulus*, and their F1 hybrid. *Canadian Journal of Forest Research* **27**, 750-759.

Dungey HS (2001) Pine hybrids-a review of their use performance and genetics. *Forest ecology and management* **148**, 243-258.

Dutkowski GW, Potts BM (1999) Geographic patterns of genetic variation in *Eucalyptus globulus* ssp. *globulus* and a revised racial classification. *Australian Journal of Botany* **47**, 237.

Dutkowski GW, Potts BM (2011) Drought tolerant *Eucalyptus globulus* provenances have thick bark, high basic density and grow poorly on wet sites. In 'Improvement and culture of eucalypts. ' pp. 582. (Escola Superior de Agricultura “Luiz de Queiroz”: Porto Seguro, Bahia State, Brazil)

Dutkowski GW, Potts BM (2012) Genetic variation in the susceptibility of *Eucalyptus globulus* to drought damage. *Tree Genetics & Genomes* **8**, 757-773.

Eckert C, Samis K, Lougheed S (2008) Genetic variation across species’ geographical ranges: the central–marginal hypothesis and beyond. *Molecular ecology* **17**, 1170-1188.

Edelaar P, Björklund M (2011) If F_{ST} does not measure neutral genetic differentiation, then comparing it with Q_{ST} is misleading. Or is it? *Molecular Ecology* **20**, 1805-1812.

Edmands S (1999) Heterosis and outbreeding depression in interpopulation crosses spanning a wide range of divergence. *Evolution* **53**, 1757-1768.

Edmands S (2007) Between a rock and a hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management. *Molecular ecology* **16**, 463-475.

El-Dien OG, Ratcliffe B, Klápště J, Porth I, Chen C, El-Kassaby YA (2016) Implementation of the realized genomic relationship matrix to open-pollinated white spruce family testing for disentangling additive from nonadditive genetic effects. *G3: Genes, Genomes, Genetics* **6**, 743-753.

Eldridge KG, Davidson J, Harwood C, Wyk Gv (1993) 'Eucalypt domestication and breeding.' (Oxford University press Inc.: New York).

Ellstrand NC, Elam DR (1993) Population genetic consequences of small population size: implications for plant conservation. *Annual review of Ecology and Systematics* **24**, 217-242.

Evans R, Ilic J (2001) Rapid prediction of wood stiffness from microfibril angle and density. *Forest Products Journal* **51**, 53-57.

Falconer DS (1952) The problem of environment and selection. *The American Naturalist* **86**, 293-298.

Falconer DS, Mackay TFC (1996) 'Introduction to quantitative genetics.' 4th edn. (Longman group Ltd.: Essex, England).

FAO (2010) Global forest resources assessment 2010. Main report. Food and Agriculture Organization of the United Nations, Rome.

Farrell R, Innes TC, Harwood CE (2012) Sorting *Eucalyptus nitens* plantation logs using acoustic wave velocity. *Australian Forestry* **75**, 22-30.

Fenster CB, Galloway LF (2000) Inbreeding and Outbreeding Depression in Natural Populations of *Chamaecrista fasciculata* (Fabaceae). *Conservation Biology* **14**, 1406-1412.

Florence RG (2004) 'Ecology and silviculture of eucalypt forests.' (CSIRO publishing: Collingwood, Victoria, Australia).

Foelkel C (2009) 'Papermaking properties of *Eucalyptus* trees, woods, and pulp fibers.' In *Eucalyptus* Online Book & Newsletter Available at http://www.eucalyptus.com.br/eucaliptos/ENG14.pdf

Foster G, Shaw D (1988) Using clonal replicates to explore genetic variation in a perennial plant species. *Theoretical and Applied Genetics* **76**, 788-794.

Fox CW, Reed DH (2011) Inbreeding depression increases with environmental stress: an experimental study and meta-analysis. *Evolution* **65**, 246-258.

Frankham R (2015) Genetic rescue of small inbred populations: Meta‐analysis reveals large and consistent benefits of gene flow. *Molecular ecology* **24**, 2610-2618.

Frankham R, Ballou JD, Eldridge MD, Lacy RC, Ralls K, Dudash MR, Fenster CB (2011) Predicting the probability of outbreeding depression. *Conservation Biology* **25**, 465-475.

Freeman JS, Potts BM, Downes GM, Pilbeam D, Thavamanikumar S, Vaillancourt RE (2013) Stability of quantitative trait loci for growth and wood properties across multiple pedigrees and environments in *Eucalyptus globulus*. *New Phytologist* **198**, 1121-1134.

Fundova I, Funda T, Wu HX (2018) Non-destructive wood density assessment of Scots pine (*Pinus sylvestris* L.) using Resistograph and Pilodyn. *PLOS ONE* **13**, e0204518.

Gardiner C (1989) Collaborative seed collections of *Eucalyptus globulus* subsp. *globulus* Labill for tree improvement purposes. CSIRO division of forestry and forest products, Australia.

Gaspar MJ, Borralho N, Gomes AL (2005) Comparison between field performance of cuttings and seedlings of *Eucalyptus globulus*. *Annals of forest science* **62**, 837-841.

Gauli A, Vaillancourt RE, Bailey TG, Steane DA, Potts BM (2015) Evidence for local climate adaptation in early-life traits of Tasmanian populations of *Eucalyptus pauciflora*. *Tree Genetics & Genomes* **11**, 104.

Gill RMA (1992) A review of damage by mammals in north temperate forests: 1. Deer. *Forestry* **65**, 145-169.

Gilmour A, Gogel B, Cullis B, Welham S, Thompson R (2015a) 'ASReml user guide ' (VSN International Ltd: Hemel Hempstead UK).

Gilmour A, Gogel B, Cullis B, Welham S, Thompson R (2015b) 'ASReml user guide release 4.1 functional specification.' (VSN International Ltd: Hemel Hempstead, UK).

Ginwal HS (2010) Inbreeding depression in *Eucalyptus tereticornis* Sm. due to cleistogamous flowering. *New Forests* **40**, 205-212.

González-Orozco CE, Pollock LJ*, et al.* (2016) Phylogenetic approaches reveal biodiversity threats under climate change. *Nature Climate Change* **6**, 1110-1114.

Goodwillie C, Kalisz S, Eckert CG (2005) The evolutionary enigma of mixed mating systems in plants: occurrence, theoretical explanations, and empirical evidence. *Annual Review of Ecology, Evolution, and Systematics* **36**, 47-79.

Gore P, Potts B (1995) The genetic control of flowering time in *Eucalyptus globulus*, *E. nitens* and their F1 hybrids. In 'Eucalypt Plantations: Improving Fibre Yield and Quality', 1995, Hobart, Tasmania. (Eds BM Potts, Borralho NMG, Reid JB, Cromer RN, T WN and R CA), pp. 241-242

Goto S, Iijima H, Ogawa H, Ohya K (2011) Outbreeding depression caused by intraspecific hybridization between local and nonlocal genotypes in *Abies sachalinensis*. *Restoration Ecology* **19**, 243-250.

Grattapaglia D, Vaillancourt RE, Shepherd M, Thumma BR, Foley W, Külheim C, Potts BM, Myburg AA (2012) Progress in Myrtaceae genetics and genomics: *Eucalyptus* as the pivotal genus. *Tree Genetics & Genomes* **8**, 463-508.

Greaves BL, Borralho NM, Raymond CA (1997) Breeding objective for plantation eucalypts grown for production of kraft pulp. *Forest Science* **43**, 465-472.

