Understanding climate adaptation and its implications for revegetation in *Eucalyptus microcarpa*: linking genomic and phenotypic variation

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SUMMARY

Trees are a keystone species in many ecosystems and a critical component of ecological restoration. As seed sourcing shifts from the traditional use of 'local-provenancing' toward incorporating genetic material from populations already adapted to projected future climates, understanding adaptation to climate will be necessary for determining appropriate seed sources under climate change. This project aimed to increase knowledge of climate adaptation in *Eucalyptus microcarpa*, an important restoration tree species in south-eastern Australia. With support from the Australian Flora Foundation, this project was able to combine new genomic approaches with traditional trait based analyses to gain a greater understanding of climate adaptation in *E. microcarpa*. A panel of single nucleotide polymorphisms (SNPs), previously identified as putatively climate-adaptive, were tested and 13 of these SNPs were found to be associated with growth and leaf traits. They suggest potential roles of growth, development, and stress response genes as well as gene regulation underpinning adaptive trait variation in *E. microcarpa*. Drawing on previous SNP-climate and trait-climate association results, several associations were identified between all three comparisons of phenotype, genotype and climate. These multiple and independent analyses provide strong evidence of climate adaptation in *E. microcarpa*. They also highlight several traits, such as leaf length, and climate variables such as mean annual temperature, aridity and winter precipitation, as potentially important factors in climate adaptation in this species. Together these results increase knowledge of climate adaptation in *E. microcarpa*, including potential traits and underlying genetic variants that warrant further in depth investigation, as well as information that can help inform restoration seed sourcing under climate change.

INTRODUCTION

Trees are a critical components of ecological restoration to mitigate effects of habitat loss and fragmentation (Prober *et al.*, 2016). Despite generally wide climate tolerances, recent environmental changes have had notable impacts on tree populations, including die-back (Calder & Kirkpatrick, 2008; Matusick *et al.*, 2013) and shifts in distribution (Wearne & Morgan, 2001). How trees respond to potential changes in climate beyond the conditions of local populations could therefore have significant implications for their wider ecosystems. Consequently, understanding the distribution of adaptation in trees underpins the design of

strategies to manage evolutionary potential in natural and restoration tree populations under climate change.

Understanding adaptive variation within species is a major goal for forest tree research, past and present (Neale & Kremer, 2011). Common garden trials have been extensively used to investigate adaptation via assessments of genetic variation in traits (Kremer *et al.*, 2014; Aitken & Bemmels, 2015). These have demonstrated significant genetic variation in traits within species along environmental clines (Vitasse *et al.*, 2014; McLean *et al.*, 2014; McKown *et al.*, 2014), including variation across climatic gradients (Holliday *et al.*, 2010; McLean *et al.*, 2014; Gauli *et al.*, 2015). More recently, genomic technologies are emerging as a complementary approach for assessing adaptation (Stapley *et al.*, 2010; Holliday *et al.*, 2016). They enable investigations in shorter time frames and without the logistical investments required for common garden experiments (Neale & Kremer, 2011; Sork *et al.*, 2013). In addition, genomics can be used to identify genes and genomic regions that have responded to selection (Dillon *et al.*, 2014; Yeaman *et al.*, 2016; Sork *et al.*, 2016).

Whilst genomics is rapidly becoming an invaluable tool for climate adaptation studies in tree species (Holliday *et al.*, 2016), combining phenotypic and genomic analyses can bolster evidence of local adaptation (Sork *et al.*, 2013; Rellstab *et al.*, 2015; de Villemereuil *et al.*, 2016). Such an approach links climate-adapted traits with underlying adaptive genetics, providing a mechanistic validation of genomic signatures of selection via insights into their role, and potential function in mediating phenotypes under selection (Neale & Kremer, 2011; Rellstab *et al.*, 2015; de Villemereuil *et al.*, 2016).

Eucalypts are the dominant trees in the majority of Australian forests and woodlands (Williams & Brooker, 1997) and are widely used in restoration plantings (Prober *et al.*, 2016). For many eucalypt species, climate change is projected to decrease the extent of climate-suitable habitat (Hughes *et al.*, 1996; Butt *et al.*, 2013), with migration in trees unlikely to match rates of climate change, hindered by long generation times, altered land use and low topographic relief in Australia (Aitken *et al.*, 2008; Hughes, 2011). Understanding current climate adaptation will be important for utilising adaptive variation to enhance evolutionary potential in both natural and restored sites for these keystone species.

In this study, we investigated associations between quantitative traits, putatively adaptive SNPs and climate variables, to infer evidence of climate adaptation in *Eucalyptus microcarpa* (Maiden) Maiden (Grey Box). *Eucalyptus microcarpa* is an important restoration species in

south-eastern Australia, used to mitigate widespread habitat loss across the species' distribution due to clearing for agriculture. Previous landscape genomic analyses on *E. microcarpa* identified genomic signatures of climate adaptation, including genomic variants associated with temperature, precipitation and aridity (Jordan *et al.*, 2017). Furthermore, quantitative trait analyses demonstrated heritable, genetic variation in traits that was also associated with climate (Jordan *et al.* in preparation). Building on these results, this study aimed to bolster evidence for climate adaptation as well as gain greater insight into potential mechanisms involved in climate adaptation by combining these independent genotypic and phenotypic analyses. In particular, this study asked 1) Are putatively adaptive genomic variants associated with quantitative traits, 2) do these associations match previous independent analyses and 3) does this support evidence of climate adaptation in *E. microcarpa*?

METHODS

To look for links between potentially adaptive genomic variation and quantitative traits, a set of 40 putatively adaptive candidate single nucleotide polymorphisms (SNPs) were genotyped in 422 individuals, for which both growth and leaf trait data were collected (Table 1).

Trait data

Growth and leaf measurements were drawn from a previous study examining quantitative trait variation in *E. microcarpa* (Jordan *et al.* in preparation). Briefly, an *E. microcarpa* trial site containing 12 provenances from across its distribution was established near Collie, Western Australia in 1988 by the Western Australian Department of Conservation and Land Management. The trial site was planted in a randomised complete block design with four replicates. Nine growth and leaf traits were measured in spring 2014; three growth traits – Diameter at Breast Height (DBH), Height and Size Ratio (Height:DBH), and six leaf traits – Leaf Area, Leaf Length, Leaf Thickness, Leaf Weight, Leaf Density and Specific Leaf Area (SLA). Data for 422 trees from 7 provenances across Victoria and New South Wales were used in this study (Table 1, Figure 1). These provenances matched the sampling distribution of the landscape genomic study from which the SNP data in this study is drawn (Jordan *et al.*, 2017).

Table 1 Information on the seven provenances studied in the *E. microcarpa* provenance trial including the number of trees genotyped from the trial site, and climate data of original provenance locations. Climate data from Atlas of Living Australia (http://www.ala.org.au).

Provenance	Number of trees genotyped (*n*)	Aridity ratio [1]		Precipitation (mm)					Temperature (ºC)		
		Mean annual	Maximum month	Annual (Bio12)	Summer	Winter	Driest period (Bio14)	Wettest period (Bio13)	Annual mean (Bio01)	Max. month abs. mean max.	Warmest period max. (Bio05)
VICTORIA											
Avoca	58	0.740	1.797	529	103	176	6	14	13.7	43	28.8
Benalla	58	0.698	1.745	550	108	174	7	16	15.4	44	30.9
Bendigo	66	0.640	1.613	489	88	154	5	13	14.4	44	29.9
NEW SOUTH WALES											
Deniliquin	60	0.394	0.989	374	85	109	5	10	15.6	46	31.5
Forbes	57	0.479	1.017	556	158	134	8	13	16.8	45	33.1
Wagga	61	0.487	1.189	489	110	133	7	12	16.4	45	33
West Wyalong	62	0.427	1.010	466	124	113	7	12	16.4	45	32.8
Total	422										
Minimum		*0.394*	*0.989*	*374*	*85*	*109*	*5*	*10*	*13.7*	*43*	*28.8*
Maximum		*0.740*	*1.797*	*556*	*158*	*176*	*8*	*16*	*16.8*	*46*	*33.1*
Trial site (WA)		0.796	2.445	587	42	307	2	24	15.2	45	30.6

[1] Ratio precipitation to potential evaporation (pan, free-water surface)
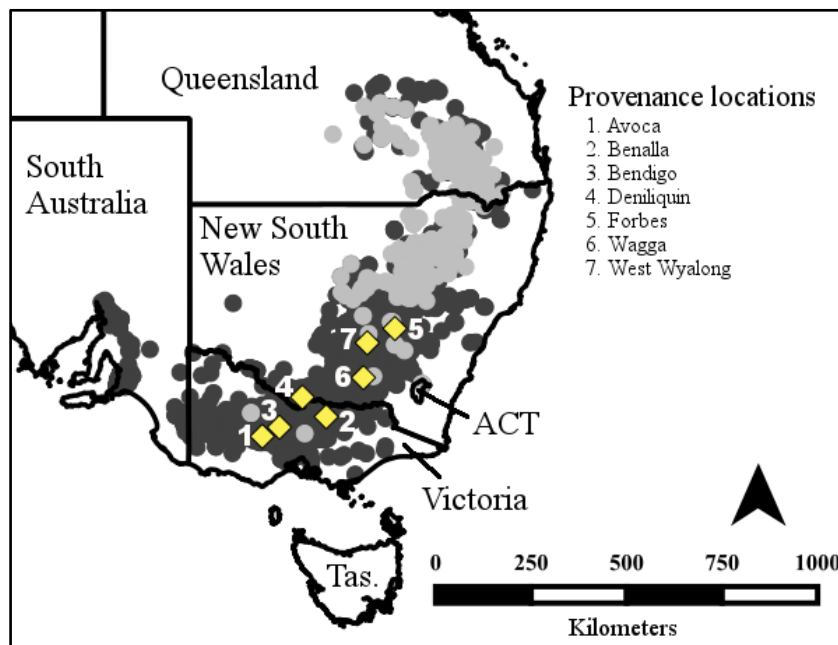Max. = maximum; Abs. = absolute

Figure 1 Original provenance locations of the seven *Eucalyptus microcarpa* provenances used in the trial planting site and this study. Grey dots indicate recorded occurrences of *E. microcarpa* (dark) and *E. woollsiana* (light) respectively (data from Atlas of Living Australia; http://www.ala.org.au), providing an indication of the species' distribution. ACT = Australian Capital Territory. Tas. = Tasmania.

Genotyping

Candidate adaptive SNPs were chosen from previous genomic analyses of climate adaptation in *E. microcarpa* (Jordan *et al.*, 2017). Candidate SNPs were selected based on identification as an F_{ST} outlier in at least one of the four analyses – BayeScan (Foll & Gaggiotti, 2008), hierarchical FDIST2 (Excoffier *et al.*, 2009), FDIST2 (Beaumont & Nichols, 1996; Antao *et al.*, 2008) or Bayenv2 $X^T X$ (Günther & Coop, 2013) or identification of a strong association with at least one of the ten climate variables tested (Bayenv2, BF > 20; Coop *et al.* 2010; Günther & Coop 2013). In the previous analyses, environmental association analyses were only performed on SNPs identified as F_{ST} outliers. To expand the list of potential candidate SNPs for genotyping in this study, environmental associations were performed in Bayenv2 for all 4,218 SNPs, using the same methodology as the previous analysis (refer to Jordan *et al.*, 2017). Potential candidate SNPs were filtered to those with a minor allele frequency of greater than 0.05 in at least six of the seven original provenance sites and to SNPs within 2,000 bp of a putative *E. grandis* gene with an *Arabidopsis thaliana* orthologue (TAIR10) based on *E. grandis* v 1.1 annotation (Myburg *et al.*, 2014).