Greaves BL, Hamilton MG, Pilbeam D, Dutkowski GW (2004) Genetic variation in commercial properties of six and fifteen year-old *Eucalyptus globulus*. In '*Eucalyptus* in a Changing World. Proceedings of the IUFRO Conference. ' (Eds NMG Borralho, JS Pereira, C Marques, J Coutinho, M Madeira and M Tome): RAIZ, Instituto Investigac;ao da Floresta e Papel, Aveiro, Portugal)

Greenwood S, Ruiz‐Benito P*, et al.* (2017) Tree mortality across biomes is promoted by drought intensity, lower wood density and higher specific leaf area. *Ecology Letters* **20**, 539-553.

Griffin A (2014) Clones or improved seedlings of *Eucalyptus*? Not a simple choice. *International Forestry Review* **16**, 216-224.

Griffin A, Cotterill P (1988) Genetic variation in growth of outcrossed, selfed and open-pollinated progenies of *Eucalyptus regnans* and some implications for breeding strategy. *Silvae Genet* **37**, 124-131.

Griffin AR, Potts BM, Vaillancourt RE, Bell JC (2019) Life cycle expression of inbreeding depression in *Eucalyptus regnans* and inter-generational stability of its mixed mating system. *Annals of Botany* **124**, 179-187.

Grindeland JM (2008) Inbreeding depression and outbreeding depression in *Digitalis purpurea*: optimal outcrossing distance in a tetraploid. *Journal of Evolutionary Biology* **21**, 716-726.

Grossnickle SC (2012) Why seedlings survive: influence of plant attributes. *New Forests* **43**, 711-738.

Hacke UG, Sperry JS, Pockman WT, Davis SD, McCulloh KA (2001) Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. *Oecologia* **126**, 457-461.

Hagemann R (2004) The sexual inheritance of plant organelles. In 'Molecular biology and biotechnology of plant organelles. (Eds H Daniell and C Chase) pp. 93-113. (Springer: Dordrecht, Netherlands).

Hai PH, Duong LA, Toan NQ, Ha TTT (2015) Genetic variation in growth, stem straightness, pilodyn and dynamic modulus of elasticity in second-generation progeny tests of *Acacia mangium* at three sites in Vietnam. *New Forests* **46**, 577-591.

Hallander J, Waldmann P (2009) Optimum contribution selection in large general tree breeding populations with an application to Scots pine. *Theoretical and Applied Genetics* **118**, 1133-1142.

Hamilton JA, Royauté R, Wright JW, Hodgskiss P, Ledig FT (2017a) Genetic conservation and management of the California endemic, Torrey pine (*Pinus torreyana* Parry): Implications of genetic rescue in a genetically depauperate species. *Ecology and evolution* **7**, 7370-7381.

Hamilton M, Potts B (2008) *Eucalyptus nitens* genetic parameters. *New Zealand Journal of Forestry Science* **38**, 102-119.

Hamilton MG, Acuna M, Wiedemann JC, Mitchell R, Pilbeam DJ, Brown MW, Potts BM (2015a) Genetic control of *Eucalyptus globulus* harvest traits. *Canadian Journal of Forest Research* **45**, 615-624.

Hamilton MG, Blackburn DP, McGavin RL, Baillères H, Vega M, Potts BM (2015b) Factors affecting log traits and green rotary-peeled veneer recovery from temperate eucalypt plantations. *Annals of Forest Science* **72**, 357-365.

Hamilton MG, Freeman JS, Blackburn DP, Downes GM, Pilbeam DJ, Potts BM (2017b) Independent lines of evidence of a genetic relationship between acoustic wave velocity and kraft pulp yield in *Eucalyptus globulus*. *Annals of Forest Science* **74**, 10.

Hamilton MG, Greaves BL, Potts BM, Dutkowski GW (2007) Patterns of longitudinal within-tree variation in pulpwood and solidwood traits differ among *Eucalyptus globulus* genotypes. *Annals of Forest Science* **64**, 831-837.

Hamilton MG, Harwood CE, Potts BM (2009a) The effects of drying temperature and method of assessment on the expression of genetic variation in gross shrinkage of *Eucalyptus globulus* wood samples. *Silvae Genetica* **58**, 252-261.

Hamilton MG, Potts BM, Greaves BL, Dutkowski GW (2010) Genetic correlations between pulpwood and solid-wood selection and objective traits in *Eucalyptus globulus*. *Annals of Forest Science* **67**, 10.

Hamilton MG, Raymond CA, Harwood CE, Potts BM (2009b) Genetic variation in *Eucalyptus nitens* pulpwood and wood shrinkage traits. *Tree Genetics & Genomes* **5**, 307-316.

Hamilton MG, Williams DR, Tilyard PA, Pinkard EA, Wardlaw TJ, Glen M, Vaillancourt RE, Potts BM (2013) A latitudinal cline in disease resistance of a host tree. *Heredity* **110**, 372-379.

Hampe A, Petit RJ (2005) Conserving biodiversity under climate change: the rear edge matters. *Ecology Letters* **8**, 461-467.

Hamzeh M, Sawchyn C, Perinet P, Dayanandan S (2007) Asymmetrical natural hybridization between *Populus deltoides* and *P. balsamifera* (Salicaceae). *Botany* **85**, 1227-1232.

Hannrup B, Cahalan C*, et al.* (2004) Genetic parameters of growth and wood quality traits in *Picea abies*. *Scandinavian Journal of Forest Research* **19**, 14-29.

Harbard J, Griffin R, Espejo J, Centurion C, Russell J (2000) One stop pollination - a new technology developed by Shell Forestry technology unit. In 'Proceedings of QFRI/CRC–SPF Symposium: Hybrid Breeding and Genetics of Forest Trees', 9-4 April 2000, pp. 430-434. (Brisbane).

Hardner C, Tibbits W (1998) Inbreeding depression for growth, wood and fecundity traits in *Eucalyptus nitens*. *Forest Genetics* **5**, 11-20.

Hardner CM, Potts BM (1995) Inbreeding depression and changes in variation after selfing in *Eucalyptus globulus* ssp. *globulus*. *Silvae Genetica* **44**, 46-54.

Hardner CM, Potts BM (1997) Postdispersal selection following mixed mating in *Eucalyptus regnans*. *Evolution* **51**, 103-111.

Hardner CM, Potts BM, Gore PL (1998) The relationship between cross success and spatial proximity of *Eucalyptus globulus* ssp. *globulus* parents. *Evolution* **52**, 614-618.

Hardner CM, Vaillancourt RE, Potts BM (1996) Stand density influences outcrossing rate and growth of open-pollinated families of *Eucalyptus globulus*. *Silvae Genetica* **45**, 226-228.

Harfouche A, Bahrman N, Baradat P, Guyon JP, Petit RJ, Kremer A (2000) Provenance hybridization in a diallel mating scheme of maritime pine (*Pinus pinaster*). II. Heterosis. *Canadian journal of forest research* **30**, 10-16.

Harfouche A, Baradat P, Durel C (1995a) Intraspecific variability in maritime pine (*Pinus pinaster* Ait) in the south-east of France. 1. Variability in autochthonous populations and in the whole range of the species. *Annales des Sciences Forestieres (France)* **52**, 307-328.

Harfouche A, Baradat P, Kremer A (1995b) Intraspecific variability in maritime pine (*Pinus pinaster* Ait) in the south-east of France. 2. Heterosis and combination of characters in interracial hybrids. *Annales des Sciences Forestieres (France)* **52**, 329-346.

Harfouche A, Kremer A (2000) Provenance hybridization in a diallel mating scheme of maritime pine (*Pinus pinaster*). I. Means and variance components. *Canadian journal of forest research* **30**, 1-9.

Harfouche A, Meilan R, Kirst M, Morgante M, Boerjan W, Sabatti M, Scarascia Mugnozza G (2012) Accelerating the domestication of forest trees in a changing world. *Trends in Plant Science* **17**, 64-72.

Hargreaves GH (1994) Defining and using reference evapotranspiration. *Journal of Irrigation and Drainage Engineering* **120**, 1132-1139.