An additional 25 non-adaptive SNPs were included to account for population structure. Non-adaptive SNPs were selected from those not significant in all four F_{ST} outlier tests – BayeScan ($q > 0.2$), hierarchical FDIST2 ($p > 0.1$), FDIST2 ($q > 0.2$) and $X^T X$ (outside top 10% of 3 runs), not strongly association with any of the 10 climate variables tested (Bayenv2, BF < 20), and with a minor allele frequency greater than 0.05 in at least six of seven original provenance sites. Despite the smaller dataset, the final dataset of 65 SNPs provided a fair representation of the population structure found previously in the full dataset of 4,218 SNPs (Jordan *et al.*, 2017) – there was a correlation of $r = 0.69$ for provenance-level pairwise F_{ST} estimates of the 65 genotyped SNPs within the provenance trial compared to the full dataset of 4,218 SNPs for the seven original natural sites (pairwise F_{ST} calculated in Arlequin v 3.5.1.2; Excoffier *et al.*, 2005).

Flanking sequences for each SNP were extracted from genomic read data. Firstly, an *E. microcarpa* consensus sequence was created from genomic read data flanking the SNPs of interest by modifying the *E. grandis* v 1.1 sequence using samtools mpileup v 1.2-10 and bcftools v 1.3 (Li *et al.*, 2009). *E. microcarpa* sequences for each SNP locus were then created by extracting flanking regions covered by known *E. microcarpa* reads from the "whole genome" consensus.

To genotype the SNPs, approximately 25 mm^2 of dried leaf material per tree and fasta sequences of SNP loci were sent to Diversity Arrays Technology Pty Ltd for DNA extraction and subsequent SNP genotyping via DArTmp multiplex PCR. Genotypes were called from allele read counts using the maximum likelihood method described by Blischak *et al.* (2016) in R (v 3.2.1; R Core Team, 2015), using an error rate of 0.05 and only scoring genotypes with a minimum total read depth of 20. Data was filtered to SNPs and individuals with < 50% missing data. Linkage between genotyped SNPs was calculated using the 'r' function in PLINK (v 1.90b3p; Purcell *et al.*, 2007). Little linkage was found between all SNPs (average $r^2 = 0.003 \pm$ s.d. 0.005), the adaptive subset or neutral SNP subset ($r^2 = 0.004 \pm 0.006$ and $r^2 = 0.004 \pm 0.005$, respectively).

Genotype-phenotype associations

SNP-trait associations between the 40 putatively adaptive SNPs and nine scored traits were performed on 422 individuals in TASSEL (v 5; Bradbury *et al.*, 2007). To account for population structure, a covariance PCA was performed in TASSEL using all 65 SNPs. Overall genetic variation, estimated via AMOVA in Arlequin using all 65 SNPs, found 3% of the variation occurred between populations. Therefore, the first PC accounting for variance found between populations (PC1 = 4.1%) was retained in all analyses.

To account for relatedness between individuals, a kinship matrix was created in Coancestry (v 1.0.0.1; Wang 2011). The coefficient of relatedness (2θ) was calculated using the triadic likelihood estimator (Wang 2007) and using 100 reference individuals. This estimator can account for potential inbreeding when estimating relatedness, an important consideration given the mixed mating system of eucalypts (House 1997). Inbreeding (*f*) was calculated using the estimator of Lynch & Ritland (1999), which bounds inbreeding coefficient estimates between 0 and 1. Two different kinship matrices were tested. The first used the individual kinship estimates, allowing for variation in the degree of relatedness between all individuals, including across families and provenances. As the small number of SNPs used in this study may result in large error rates, an alternative matrix was created using the average family-level relatedness within and between families. This can reduce the effects of individual spurious estimates, especially between provenances, however it may reduce genuine unique relationships. The average relatedness (2θ) within and between each family and the average inbreeding (*f*) within each family was calculated and an individual matrix created using family-level averages.

SNP-trait associations were tested using two alternative linear models – 1) a generalised linear model (GLM) including a single PC fitted as a fixed effect to account for population structure, and 2) a mixed linear model (MLM), also including one PC as a fixed term with kinship included as a random effect to account for relatedness between individuals. Both the raw individual kinship matrix and family-average kinship matrix were tested in the MLM.

To provide additional support for climate adaptation in *E. microcarpa*, and possible underlying genetic mechanisms, we compared results from the three independent pairwise associations of genotype, phenotype and climate performed in this and previous analyses. Results of SNP-climate (Jordan *et al.* 2017), and trait-climate (Jordan *et al.* in preparation) associations were compared to SNP-trait associations in this study. In trait-climate associations, growth and leaf traits were reduced to principal components to reduce correlations between traits. For comparisons in this study, climate association results for the principal component(s) to which the individual traits loaded were used (Appendix, Table A.1)

RESULTS

Genotype-phenotype associations

Associations between putatively adaptive SNPs and quantitative trait variation supported a genetic basis for trait variation, providing insights into possible genomic regulatory factors, as well as validating results of previous genomic adaptation analyses. Thirteen putatively adaptive SNPs were significantly associated with at least one of the nine measured traits (Appendix, Table A.2). For growth traits, three significant SNP-trait associations ($p < 0.05$) were identified, both with and without accounting for kinship. The three SNPs each explained approximately 0.9 – 1.9% of trait variation. For leaf traits, 17 significant associations were identified (Table A.2). Six leaf SNP-trait associations were identified using only the GLM (no kinship), whilst three were found only when kinship was accounted for using a MLM. The remaining eight associations were identified both with and without accounting for kinship. The amount of leaf trait variance explained by an individual SNP varied from 0.6 – 3.3%. No SNP-trait associations were significant after correction for multiple testing across SNP-trait comparisons within a model (GLM or MLM).

Based on *Arabidopsis thaliana* orthologues of putative *E. grandis* genes (TAIR10; Table A.2; TAIR (2017)), variation in growth traits may be influenced by genes related to auxin-activated signalling (At3g13980, TAIR (2017)) and development (CHC1, TAIR (2017)). Leaf traits were associated with TAIR10 orthologue genes found to be associated with response to stress (PRK5, Yoon *et al.* (2014); MGL, Less & Galili (2008); MYB78, Yanhui *et al.* (2006)), transcription (MYB78; ZPR2, Wenkel *et al.* (2007)) and biotinylation of carboxylases (HCS1, Chen *et al.* (2013)) in *A. thaliana* and other plant species.

Seven climate-related associations were corroborated in several independent analyses, namely previous SNP-climate associations (Jordan *et al.*, 2017), trait-climate associations (Jordan *et al.*) and finally SNP-trait from this study (examples in Figure 2). As such these results provide stronger evidence of genetically based climate adaptation in *E. microcarpa* including potential genes underlying climate-related trait variation.
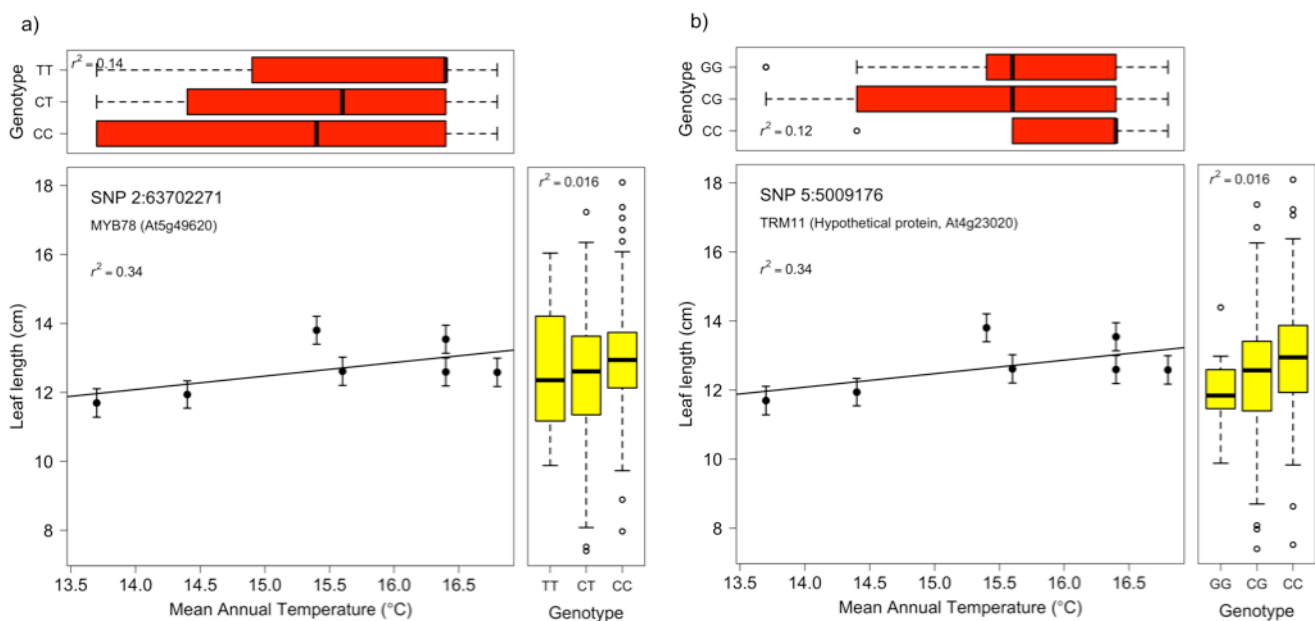

Figure 2 Examples of corroboration between three independent association analyses in *Eucalyptus microcarpa* − genotype (SNP; a) SNP 2:63702271, b) SNP 5:5009176), phenotype (leaf length) and climate (mean annual temperature). Main plot = trait (provenance level Best Unbiased Linear Estimates, BLUEs) vs climate. Box plot (right, yellow) = SNP vs trait (provenance BLUEs). Box plot (top, red) = SNP vs climate. Error bars in main plot = 95% confidence interval. Box plot whiskers extend to 1.5 × interquartile range.

Leaf length was associated with five SNPs (SNP 1:17805026, 2:57262814, 2:63702271, 5:5009176 and 6:39617441; examples in Figure 2) sharing a number of climate associations, suggesting several possible genes involved in climate-associated variation in leaf length. Leaf

length correlated primarily with the first, but also the second leaf trait axis. Both leaf length, via its correlation to the second leaf trait axis and all five SNPs were associated with mean annual temperature and all except SNP 5:5009176 were also associated with warmest period maximum temperature. In addition, the second leaf trait axis and SNPs 1:17805026, 2:57262814 and 5:5009176 were associated with aridity, and the second leaf trait axis and SNP 2:57262814 were associated with winter precipitation. Based on *A. thaliana* orthologues of predicted *E. grandis* genes, possible genes or functions that may influence climate-related variation in leaf length include an MYB domain protein (MYB78; SNP 2:63702271), an alpha/beta-Hydrolases superfamily protein (SNP 2:57262814), a C2 calcium/lipid-binding plant phosphoribosyltransferase family protein (SNP 6:39617441) and proteins of unknown function or hypothetical proteins (SNP 1:17805026 and SNP 5:5009176; refer to Table A.2).

Results for leaf density suggest a possible link to SNP 6:39617441 (putative *A. thaliana* orthologue, C2 calcium/lipid-binding plant phosphoribosyltransferase family protein; Table A.2) and temperature, with both the SNP and leaf density, via its correlation with the second leaf trait axis, associated with mean annual temperature and warmest period maximum temperature.