Hart PW, Santos RB (2015) Changing the face of short fiber - A review of the *Eucalyptus* revolution. *Tappi Journal* **14**, 353-359.

Harwood C (2011) New introductions-doing it right. In 'Developing a eucalypt resource: learning from Australia and elsewhere. ' Ed. J Walker) pp. 43-54: Wood Technology Research Centre, University of Canterbury, Christchurch, New Zealand)

He Y, Li Z (2018) Epigenetic environmental memories in plants: establishment, maintenance, and reprogramming. *Trends in Genetics* **34**, 856-866.

Hedrick PW, Garcia-Dorado A (2016) Understanding inbreeding depression, purging, and genetic rescue. *Trends in Ecology & Evolution* **31**, 940-952.

Hedrick PW, Hellsten U, Grattapaglia D (2016) Examining the cause of high inbreeding depression: analysis of whole-genome sequence data in 28 selfed progeny of *Eucalyptus grandis*. *New Phytologist* **209**, 600-611.

Hickey J, Kostoglou P, Sargison G (2000) Tasmania's tallest trees. *Tasforests* **12**, 105-122.

Hill WG, Goddard ME, Visscher PM (2008) Data and theory point to mainly additive genetic variance for complex traits. *PLoS genetics* **4**, e1000008.

Hingston AB, Potts BM, McQuillan PB (2004) The swift parrot, *Lathamus discolor* (Psittacidae), social bees (Apidae) and native insects as pollinators of *Eucalyptus globulus* ssp *globulus* (Myrtaceae). *Australian Journal of Botany* **52**, 371-379.

Hodge GR, Volker PW, Potts BM, Owen JV (1996) A comparison of genetic information from open-pollinated and control-pollinated progeny tests in two eucalypt species. *Theoretical and Applied Genetics* **92**, 53-63.

Holeski LM, Jander G, Agrawal AA (2012) Transgenerational defense induction and epigenetic inheritance in plants. *Trends in ecology & evolution* **27**, 618-626.

Hothorn T, Bretz F, Hothorn MT (2009) The multcomp package. Technical Report 1.0-6, The R Project for Statistical Computing, www. r-project. org, Version 1.0.143.

Hu Y, Thomas BR (2019) Hormones and Heterosis in Hybrid Balsam Poplar (*Populus balsamifera* L.). *Forests* **10**, 143.

Husband BC, Schemske DW (1996) Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution* **50**, 54-70.

Husband BC, Schemske DW (1997) The effect of inbreeding in diploid and tetraploid populations of *Epilobium angustifolium* (*onagraceae*): Implications for the genetic basis of inbreeding depression. *Evolution* **51**, 737-746.

Husch B, Beers TW, Kershaw Jr JA (2002) 'Forest mensuration.' 4 edn. (John Wiley & Sons: Hoboken, New Jersy).

Ingvarsson PK, Dahlberg H (2019) The effects of clonal forestry on genetic diversity in wild and domesticated stands of forest trees. *Scandinavian Journal of Forest Research* **34**, 370-379.

Isik F, Holland J, Maltecca C (2017) 'Genetic data analysis for plant and animal breeding.' (Springer International Publishing AG: Cham, Switzerland).

Isik F, Li B (2003) Rapid assessment of wood density of live trees using the Resistograph for selection in tree improvement programs. *Canadian Journal of Forest Research* **33**, 2426-2435.

Isik F, Li B, Frampton J (2003) Estimates of additive, dominance and epistatic genetic variances from a clonally replicated test of loblolly pine. *Forest Science* **49**, 77-88.

Jansson G, Li B (2004) Genetic gains of full-sib families from disconnected diallels in loblolly pine. *Silvae genetica* **53**, 60-64.

Jarvis S, Borralho N, Potts B (1995) Implementation of a multivariate BLUP model for genetic evaluation of *Eucalyptus globulus* in Australia. In 'CRC for Temperate Hardwood Forestry IUFRO '. (Eds BM Potts, NMG Borralho, JB Reid, RN Cromer, WN Tibbits and CA Raymond) pp. 212-219: Hobart, Tasmania, Australia)

Johnston AJ (2001) Performance and genetics of provenance hybrids of *Pinus caribaea* var. *hondurensis* in Queensland. University of the Sunshine Coast, Queensland, Australia

Johnstone DM, Ades PK, Moore GM, Smith IW (2007) Predicting wood decay in eucalypts using an expert system and the IML-Resistograph drill. *Arboriculture and Urban Forestry* **33**, 76.

Jonas E, de Koning D-J (2013) Does genomic selection have a future in plant breeding? *Trends in Biotechnology* **31**, 497-504.

Jones DA, Wang W, Fawcett R (2009) High-quality spatial climate data-sets for Australia. *Australian Meteorological and Oceanographic Journal* **58**, 233.

Jones RC, Steane DA, Lavery M, Vaillancourt RE, Potts BM (2012) Multiple evolutionary processes drive the patterns of genetic differentiation in a forest tree species complex. *Ecology and Evolution* **3**, 1-17.

Jones RC, Steane DA, Potts BM, Vaillancourt RE (2002) Microsatellite and morphological analysis of *Eucalyptus globulus* populations. *Canadian Journal of Forest Research* **32**, 59-66.

Jones RC, Vaillancourt RE, Gore PL, Potts BM (2011) Genetic control of flowering time in *Eucalyptus globulus* ssp. *globulus*. *Tree Genetics & Genomes* **7**, 1209-1218.

Jones TH, Vaillancourt RE, Potts BM (2007) Detection and visualization of spatial genetic structure in continuous *Eucalyptus globulus* forest. *Molecular Ecology* **16**, 697-707.

Jordan G, Potts B, Kirkpatrick J, Gardiner C (1993) Variation in the *Eucalyptus globulus* complex revisited. *Australian Journal of Botany* **41**, 763-785.

Jordan GJ, Potts BM, Clarke AR (2002) Susceptibility of *Eucalyptus globulus* ssp. *globulus* to sawfly (*Perga affinis* ssp. *insularis*) attack and its potential impact on plantation productivity. *Forest Ecology and Management* **160**, 189-199.

Jordan GJ, Potts BM, Wiltshire RJ (1999) Strong, independent, quantitative genetic control of the timing of vegetative phase change and first flowering in *Eucalyptus globulus* ssp*. globulus* (Tasmanian blue gum). *Heredity* **83**, 179-187.

Joseph AA, Dieters MJ, Dungey HS, Wallace HM, Nikles G (2000) Provenance hybridisation in *Pinus caribaea* var hondurensis in Queensland. In ''Hybrid Breeding and Genetics of Forest Trees. Proceedings of QFRI/CRC-SPF Symposium. ' (Eds DH S, DM J and ND G) pp. 153-159. (Department of Primary Industries, Brisbane: Noosa, Queensland, Australia)

Kahrood HV, Spokevicius AV, Rigault P, Hung A, Ades PK, Bossinger G, Tibbits JFG (2019) Adaptive evolution of cpDNA in Eucalyptus: rbcL, a pivotal gene, shows nucleotide signature of selection. In 'Eucalypt genetics: fundamental and applied research in a post-genome era. ' (Eds R Jones, D Steane, P Tilyard, R Vaillancourt, R Andrew, P Rymer and B Potts) pp. 12: University of Tasmania, Hobart, Tasmania)

Kardos M, Taylor HR, Ellegren H, Luikart G, Allendorf FW (2016) Genomics advances the study of inbreeding depression in the wild. *Evolutionary Applications* **9**, 1205-1218.

Kärkkäinen K, Koski V, Savolainen O (1996) Geographical variation in the inbreeding depression of Scots pine. *Evolution* **50**, 111-119.