Finally, a potential link was found between size ratio and SNP 10:29282238, supported by both the trait, via correlation with the second growth trait axis, and the SNP associated with mean annual temperature and warmest period maximum temperature. Based on *A. thaliana* orthologues of predicted *E. grandis* genes, a possible SWIB/MDM2 domain or galactose oxidase/kelch repeat superfamily protein may be involved in temperature related variation in size ratio (Table A.2).

DISCUSSION

This study found increased evidence of not only local adaptation to climate in *E. microcarpa*, but also potential mechanisms involved in climate adaptation. These results suggest important climate-related genetic variation in this species, and highlight potentially important climate variables that may be useful for restoration seed sourcing. Whilst this is a small study, and results should be interpreted cautiously, the findings are bolstered by agreement between multiple lines of evidence for local adaptation based on genotype, phenotype and environmental associations. The results are therefore presented as suggestive of adaptation but warranting further investigation.

Linking genotype & phenotype

Greater support for climate adaptation may be achieved by combining multiple independent lines of enquiry (Sork *et al.*, 2013; Eckert *et al.*, 2013; de Villemereuil *et al.*, 2016). SNP-trait associations in this study linked previous genomic analyses (Jordan *et al.*, 2017) and quantitative analyses (Jordan *et al.* in preparation) of climate adaptation in *E. microcarpa*, validating earlier results, providing stronger evidence of climate as a driver of local adaption and highlighting potential genes or genic regions underlying quantitative traits.

The study firstly demonstrated that putatively adaptive genomic markers explained, individually, a small proportion of genetic variation in quantitative traits. This, along with the fact that multiple SNPs associated with individual traits, is in line with the expectations for polygenic traits, and characteristic of associations studies in trees (Kremer *et al.*, 2014; Yeaman *et al.*, 2016). Indeed, variance estimates found here are comparable to other SNP-trait association studies in trees (Eckert *et al.* 2009; Holliday *et al.* 2010; Alberto *et al.* 2013). The relatively small number of SNP-trait associations found here is likely due to the small size and power of this study, along with the small effect sizes associated with the markers (Le Corre & Kremer, 2012; Eckert *et al.*, 2013). Future analyses are warranted to test conclusions drawn from these results, as well as to repeat these analyses with more genomic markers and assessing a wider range of traits, including phenological and physiological traits. Multi-species analysis may also provide additional evidence for genomic regions and mechanisms underlying climate adaptation (e.g. Yeaman *et al.* 2016).

Secondly, as found in other studies (Eckert *et al.*, 2009; De Kort *et al.*, 2014), the results demonstrated the power of a combined approach for validating genomic signatures of adaptation, via associations between putatively adaptive SNPs and phenotypic variation, and by validating associations with climate. This combined approach strengthened evidence of local adaptation as a driver of genetic differences, despite the small dataset. For example, in this study, associations between leaf length, two independent SNPs and mean annual temperature corroborate genomic adaptation analyses and highlight potential genes underlying temperature-related leaf variation, based on *Arabidopsis thaliana* (TAIR10) orthologues of *E. grandis* genes. Results of this study therefore support the combination of traditional common garden trials with modern genomic technologies as a method for demonstrating a genetic basis for trait variation and local adaptation in trees (Sork *et al.*, 2013; de Villemereuil *et al.*, 2016).

Conservation & restoration under climate change

Incorporating evolutionary potential into conservation management will be essential for long-term sustainability in natural systems under environmental change (Sgrò *et al.*, 2011; Hoffmann *et al.*, 2015). As restoration seed sourcing strategies move away from 'local' and toward capturing evolutionary potential, especially pre-adapted climate-related diversity (Prober *et al.*, 2015), understanding climate-related variation across species' distributions will help inform effective seed sourcing for long-term sustainability of populations under climate change. Results of this study suggest the presence of climate-associated genetic variation within *E. microcarpa* that could be utilised to enhance diversity and pre-adapted climate variation within restoration plantings and the wider landscape. In particular, temperature, aridity and winter precipitation appear to be important climate drivers of adaptation in *E. microcarpa*, corroborating results of previous genomic analyses (Jordan *et al.*, 2017). Sourcing seed along these climatic gradients, towards projected future climates, may therefore enhance the long-term potential of restoration sites as well as support adaptation in the wider landscape through the introduction of pre-adapted genetic variation.

CONCLUSION

This study found evidence of climate adaptation in *E. microcarpa*. A combined approach, employing multiple independent lines of evidence, proved an effective method for corroborating previous results and providing greater support for climate as a driver of local adaptation in *E. microcarpa*. Given the small size of this study, results presented here are suggestive, highlighting candidate traits and climatic variables for further investigations of adaptation. Exploring additional genomic variants, environmental variables and traits would also improve knowledge of important adaptive genes and traits in *E. microcarpa*. Furthermore, additional common garden sites, in contrasting environments, would assist in separating plastic from genetic responses as well as identifying gene-by-environment interactions that were not able to be separated in this study.

REFERENCES

Aitken SN, Bemmels JB. **2015**. Time to get moving: assisted gene flow of forest trees. *Evolutionary Applications* **9**: 271–290.

Aitken SN, Yeaman S, Holliday JA, Wang T, Curtis-McLane S. **2008**. Adaptation, migration or extirpation: climate change outcomes for tree populations. *Evolutionary Applications* **1**: 95–111.

Alberto FJ, Aitken SN, Alía R, González-Martínez SC, Hänninen H, Kremer A, Lefèvre F, Lenormand T, Yeaman S, Whetten R, *et al.* 2013. Potential for evolutionary responses to climate change - evidence from tree populations. *Global Change Biology* **19**: 1645–1661.

Antao T, Lopes A, Lopes RJ, Beja-Pereira A, Luikart G. **2008**. LOSITAN: a workbench to detect molecular adaptation based on a F_{ST}-outlier method. *BMC Bioinformatics* **9**: 323.

Beaumont MA, Nichols RA. **1996**. Evaluating loci for use in the genetic analysis of population structure. *Proceedings of the Royal Society B: Biological Sciences* **263**: 1619–1626.

Blischak PD, Kubatko LS, Wolfe AD. **2016**. Accounting for genotype uncertainty in the estimation of allele frequencies in autopolyploids. *Molecular Ecology Resources* **16**: 742–754.

Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y, Buckler ES. **2007**. TASSEL: software for association mapping of complex traits in diverse samples. *Bioinformatics* **23**: 2633–2635.

Butt N, Pollock LJ, McAlpine CA. **2013**. Eucalypts face increasing climate stress. *Ecology and Evolution* **3**: 5011–5022.

Calder JA, Kirkpatrick JB. **2008**. Climate change and other factors influencing the decline of the Tasmanian cider gum (*Eucalyptus gunnii*). *Australian Journal of Botany* **56**: 684–692.

Chen X, Chou H, Wurtele ES. **2013**. Holocarboxylase Synthetase 1 physically interacts with histone H3 in Arabidopsis. *Scientifica* **2013**.

Coop G, Witonsky D, Di Rienzo A, Pritchard JK. **2010**. Using environmental correlations to identify loci underlying local adaptation. *Genetics* **185**: 1411–1423.

Le Corre V, Kremer A. **2012**. The genetic differentiation at quantitative trait loci under local adaptation. *Molecular Ecology* **21**: 1548–1566.

Dillon S, McEvoy R, Baldwin DS, Rees GN, Parsons Y, Southerton S. **2014**. Characterisation of adaptive genetic diversity in environmentally contrasted populations of *Eucalyptus camaldulensis* Dehnh. (River Red Gum). *PLoS One* **9**: e103515.

Eckert AJ, Bower AD, Wegrzyn JL, Pande B, Jermstad KD, Krutovsky K V, St Clair JB, Neale DB. **2009**. Association genetics of coastal Douglas fir (*Pseudotsuga menziesii* var. *menziesii*, Pinaceae). I. Cold-hardiness related traits. *Genetics* **182**: 1289–1302.

Eckert AJ, Wegrzyn JL, Liechty JD, Lee JM, Cumbie WP, Davis JM, Goldfarb B, Loopstra CA, Palle SR, Quesada T, *et al.* 2013. The evolutionary genetics of the genes underlying phenotypic associations for loblolly pine (*Pinus taeda*, Pinaceae). *Genetics* **195**: 1353–1372.

Excoffier L, Hofer T, Foll M. **2009**. Detecting loci under selection in a hierarchically structured population. *Heredity* **103**: 285–298.

Excoffier L, Laval G, Schneider S. **2005**. Arlequin (version 3.0): An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics* **1**: 47–50.

Foll M, Gaggiotti O. **2008**. A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: a Bayesian perspective. *Genetics* **180**: 977–993.

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 pauciflor*a. *Tree Genetics & Genomes* **11**: 104.

Günther T, Coop G. **2013**. Robust identification of local adaptation from allele frequencies. *Genetics* **195**: 205–220.

Hoffmann A, Griffin P, Dillon S, Catullo R, Rane R, Byrne M, Jordan R, Oakeshott J, Weeks A, Joseph L, *et al.* 2015. A framework for incorporating evolutionary genomics into biodiversity conservation and management. *Climate Change Responses* **2**: 1.

Holliday JA, Aitken SN, Cooke JEK, Fady B, González-Martínez SC, Heuertz M, Jaramillo-Correa J-P, Lexer C, Staton M, Whetten RW, *et al.* 2016. Advances in ecological genomics in forest trees and applications to genetic resources conservation and breeding. *Molecular Ecology* **26**: 706–717.

Holliday JA, Ritland K, Aitken SN. **2010**. Widespread, ecologically relevant genetic markers developed from association mapping of climate-related traits in Sitka spruce (*Picea sitchensis*). *New Phytologist* **188**: 501–514.

House SM. **1997**. Reproductive biology of eucalypts. In: Williams JE, Woinarski J, eds. Eucalypt Ecology. Cambridge, United Kingdom: Cambridge University Press, 30–55.

Hughes L. **2011**. Climate change and Australia: key vulnerable regions. *Regional Environmental Change* **11**: 189–195.

Hughes L, Cawsey EM, Westoby M. **1996**. Climatic range sizes of Eucalyptus species in relation to future climate change. *Global Ecology and Biogeography Letters* **5**: 23–29.

Jordan R, Hoffmann AA, Dillon SK, Prober SM. **2017**. Evidence of genomic adaptation to climate in *Eucalyptus microcarpa*: Implications for adaptive potential to projected climate change. *Molecular Ecology* **26**: 6002–6020.

Jordan R, Prober SM, Hoffmann AA, Dillon SK. Phenotype, genotype and climate associations implicate local adaptation as a driver of diversity in *Eucalyptus microcarpa*. *In preparation*.

De Kort H, Vandepitte K, Bruun HH, Closset-Kopp D, Honnay O, Mergeay J. **2014**. Landscape genomics and a common garden trial reveal adaptive differentiation to temperature across Europe in the tree species *Alnus glutinosa*. *Molecular Ecology* **23**: 4709–4721.

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

Less H, Galili G. **2008**. Principal transcriptional programs regulating plant amino acid metabolism in response to abiotic stress. *Plant Physiology* **147**: 316–330.

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R. **2009**. The Sequence Alignment/Map format and SAMtools. *Bioinformatics* **25**: 2078–2079.

Lynch M, Ritland K. **1999**. Estimation of pairwise relatedness with molecular markers. *Genetics* **152**: 1753–1766.

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.

McKown AD, Guy RD, Klápště J, Geraldes A, Friedmann M, Cronk QCB, El-Kassaby YA, Mansfield SD, Douglas CJ. **2014**. Geographical and environmental gradients shape phenotypic trait variation and genetic structure in *Populus trichocarpa*. *New Phytologist* **201**: 1263–1276.