Kerr R, Tier B, McRae T, Dutkowski G, Apiolaza L (2001) TREEPLAN® - a genetic evaluation system for forest tree improvement. In 'IUFRO Symposium on developing the eucalypt of the future. ' Valdivia, Chile)

Kien ND, Jansson G, Harwood C, Almqvist C, Thinh H (2008) Genetic variation in wood basic density and pilodyn penetration and their relationships with growth, stem straightness, and branch size for *Eucalyptus urophylla* in northern Vietnam. *New Zealand Journal of Forestry Science* **38**, 160-175.

Kirkpatrick J (1975) Geographical variation in *Eucalyptus globlus*. Australian Government Publishing Service, Canberra.

Kirkpatrick J, Marks F (1985) Observations on drought damage to some native plant species in eucalypt forests and woodlands near Hobart, Tasmania. In 'Papers and Proceedings of the Royal Society of Tasmania. Vol. 119'. pp. 15-21)

Klápště J, Lstibůrek M, El-Kassaby YA (2014) Estimates of genetic parameters and breeding values from western larch open-pollinated families using marker-based relationship. *Tree Genetics & Genomes* **10**, 241-249.

Koch GW, Sillett SC, Jennings GM, Davis SD (2004) The limits to tree height. *Nature* **428**, 851-854.

Koelewijn HP, Koski V, Savolainen O (1999) Magnitude and timing of inbreeding depression in Scots pine (*Pinus sylvestris* L.). *Evolution* **53**, 758-768.

Kremer A, Potts BM, Delzon S (2014) Genetic divergence in forest trees: understanding the consequences of climate change. *Functional Ecology* **28**, 22-36.

Kriebel HB (1954) Bark thickness as a factor in resistance to White Pine weevil injury. *Journal of Forestry* **52**, 842-5.

Kube P, Raymond C (2001) Genetic parameters for *Eucalyptus nitens* solid wood traits and relationships with pulpwood traits. In 'IUFRO International Symposium on Developing the Eucalypt of the Future', 2001, Valdivia, Chile, p. 184

Kube PD, Raymond CA (2005) Breeding to minimise the effects of collapse in *Eucalyptus nitens* sawn timber. *Forest Genetics* **12**, 23-34.

Kuznetsova A, Brockhoff PB, Christensen RHB (2015) lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software* **82**, 1-26.

Ladiges PY, Udovicic F, Nelson G (2003) Australian biogeographical connections and the phylogeny of large genera in the plant family Myrtaceae. *Journal of Biogeography* **30**, 989-998.

Lanuza-Garay A, Barrios H (2018) Host specificity and wood density-based host choice by longhorn beetles (Coleoptera: *Cerambycidae*) in a panamanian lowland rainforest. *The Coleopterists Bulletin* **72**, 590-597.

Larcombe MJ, Barbour RC, Vaillancourt RE, Potts BM (2014) Assessing the risk of exotic gene flow from *Eucalyptus globulus* plantations to native *E. ovata* forests. *Forest Ecology and Management* **312**, 193-202.

Latta RG (1998) Differentiation of allelic frequencies at quantitative trait loci affecting locally adaptive traits. *The American Naturalist* **151**, 283-292.

Lawes MJ, Adie H, Russell-Smith J, Murphy B, Midgley JJ (2011) How do small savanna trees avoid stem mortality by fire? The roles of stem diameter, height and bark thickness. *Ecosphere* **2**, 1-13.

Lawes MJ, Midgley JJ, Clarke PJ (2013) Costs and benefits of relative bark thickness in relation to fire damage: a savanna/forest contrast. *Journal of Ecology* **101**, 517-524.

Leimu R, Fischer M (2008) A meta-analysis of local adaptation in plants. *PloS one* **3**, e4010.

Leimu R, Vergeer P, Angeloni F, Ouborg N (2010) Habitat fragmentation, climate change, and inbreeding in plants. *Annals of the New York Academy of Sciences* **1195**, 84-98.

Leinonen T, McCairns RS, O'hara RB, Merilä J (2013) Q_{ST}–F_{ST} comparisons: evolutionary and ecological insights from genomic heterogeneity. *Nature Reviews Genetics* **14**, 179.

Lenoir J, Gegout JC, Marquet PA, de Ruffray P, Brisse H (2008) A significant upward shift in plant species optimum elevation during the 20th century. *Science* **320**, 1768-1771.

Lepoittevin C, Rousseau J-P, Guillemin A, Gauvrit C, Besson F, Hubert F, da Silva Perez D, Harvengt L, Plomion C (2011) Genetic parameters of growth, straightness and wood chemistry traits in *Pinus pinaster*. *Annals of Forest Science* **68**, 873-884.

Levin DA (2011) Mating system shifts on the trailing edge. *Annals of Botany* **109**, 613-620.

Lewin-Koh NJ (2010) maptools: Tools for reading and handling spatial objects, R package version 0.7-38. In 'http://cran.r-project.org/package=maptools. ')

Li CR, Weng QJ*, et al.* (2017a) Genetic parameters for growth and wood mechanical properties in *Eucalyptus cloeziana* F. Muell. *New Forests* **48**, 33-49.

Li Y, Dutkowski GW, Apiolaza LA, Pilbeam D, Costa e Silva J, Potts BM (2007) The genetic architecture of a *Eucalyptus globulus* full-sib breeding population in Australia. *Forest Genetics* **12**, 167-179.

Li Y, Suontama M, Burdon RD, Dungey HS (2017b) Genotype by environment interactions in forest tree breeding: review of methodology and perspectives on research and application. *Tree Genetics & Genomes* **13**.

Lindgren D, Wei R-P (1994) Effects of maternal environment on mortality and growth in young *Pinus sylvestris* in field trials. *Tree Physiology* **14**, 323-327.

Lippman ZB, Zamir D (2007) Heterosis: revisiting the magic. *Trends in genetics* **23**, 60-66.

Lonsdale W (1990) The self-thinning rule: dead or alive? *Ecology* **71**, 1373-1388.

López G, Cañas I, Ruiz F (2010) Vegetative propagation techniques and genetic improvement in *Eucalyptus globulus*. In '*Eucalyptus* Species Management, History, Status and Trends in Ethiopia', 15-17 September 2010, Addis Ababa, Ethiopia. (Eds Gil L, Tadesse W, Tolosana E and López R),

López GA, Potts B, Tilyard P (2000a) The performance of F1 hybrids between *Eucalyptus ovata* and *E. globulus* compared to inbred and outcrossed controls. In 'QFRI/CRC-SPF Symposium. ' pp. 457-461)

López GA, Potts BM, Dutkowski GW, Apiolaza LA, Gelid P (2002) Genetic variation and inter-trait correlations in *Eucalyptus globulus* base population trials in Argentina. *Forest Genetics* **9**, 217-231.

López GA, Potts BM, Dutkowski GW, Rodriguez Traverso J (2001) Quantitative genetics of *Eucalyptus globulus:* affinities of land race and native stand localities. *Silvae Genetica* **50**, 244-252.

López GA, Potts BM, Tilyard PA (2000b) F_1 hybrid inviability in *Eucalyptus*: the case of *E. ovata* x *E. globulus*. *Heredity* **85**, 242-250.

Lopez GA, Potts BM, Vaillancourt RE, Apiolaza LA (2003) Maternal and carryover effects on early growth of *Eucalyptus globulus*. *Canadian Journal of Forest Research* **33**, 2108-2115.

López GA, Potts BM, Vaillancourt RE, Apiolaza LA (2003) Maternal and carryover effects on early growth of *Eucalyptus globulus*. *Canadian Journal of Forest Research* **33**, 2108-2115.

Lynch M, Walsh B (1998) 'Genetics and analysis of quantitative traits.' (Sinauer Associates: Sunderland, Massachusetts, U.S.A.).

MacDonald A, Borralho N, Potts B (1997) Genetic variation for growth and wood density in *Eucalyptus globulus* ssp. *globulus* in Tasmania (Australia). *Silvae Genetica* **46**, 236-241.