McLean EH, Prober SM, Stock WD, Steane D a, Potts BM, Vaillancourt RE, Byrne M. **2014**. Plasticity of functional traits varies clinally along a rainfall gradient in *Eucalyptus tricarpa*. *Plant, Cell & Environment* **37**: 1440–1451.

Myburg AA, Grattapaglia D, Tuskan GA, Hellsten U, Hayes RD, Grimwood J, Jenkins J, Lindquist E, Tice H, Bauer D, *et al.* 2014. The genome of *Eucalyptus grandis*. *Nature* **510**: 356–362.

Neale DB, Kremer A. **2011**. Forest tree genomics: growing resources and applications. *Nature Reviews Genetics* **12**: 111–122.

Prober SM, Byrne M, McLean EH, Steane DA, Potts BM, Vaillancourt RE, Stock WD. **2015**. Climate-adjusted provenancing: a strategy for climate-resilient ecological restoration. *Frontiers in Ecology and Evolution* **3**: 65.

Prober SM, Potts BM, Bailey T, Byrne M, Dillon S, Harrison PA, Hoffmann AA, Jordan R, McLean EH, Steane DA, *et al.* 2016. Climate adaptation and ecological restoration in eucalypts. *Proceedings of the Royal Society of Victoria* **128**: 40–53.

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, Maller J, Sklar P, De Bakker PIW, Daly MJ, *et al.* 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. *American Journal of Human Genetics* **81**: 559–575.

R Core Team. **2015**. *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. URL: http://www.r-project.org/ [accessed 29 July 2015].

Rellstab C, Gugerli F, Eckert AJ, Hancock AM, Holderegger R. **2015**. A practical guide to environmental association analysis in landscape genomics. *Molecular Ecology* **24**: 4348–4370.

Sgrò CM, Lowe AJ, Hoffmann AA. **2011**. Building evolutionary resilience for conserving biodiversity under climate change. *Evolutionary Applications* **4**: 326–337.

Sork VL, Aitken SN, Dyer RJ, Eckert AJ, Legendre P, Neale DB. **2013**. Putting the landscape into the genomics of trees: approaches for understanding local adaptation and population responses to changing climate. *Tree Genetics & Genomes* **9**: 901–911.

Sork VL, Squire K, Gugger PF, Steele SE, Levy ED, Eckert AJ. **2016**. Landscape genomic analysis of candidate genes for climate adaptation in a California endemic oak, *Quercus lobata*. *American Journal of Botany* **103**: 33–46.

Stapley J, Reger J, Feulner PGD, Smadja C, Galindo J, Ekblom R, Bennison C, Ball AD, Beckerman AP, Slate J. **2010**. Adaptation genomics: the next generation. *Trends in Ecology & Evolution* **25**: 705–712.

TAIR. **2017**. *The Arabidopsis Information Resource (TAIR)*. URL: https://www.arabidopsis.org [accessed March 2017].

De Villemereuil P, Gaggiotti OE, Mouterde M, Till-Bottraud I. **2016**. Common garden experiments in the genomic era: new perspectives and opportunities. *Heredity* **116**: 249–254.

Vitasse Y, Lenz A, Kollas C, Randin CF, Hoch G, Körner C. **2014**. Genetic vs. non-genetic responses of leaf morphology and growth to elevation in temperate tree species. *Functional Ecology* **28**: 243–252.

Wang J. **2007**. Triadic IBD coefficients and applications to estimating pairwise relatedness. *Genetical research* **89**: 135–153.

Wang J. **2011**. COANCESTRY: a program for simulating, estimating and analysing relatedness and inbreeding coefficients. *Molecular Ecology Resources* **11**: 141–145.

Wearne LJ, Morgan JW. **2001**. Recent forest encroachment into subalpine grasslands near Mount Hotham, Victoria, Australia. *Arctic, Antarctic and Alpine Research* **33**: 369–377.

Wenkel S, Emery J, Hou B, Evans MMS, Barton MK. **2007**. A feedback regulatory module formed by LITTLE ZIPPER and HD-ZIPIII genes. *The Plant Cell* **19**: 3379–3390.

Williams JE, Brooker I. **1997**. Eucalypts: an introduction. In: Williams JE, Woinarski J, eds. Eucalypt Ecology. Cambridge, United Kingdom: Cambridge University Press, 1–15.

Yanhui C, Xiaoyuan Y, Kun H, Meihua L, Jigang L, Zhaofeng G, Zhiqiang L, Yunfei Z, Xiaoxiao W, Xiaoming Q, *et al.* 2006. The MYB transcription factor superfamily of Arabidopsis: expression analysis and phylogenetic comparison with the rice MYB family. *Plant Molecular Biology* **60**: 107–124.

Yeaman S, Hodgins KA, Lotterhos KE, Suren H, Nadeau S, Degner JC, Nurkowski KA, Smets P, Wang T, Gray LK, *et al.* 2016. Convergent local adaptation to climate in distantly related conifers. *Science* **353**: 23–26.

Yoon S-K, Park E-J, Choi Y-I, Bae E-K, Kim J-H, Park S-Y, Kang K-S, Lee H. **2014**. Response to drought and salt stress in leaves of poplar (*Populus alba* × *Populus glandulosa*): expression profiling by oligonucleotide microarray analysis. *Plant Physiology and Biochemistry* **84**: 158–168.