MacDonald E, Mochan S, Connolly T (2009) Validation of a stem straightness scoring system for Sitka spruce (*Picea sitchensis* (Bong.) Carr.). *Forestry* **82**, 419-429.

Magnussen S, Yeatman C (1988) Height growth and survival of within-and between-provenance crosses in jack pine. *Canadian Journal of Forest Research* **18**, 1145-1151.

Mahmood K, Marcar NE, Naqvi MH, Arnold RJ, Crawford DF, Iqbal S, Aken KM (2003) Genetic variation in *Eucalyptus camaldulensis* Dehnh. for growth and stem straightness in a provenance–family trial on saltland in Pakistan. *Forest Ecology and Management* **176**, 405-416.

Martinsen GD, Whitham TG, Turek RJ, Keim P (2001) Hybrid populations selectively filter gene introgression between species. *Evolution* **55**, 1325-1335.

Matusick G, Ruthrof KX, Brouwers NC, Dell B, Hardy GSJ (2013) Sudden forest canopy collapse corresponding with extreme drought and heat in a mediterranean-type eucalypt forest in southwestern Australia. *European Journal of Forest Research* **132**, 497-510.

McGavin RL, Bailleres H, Hamilton M, Blackburn D, Vega M, Ozarska B (2015) Variation in rotary veneer recovery from Australian plantation *Eucalyptus globulus* and *Eucalyptus nitens*. *BioResources* **10**, 313-329.

McGavin RL, Bailleres H, Lane F, Fehrmann J, Ozarska B (2014) Veneer grade analysis of early to mid-rotation plantation *Eucalyptus* species in Australia. *BioResources* **9**, 6562-6581.

McGowen M, Potts B, Vaillancourt R, Gore P, Williams D, Pilbeam D (2004) The genetic control of sexual reproduction in *Eucalyptus globulus*. In '*Eucalyptus* in a Changing World. International IUFRO Conference', 11-15 October 2004, Aveiro, Portugal,

McGowen MH, Vaillancourt RE, Pilbeam DJ, Potts BM (2010) Sources of variation in self-incompatibility in the Australian forest tree, *Eucalyptus globulus*. *Annals of Botany* **105**, 737-745.

McKinnon GE, Vaillancourt RE, Tilyard PA, Potts BM (2001) Maternal inheritance of the chloroplast genome in *Eucalyptus globulus* and interspecific hybrids. *Genome* **44**, 831-835.

McRae T, Pilbeam D, Gore P, Dutkowski G, Apiolaza L (2001) Australian cooperative tree improvement strategy for Eucalyptus globulus. In 'IUFRO International symposium on developing the eucalypt of the future. ' pp. 184. (International Union of Forestry Research Organizations: Chile)

Meder R (2015) The magnitude of tree breeding and the role of near infrared spectroscopy. *NIR news* **26**, 8-10.

Meder R, Downes GM, Brawner JT, Ebdon N (2010) Understanding radial variation to aid development of methods for in-field NIR assessment of Kraft pulp yield. In '2010 TAPPI PEERS conference and 9th research forum on recycling '. Norfolk, Virginia, USA)

Meirmans PG, Hedrick PW (2011) Assessing population structure: F_{ST} and related measures. *Molecular ecology resources* **11**, 5-18.

Miguez-Soto B, Lopez-Villamor A, Fernandez-Lopez J (2016) Additive and non-additive genetic parameters for multipurpose traits in a clonally replicated incomplete factorial test of *Castanea spp*. *Tree Genetics & Genomes* **12**, 14.

Mihai G, Mirancea I, Duta C (2014) Variation of the quantitative traits in a progeny test of *Abies alba* (Mill.) at the nursery stage. *Silvae Genetica* **63**, 275-284.

Mimura M, Barbour RC, Potts BM, Vaillancourt RE, Watanabe KN (2009) Comparison of contemporary mating patterns in continuous and fragmented *Eucalyptus globulus* native forests. *Molecular Ecology* **18**, 4180-4192.

Miranda I, Almeida MH, Pereira H (2001) Provenance and site variation of wood density in *Eucalyptus globulus* Labill. at harvest age and its relation to a non-destructive early assessment. *Forest Ecology and Management* **149**, 235-240.

Misztal I (1997) Estimation of variance components with large-scale dominance models. *Journal of Dairy Science* **80**, 965-974.

Mitchell PJ, O'Grady AP, Hayes KR, Pinkard EA (2014) Exposure of trees to drought-induced die-off is defined by a common climatic threshold across different vegetation types. *Ecology and Evolution* **4**, 1088-1101.

Moore J, Gardiner B, Sellier D (2018) Tree mechanics and wind loading. In 'Plant Biomechanics. (Eds A Geitmann and J Gril) pp. 79-106. (Springer).

Mora F, Ballesta P, Serra N (2019) Bayesian analysis of growth, stem straightness and branching quality in full-sib families of *Eucalyptus globulus*. *Bragantia* **78**, 328-336.

Mora F, Serra N (2014) Bayesian estimation of genetic parameters for growth, stem straightness, and survival in *Eucalyptus globulus* on an Andean Foothill site. *Tree Genetics & Genomes* **10**, 711-719.

Mottet MJ, DeBlois J, Perron M (2015) High genetic variation and moderate to high values for genetic parameters of *Picea abies* resistance to *Pissodes strobi*. *Tree Genetics & Genomes* **11**, 11.

Muneri A, Raymond C (2000) Genetic parameters and genotype-by-environment interactions for basic density, pilodyn penetration and stem diameter in *Eucalyptus globulus*. *Forest Genetics* **7**, 317-328.

Muñoz PR, Resende MF, Gezan SA, Resende MDV, de los Campos G, Kirst M, Huber D, Peter GF (2014) Unraveling additive from nonadditive effects using genomic relationship matrices. *Genetics* **198**, 1759-1768.

Nabais C, Hansen JK, David-Schwartz R, Klisz M, López R, Rozenberg P (2018) The effect of climate on wood density: What provenance trials tell us? *Forest Ecology and Management* **408**, 148-156.

Namkoong G (1966) Inbreeding effects on estimation of genetic additive variance. *Forest Science* **12**, 8-13.

Namkoong G, Kang HC, Brouard JS (2012) 'Tree Breeding: Principles and Strategies.' (Springer-Verlag New York).

Nicolle D (2006) 'Eucalypts of Victoria and Tasmania.' (Bloomings Books: Melbourne, Australia).

Nicolle D (2019) Classification of the eucalypts (*Angophora, Corymbia* and *Eucalyptus*) *Version 4*.

Nolan G, Greaves B, Washusen R, Parsons M, Jennings S (2005) 'Eucalypt plantations for solid wood products in Australia-a review: 'If you don't prune it, we can't use it'.' (Forest and Wood Products Research and Development Corporation: Victoria, Australia).

O'Reilly-Wapstra JM, Miller AM, Hamilton MG, Williams D, Glancy-Dean N, Potts BM (2013) Chemical variation in a dominant tree species: population divergence, selection and genetic stability across environments. *PLoS One* **8**, e58416.

Oakley CG, Ågren J, Schemske D (2015) Heterosis and outbreeding depression in crosses between natural populations of *Arabidopsis thaliana*. *Heredity* **115**, 73.

Odoom FK (2001) Promotion of valuable hardwood plantations in the tropics. A global overview. Forestry Department Food and Agriculture Organization of the United Nations, Rome, Italy.

Otieno DO, Schmidt MWT, Adiku S, Tenhunen J (2005) Physiological and morphological responses to water stress in two *Acacia* species from contrasting habitats. *Tree Physiology* **25**, 361-371.

Owens JN, Colangeli AM, Morris SJ (1990) The effect of self-, cross-, and no pollination on ovule, embryo, seed, and cone development in western red cedar (*Thuja plicata*). *Canadian Journal of Forest Research* **20**, 66-75.

Patterson B, Gore P, Potts BM, Vaillancourt RE (2004a) Advances in pollination techniques for large-scale seed production in *Eucalyptus globulus*. *Australian Journal of Botany* **52**, 781-788.

Patterson B, Vaillancourt RE, Pilbeam DJ, Potts BM (2004b) Factors affecting variation in outcrossing rate in *Eucalyptus globulus*. *Australian Journal of Botany* **52**, 773-780.

Patterson B, Vaillancourt RE, Pilbeam DJ, Potts BM (2005) Factors affecting variation in outcrossing rate in *Eucalyptus globulus*. *Australian Journal of Botany* **52**, 773-780.

Patterson B, Vaillancourt RE, Potts BM (2001) Eucalypt seed collectors: beware of sampling seedlots from low in the canopy! *Australian Forestry* **64**, 139-142.

Paul AD, Foster GS, Caldwell T, McRae J (1997) Trends in genetic and environmental parameters for height, diameter, and volume in a multilocation clonal study with loblolly pine. *Forest Science* **43**, 87-98.

Peiman KS, Robinson BW (2017) Comparative analyses of phenotypic trait covariation within and among populations. *The American Naturalist* **190**, 451-468.

Petit RJ, Hampe A (2006) Some evolutionary consequences of being a tree. *Annual Review of Ecology, Evolution, and Systematics* **37**, 187-214.

Phillips N, Ryan M, Bond B, McDowell N, Hinckley T, Čermák J (2003) Reliance on stored water increases with tree size in three species in the Pacific Northwest. *Tree Physiology* **23**, 237-245.

Pinard MA, Huffman J (1997) Fire resistance and bark properties of trees in a seasonally dry forest in eastern Bolivia. *Journal of Tropical Ecology* **13**, 727-740.

Poke FS, Potts BM, Vaillancourt RE, Raymond CA (2006) Genetic parameters for lignin, extractives and decay in *Eucalyptus globulus*. *Annals of Forest Science* **63**, 813-821.

Poorter L, McNeil A, Hurtado VH, Prins HH, Putz FE (2014) Bark traits and life‐history strategies of tropical dry‐and moist forest trees. *Functional Ecology* **28**, 232-242.

Pot D, Chantre G, Rozenberg P, Rodrigues JC, Jones GL, Pereira H, Hannrup B, Cahalan C, Plomion C (2002) Genetic control of pulp and timber properties in maritime pine (*Pinus pinaster* Ait.). *Annals of Forest Science* **59**, 563-575.

Potts B, Volker P, Dungey H (1992) Barriers to the production of interspecific hybrids in *Eucalyptus*. In 'Hybrid Breeding and Genetics of Forest Trees. ' (Eds DHS D, DM J and ND G) pp. 200–211. (Department of Primary Industries, Brisbane: Noosa, Queensland, Australia)

Potts BM, Dungey HS (2004) Interspecific hybridization of *Eucalyptus*: Key issues for breeders and geneticists. *New Forests* **27**, 115-138.

Potts BM, Hamilton MG, Blackburn DP (2011) Genetics of Eucalypts: traps and opportunities. In 'Developing a eucalypt resource: Learning from Australia and elsewhere. ' Ed. J Walker) pp. 1-29: Wood Technology Research Centre, University of Canterbury, Canterbury)

Potts BM, Hamilton MG, Pilbeam DJ (2014) Mejoramiento genético de eucaliptos de zonas templadas en Australia [Genetic improvement of temperate eucalypts in Australia]. In 'Mejoramiento Genético de Eucaliptos de en Chile. ' (Eds R Ipinza, SA Barros, BC Gutiérrez and N Borralho) pp. 411- 443 (Original English version). (INFOR Instituto Forestal: Chile)

Potts BM, McGowen MH, Williams DR, Suitor S, Jones TH, Gore PL, Vaillancourt RE (2008) Advances in reproductive biology and seed production systems of *Eucalyptus*: the case of *Eucalyptus globulus*. *Southern Forests* **70**, 145-154.

Potts BM, Vaillancourt RE*, et al.* (2004) Exploration of the *Eucalyptus globulus* gene pool. In '*Eucalyptus* in a changing world. ' Ed. N Borralho): RAIZ - Instituto de Investigacao da Floresta e Papel, Aveiro, Portugal)

Potts BM, Volker PW, Tilyard P, Joyce K (2000) The genetics of hybridisation in the temperate Eucalyptus. In 'Hybrid Breeding and Genetics of Forest Trees. ' (Eds HS Dungey, MJ Dieters and DG Nikles) pp. 200-211. (Department of Primary Industries: Noosa, Queensland, Australia)

Potts BM, Wiltshire RJ (1997) Eucalypt genetics and genecology. *Eucalypt ecology: individuals to ecosystems. Cambridge University Press, Cambridge*, 56-91.

Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O, Kunin WE (2010) Global pollinator declines: trends, impacts and drivers. *Trends in Ecology & Evolution* **25**, 345-353.

Pound L, Patterson B, Wallwork M, Potts B, Sedgley M (2003) Pollen competition does not affect the success of self-pollination in *Eucalyptus globulus* (Myrtaceae). *Australian Journal of Botany* **51**, 189-195.

Prober S, Byrne M, McLean E, Steane D, Potts B, Vaillancourt R, Stock W (2015) Climate-adjusted provenancing: a strategy for climate-resilient ecological restoration. *Frontiers in Ecology and Evolution* **3**.

Prober SM, Potts BM*, et al.* (2016) Climate adaptation and ecological restoration in eucalypts. *Proceedings of the Royal Society of Victoria* **128**, 40-53.

Quilhó T, Pereira H (2001) Within and between-tree variation of bark content and wood density of Eucalyptus globulus in commercial plantations. *Iawa Journal* **22**, 255-265.

Quilhó T, Pereira H, Richter HG (2000) Within–tree variation in phloem cell dimensions and proportions in *Eucalyptus globulus*. **21**, 31.

R Core Team (2017) R: A language and environment for statistical computing. In. ' R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/)

R Core Team (2018) R: A Language and Environment for Statistical Computing. In. ' (ISBN 3-900051-07-0: R Foundation for Statistical Computing, Austria, 2015)

Rao H-x, Patterson B, Potts B, Vaillancourt R (2008) A microsatellite study on outcrossing rates and contamination in an *Eucalyptus globulus* breeding arboretum. *Journal of Forestry Research* **19**, 136-140.

Ratcliffe B, El-Dien OG, Cappa EP, Porth I, Klápště J, Chen C, El-Kassaby YA (2017) Single-step BLUP with varying genotyping effort in open-pollinated *Picea glauca*. *G3: Genes, Genomes, Genetics* **7**, 935-942.

Raymond CA (2000) Tree breeding issues for solid wood production. In 'IUFRO Conference The future of eucalypts for solid wood products. ' pp. 265-270. (Forest Industries Association of Tasmania: Launceston, Australia)

Raymond CA (2002) Genetics of *Eucalyptus* wood properties. *Annals of Forest Science* **59**, 525-531.

Raymond CA, Apiolaza LA (2004) Incorporating wood quality and deployment traits in *Eucalyptus globulus* and *Eucalyptus nitens*. In 'Plantation forest biotechnology for the 21st Century. Vol. 5'. (Eds C Walter and M Carson) pp. 87-89. (Research Signpost: Trivandrum, Kerala, India)

Raymond CA, Schimleck LR, Muneri A, Michel A (2001) Genetic parameters and genotype-by-environment interactions for pulp-yield predicted using near infrared reflectance analysis and pulp productivity in *Eucalyptus globulus*. *Forest Genetics* **8**, 213-214.