APPENDIX

Table A.1 Trait correlations (a) and contributions (b) to trait principal component (PC) axes in *Eucalyptus microcarpa*. PC axes from a principal component analysis (PCA) of provenance level Best Unbiased Linear Estimate (BLUEs) trait data. Values in bold represent (a) highest correlation per trait and (b) two highest contributing traits to the PC axis. Note, first line for both the growth and leaf PCA indicate the percentage variance explained by the principal component axes (in *italics*).

	a) Correlation			b) Contribution (%)		
PC axis	1	2	3	1	2	3
Growth trait PCA						
% variance	*79.31*	*19.07*				
DBH	**0.91**	-0.40		**34.51**	**28.08**	
Height	**0.98**	-0.13		**40.18**	2.84	
Size ratio	**0.78**	0.63		25.30	**69.08**	
Leaf trait PCA						
% variance	*58.63*	*30.30*	*8.99*			
Area	**0.97**	-0.01	0.24	**26.58**	0.00	10.58
Length	**0.85**	0.44	-0.06	20.43	10.75	0.60
Weight	**0.85**	-0.27	0.44	**20.71**	4.02	**36.48**
Thickness	**-0.75**	0.51	0.41	16.10	14.44	**31.26**
SLA	**0.73**	0.59	-0.31	15.23	**18.83**	18.24
Density	0.18	**-0.97**	-0.12	0.96	**51.96**	2.83

Table A.2 Significant SNP-trait associations ($p < 0.05$) in *Eucalyptus microcarpa* and possible gene function of SNPs based on *Arabidopsis thaliana* orthologues (TAIR10) of predicted *E. grandis* genes (information from *E. grandis* v1.1 genome annotation). Generalised linear model (GLM) accounts for population structure, whilst the mixed linear model (MLM) accounts for population structure and kinship using a kinship matrix based on individual kinship (indv. kinship) or family-average kinship (avg. kinship).

	GLM		MLM (indv. kinship)		MLM (avg. kinship)		*Eucalyptus grandis* gene information (+/- 2000 bp)		Best TAIR10 gene orthologue		
Trait Marker	*p*	r^2	*p*	r^2	*p*	r^2	Name	Gene effect	Name	Symbol	Definition
DBH											
3:59841756	**0.026**	0.019	**0.043**	0.019	**0.049**	0.019	Eucgr.C03147	synonymous	AT3G13980.1		
Size ratio											
10:29282238	**0.014**	0.014	**0.023**	0.012	**0.015**	0.014	Eucgr.J02333	synonymous	AT3G27150.1		Galactose oxidase/kelch repeat superfamily protein
							Eucgr.J02334	upstream	AT5G14170.1	CHC1	SWIB/MDM2 domain superfamily protein
2:58822368	**0.041**	0.010	0.060	0.009	**0.044**	0.010	Eucgr.B03399	downstream	AT1G27150.1		Tetratricopeptide repeat (TPR)-like superfamily protein
Leaf area											
4:30801453	0.099	0.011	0.069	0.013	**0.037**	0.016	Eucgr.D01681	synonymous, intron	AT1G64660.1	ATMGL, MGL	methionine gamma-lyase
10:19426430	**0.040**	0.015	0.159	0.009	0.130	0.010	Eucgr.J01501	intron	AT1G67100.1	LBD40	LOB domain-containing protein 40
11:4085447	**0.005**	0.027	0.052	0.016	**0.044**	0.017	Eucgr.K00355	downstream	AT5G38280.1	PR5K	PR5-like receptor kinase
Leaf length											
1:17805026	**0.038**	0.018	0.076	0.015	0.055	0.017	Eucgr.A01148	upstream	AT5G03230.1		Protein of unknown function, DUF584
2:57262814	**0.047**	0.014	0.257	0.006	0.160	0.009	Eucgr.B03228	synonymous	AT3G27320.1		alpha/beta-Hydrolases superfamily protein
2:58822368	**0.043**	0.015	0.136	0.009	0.161	0.008	Eucgr.B03399	downstream	AT1G27150.1		Tetratricopeptide repeat (TPR)-like superfamily protein
2:63702271	0.057	0.013	**0.036**	0.016	**0.002**	0.030	Eucgr.B03985	missense	AT5G49620.1	AtMYB78, MYB78	myb domain protein 78
4:30801453	**0.013**	0.020	**0.012**	0.021	**0.004**	0.026	Eucgr.D01681	synonymous, intron	AT1G64660.1	ATMGL, MGL	methionine gamma-lyase
5:5009176	**0.001**	0.033	**0.030**	0.016	0.130	0.009	Eucgr.E00527	missense	AT4G23020.1		
6:39617441	**0.037**	0.018	0.106	0.013	0.178	0.010	Eucgr.F02999	synonymous	AT1G22610.1		C2 calcium/lipid-binding plant phosphoribosyltransferase family protein

Table 4.5 Continued from previous page

Trait Marker	GLM		MLM (indv. kinship)		MLM (avg. kinship)		*Eucalyptus grandis* gene information (+/- 2000 bp)		Best TAIR10 gene orthologue		
	p	r^2	p	r^2	p	r^2	Name	Gene effect	Name	Symbol	Definition
Leaf weight											
5:4625458	0.061	0.013	**0.028**	0.017	**0.027**	0.017	Eucgr.E00491	upstream	AT3G60890.2	ZPR2	protein binding
Leaf thickness											
5:4625458	**0.005**	0.023	**0.006**	0.023	**0.007**	0.023	Eucgr.E00491	upstream	AT3G60890.2	ZPR2	protein binding
SLA											
5:4625458	**0.028**	0.017	**0.024**	0.018	**0.017**	0.020	Eucgr.E00491	upstream	AT3G60890.2	ZPR2	protein binding
6:45708775	**0.025**	0.018	0.216	0.007	0.072	0.013	Eucgr.F03750	intron	AT2G25710.1	HCS1	holocarboxylase synthase 1
11:4085447	**0.032**	0.018	**0.020**	0.021	**0.015**	0.022	Eucgr.K00355	downstream	AT5G38280.1	PR5K	PR5-like receptor kinase
Leaf density											
5:4625458	**0.040**	0.014	0.102	0.010	**0.042**	0.014	Eucgr.E00491	upstream	AT3G60890.2	ZPR2	protein binding
6:39617441	**0.029**	0.018	0.086	0.014	**0.044**	0.018	Eucgr.F02999	synonymous	AT1G22610.1		C2 calcium/lipid-binding plant phosphoribosyltransferase family protein