Retief E, Stanger T (2009) Genetic control of wood density and bark thickness, and their correlations with diameter, in pure and hybrid populations of *Eucalyptus grandis* and *E. urophylla* in South Africa. *Southern Forests* **71**, 147-153.

Rezende GDSP, Resende MDV, Assis TF (2014) *Eucalyptus* Breeding for Clonal Forestry. In 'Challenges and Opportunities for the World's Forests in the 21st Century. (Ed. T Fenning) pp. 393-424. (Springer: Dordrecht, Netherlands).

Rhys D, Mijo G (2018) Australian plantation statistics 2018 update. Australian Bureau of Agricultural and Resource Economics and Sciences, Canberra, Australia.

Ribeiro MM, Sanchez L, Ribeiro C, Cunha F, Araujo J, Borralho NMG, Marques C (2011) A case study of *Eucalyptus globulus* fingerprinting for breeding. *Annals of Forest Science* **68**, 701-714.

Rix KD, Gracie AJ, Potts BM, Brown PH, Spurr CJ, Gore PL (2012) Paternal and maternal effects on the response of seed germination to high temperatures in *Eucalyptus globulus*. *Annals of Forest Science* **69**, 673-679.

Roach DA, Wulff RD (1987) Maternal effects in plants. *Annual review of ecology and systematics* **18**, 209-235.

Robertson A (1959) The sampling variance of the genetic correlation coefficient. *Biometrics* **15**, 469-485.

Roff DA (2002) Inbreeding depression: tests of the overdominance and partial dominance hypotheses. *Evolution* **56**, 768-75.

Rosell JA (2016) Bark thickness across the angiosperms: more than just fire. *New Phytologist* **211**, 90-102.

Rosell JA, Gleason S, Méndez-Alonzo R, Chang Y, Westoby M (2014) Bark functional ecology: evidence for tradeoffs, functional coordination, and environment producing bark diversity. *New Phytologist* **201**, 486-497.

Rossiter M (1996) Incidence and consequences of inherited environmental effects. *Annual review of ecology and systematics* **27**, 451-476.

Ruiz Diaz Britez M, Sergent A-S, Martinez Meier A, Bréda N, Rozenberg P (2014) Wood density proxies of adaptive traits linked with resistance to drought in Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco). *Trees* **28**, 1289-1304.

Russell JH, Costa e Silva J, Baltunis BS (2015) Multisite genetic parameter estimates from a Callitropsis nootkatensis diallel study with clonally replicated progeny. *Canadian Journal of Forest Research* **45**, 689-697.

Salas M, Nieto V, Perafán L, Sánchez A, Borralho NMG (2014) Genetic parameters and comparison between native and local landraces of *Eucalyptus globulus* Labill. ssp. *globulus* growing in the central highlands of Colombia. *Annals of Forest Science* **71**, 405-414.

Santini NS, Cleverly J, Faux R, Lestrange C, Rumman R, Eamus D (2016) Xylem traits and water-use efficiency of woody species co-occurring in the Ti Tree Basin arid zone. *Trees* **30**, 295.

Santos PETd, Geraldi IO, Garcia JN (2004) Estimates of genetic parameters of wood traits for sawn timber production in *Eucalyptus grandis*. *Genetics and Molecular Biology* **27**, 567-573.

Schmidtling R, Nelson C (1996) Interprovenance crosses in loblolly pine using selected parents. *Forest Genetics* **3**, 53-66.

Sharapov E, Brischke C, Militz H, Smirnova E (2019) Prediction of modulus of elasticity in static bending and density of wood at different moisture contents and feed rates by drilling resistance measurements. *European Journal of Wood and Wood Products* **77**, 833–842.

Silva CESd, Xavier CN, Santos PVd, Carvalho AMd, Latorraca JVdF, Brancalion PHS (2017) Estimation of wood density in living trees of *Cedrela fissilis Vell*. through resistographic analysis. *Scientia Forestalis* **45**, 139-144.

Skabo S, Vaillancourt RE, Potts BM (1998) Fine-scale genetic structure of *Eucalyptus globulus* ssp. g*lobulus* forest revealed by RAPDs. *Australian Journal of Botany* **46**, 583-594.

Smith DM (1954) Maximum moisture content method for determining specific gravity of small wood samples. United States Department of Agriculture, Forest Service,Report Book No. For. Prod. Lab. Report No. 2014, Madison, Wisconsin.

Stackpole DJ, Vaillancourt RE, Alves A, Rodrigues J, Potts BM (2011) Genetic variation in the chemical components of *Eucalyptus globulus* wood. *G3: GENES, GENOMES, GENETICS* **1**, 151-159.

Stackpole DJ, Vaillancourt RE, de Aguigar M, Potts BM (2010a) Age trends in genetic parameters for growth and wood density in *Eucalyptus globulus*. *Tree Genetics & Genomes* **6**, 179-193.

Stackpole DJ, Vaillancourt RE, Downes GM, Harwood CE, Potts BM (2010b) Genetic control of kraft pulp yield in *Eucalyptus globulus*. *Canadian Journal of Forest Research* **40**, 917-927.

Stacy EA (2001) Cross-fertility in two tropical tree species: evidence of inbreeding depression within populations and genetic divergence among populations. *American Journal of Botany* **88**, 1041-1051.

Stayton CL, Hoffman M (1970) Estimating sugar maple bark thickness and volume Forest Service, U.S. Department of Agriculture, St. Paul, Minnesota.

Steane DA, Conod N, Jones RC, Vaillancourt RE, Potts BM (2006) A comparative analysis of population structure of a forest tree, *Eucalyptus globulus* (Myrtaceae), using microsatellite markers and quantitative traits. *Tree Genetics & Genomes* **2**, 30-38.

Stoehr M, Ott P, Woods J (2015) Inbreeding in mid-rotation coastal Douglas-fir: implications for breeding. *Annals of forest science* **72**, 195-204.

Strauss SY, Whittall JB (2006) Non-pollinator agents of selection on floral traits. In 'Ecology and evolution of flowers. (Eds LD Harder and SCH Barrett) pp. 120-138. (Oxford University Press: Oxford, United Kingdom).

Suitor S, Pott B, McGowen M, Pilbeam D, Brown P, Gracie A, Gore P (2009a) The relative contribution of the male and female to the variation in reproductive success in *Eucalyptus globulus*. *Silvae Genetica* **58**, 129-138.

Suitor S, Potts BM, Brown PH, Gracie A, Gore PL (2009b) The relationship of the female reproductive success of *Eucalyptus globulus* to the endogenous properties of the flower. *Sexual plant reproduction* **22**, 37-44.

Suitor S, Potts BM, McGowen MH, Pilbeam DJ, Brown PH, Gracie AJ, Gore PL (2009c) The relative contribution of the male and female to the variation in reproductive success in *Eucalyptus globulus*. *Silvae Genetica* **58**, 129-138.

Tallmon DA, Luikart G, Waples RS (2004) The alluring simplicity and complex reality of genetic rescue. *Trends in Ecology & Evolution* **19**, 489-496.

Tambarussi EV, Pereira FB, da Silva PHM, Lee D, Bush D (2018) Are tree breeders properly predicting genetic gain? A case study involving *Corymbia* species. *Euphytica* **214**, 150.

Therneau T, Lumley T (2009) Survival: survival analysis, including penalised likelihood. In. ' (R package Version 2.35 -37)

Thomas RE, Bennett ND (2014) Estimating bark thicknesses of common Appalachian hardwoods. In '19th Central Hardwood Forest Conference. ' (Eds JW Groninger, EJ Holzmueller, CK Nielsen and DC Dey) pp. 283-294. (US Department of Agriculture, Forest Service, Northern Research Station, Newtown Square, PA: Carbondale, IL)

Thornhill NW (1993) 'The natural history of inbreeding and outbreeding: theoretical and empirical perspectives.' viii edn. (University of Chicago Press: Chicago, Ill).

Turner CH, Balodis V, Dean GH (1983) Variability in pulping quality of *E. globulus* from Tasmanian provenances. *Appita* **36**, 371-376.

Uyenoyama MK (1986) Inbreeding and the cost of meiosis: the evolution of selfing in populations practicing biparental inbreeding. *Evolution* **40**, 388-404.

Vaillancourt R, Potts B, Watson M, Volker P, Hodge G, Reid J, West A (1995) Detection and prediction of heterosis in *Eucalyptus globulus*. *Forest Genetics* **2**, 11-19.

Vaillancourt RE, Petty A, McKinnon GE (2004) Maternal inheritance of mitochondria in E*ucalyptus globulus*. *Journal of Heredity* **95**, 353-355.

Van den Berg GJ, Verryn SD, Chirwa PW, van Deventer F (2017) Genetic parameters and genotype by environment interaction of *Eucalyptus grandis* populations used in intraspecific hybrid production in South Africa. *Southern Forests* **79**, 287-295.

Van Wyk G (1977) Pollen Handling, Controlled Pollination and Grafting of *Eucalyptus grandis South African Forestry Journal* **101**, 47-53.

Venturas MD, Sperry JS, Hacke UG (2017) Plant xylem hydraulics: what we understand, current research, and future challenges. *Journal of integrative plant biology* **59**, 356-389.

Vicente-Serrano SM, Beguería S, López-Moreno JI (2010) A multiscalar drought index sensitive to global warming: the standardized precipitation evapotranspiration index. *Journal of Climate* **23**, 1696-1718.

Vikram V, Cherry ML, Briggs D, Cress DW, Evans R, Howe GT (2011) Stiffness of Douglas-fir lumber: effects of wood properties and genetics. *Canadian Journal of Forest Research* **41**, 1160-1173.

Vivas M, Kemler M, Mphahlele MM, Wingfield MJ, Slippers B (2017) Maternal effects on phenotype, resistance and the structuring of fungal communities in *Eucalyptus grandis*. *Environmental and Experimental Botany* **140**, 120-127.

Vivas M, Rolo V, Wingfield MJ, Slippers B (2019) Maternal environment regulates morphological and physiological traits in *Eucalyptus grandis*. *Forest ecology and management* **432**, 631-636.

Volker P, Dean C, Tibbits W, Ravenwood I (1990) Genetic parameters and gains expected from selection in *Eucalyptus globulus* in Tasmania. *Silvae Genetica* **39**, 18-21.

Volker PW (2002) Quantitative genetics of *Eucalyptus globulus, E. nitens* and their F1 hybrid. University of Tasmania Tasmania, Australia

Volker PW, Orme RK (1988) Provenance trials of *Eucalyptus globulus* and related species in Tasmania. *Australian Forestry* **51**, 257-265.

Volker PW, Potts BM, Borralho NM (2008) Genetic parameters of intra-and inter-specific hybrids of *Eucalyptus globulus* and *E. nitens*. *Tree Genetics & Genomes* **4**, 445-460.

Waddington KD (1983) Pollen flow and optimal outcrossing distance. *The American Naturalist* **122**, 147-151.

Waldmann P, Hallander J, Hoti F, Sillanpää MJ (2008) Efficient Markov chain Monte Carlo implementation of Bayesian analysis of additive and dominance genetic variances in noninbred pedigrees. *Genetics* **179**, 1101-1112.

Walsh B (2005) The struggle to exploit non-additive variation. *Australian Journal of Agricultural Research* **56**, 873-881.

Waser NM (1993) Population structure, optimal outbreeding, and assortative mating in angiosperms. In 'The Natural History of Inbreeding and Outbreeding: Theoretical and Empirical Perspectives. (Ed. NW Thornhill) pp. 173-199. (University of Chicago Press: London).

Waser NM, Price MV (1989) Optimal outcrossing in *Ipomopsis aggregata*: Seed set and offspring fitnes. *Evolution* **43**, 1097-1109.

Waser NM, Price MV (1994) Crossing-distance effects in *Delphinium nelsonii*: Outbreeding and inbreeding depression in progeny fitness. *Evolution* **48**, 842-852.

Wei X, Borralho N (1997) Genetic control of wood basic density and bark thickness and their relationships with growth traits of *Eucalyptus urophylla* in south east China. *Silvae genetica* **46**, 245-249.

White TL, Adams WT, Neale DB (2007) 'Forest genetics.' (CABI: UK).

Whitham TG, Bailey JK*, et al.* (2006) A framework for community and ecosystem genetics: from genes to ecosystems. *Nature Reviews Genetics* **7**, 510-523.

Willi Y, Van Buskirk J, Hoffmann AA (2006) Limits to the adaptive potential of small populations. *Annual Review of Ecology, Evolution, and Systematics* **37**, 433-458.

Williams D, Potts B, Black P (1999) Testing single visit pollination procedures for *Eucalyptus globulus* and *E. nitens*. *Australian Forestry* **62**, 346-352.

Williams ER, Matheson AC, Harwood CE (2002) 'Experimental design and analysis for tree improvement.' 2 edn. (CSIRO publishing: Collingwood, VIictoria, Australia).

Williams K, Potts B (1996) The natural distribution of *Eucalyptus* species in Tasmania. *Tasforests* **8**, 39-165.

Wu HX, Matheson A (2001) Reciprocal, maternal and non-maternal effects in radiata pine diallel mating experiment on four Australia sites. *Forest genetics* **8**, 205-212.

Wu HX, Matheson AC (2004) General and specific combining ability from partial diallels of radiata pine: implications for utility of SCA in breeding and deployment populations. *Theoretical and applied genetics* **108**, 1503-1512.

Wyk GV (1977) Early growth results in a diallel progeny test of *Eucalyptus grandis* (Hill) Maiden. *Silvae Genetica* **26**, 44-50.

Xu T, Hutchinson MF (2013) New developments and applications in the ANUCLIM spatial climatic and bioclimatic modelling package. *Environmental Modelling & Software* **40**, 267-279.

Yang J, Evans R (2003) Prediction of MOE of eucalypt wood from microfibril angle and density. *European Journal of Wood and Wood Products* **61**, 449-452.

Yang R-C, Yeh FC, Yanchuk AD (1996) A comparison of isozyme and quantitative genetic variation in *Pinus contorta* ssp*. latifolia* by F_{ST}. *Genetics* **142**, 1045-1052.

Yeoh SH, Bell JC, Foley WJ, Wallis IR, Moran GF (2012) Estimating population boundaries using regional and local-scale spatial genetic structure: an example in *Eucalyptus globulus*. *Tree genetics & genomes* **8**, 695-708.

Ying C (1978) Height growth of interprovenance crosses in white spruce, *Picea glauca* (Moench) Voss. *Silvae genetica* **27**, 226-229.

Zar JH (1999) Multiple regression and correlation. In 'Biostatistical analysis. ' 5 edn. pp. 663. (Pearson Education London).

Zas R, Cendán C, Sampedro L (2013) Mediation of seed provisioning in the transmission of environmental maternal effects in Maritime pine (*Pinus pinaster* Aiton). *Heredity* **111**, 248.

Zhu Ya, Wu S, Xu J, Lu Z, Li G, Hu Y, Yang X, Bush D (2017) Genetic parameters for growth traits and stem-straightness in *Eucalyptus urophylla* × *E. camaldulensis* hybrids from a reciprocal mating design. *Euphytica* **213**, 142.

Zobel BJ, Van Buijtenen JP (1989) Wood variation and wood properties. In 'Wood Variation. pp. 1-32. (Springer: Berlin, Germany).

Zuur AF, Ieno EN, Elphick CS (2010) A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution* **1**, 3-14.