Signatures of natural selection in a foundation tree along Mediterranean climatic gradients

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September 25, 2021

Abstract

Temperature and precipitation regimes are rapidly changing, resulting in forest dieback and local extinction events, particularly in Mediterranean-type climates. Strategic forest management approaches that enhance forests' resilience to future climates are urgently required, however adaptation to climates in heterogeneous landscapes with multiple selection pressures may be complex. For widespread trees in Mediterranean-type climates we hypothesized that patterns of local adaptation are associated with climate; precipitation is a stronger factor of adaptation than temperature; functionally related genes show similar signatures of adaptation; and adaptive variants are independently sorting across the landscape. To test our hypotheses, we sampled 28 populations across the geographic and climatic distribution of Eucalyptus marginata (jarrah), in south-west Western Australia, and obtained 13,534 independent single nucleotide polymorphic (SNP) markers across the genome. While overall levels of population differentiation were low (FST=0.04), environmental association analyses found a total of 2,336 unique SNPs potentially associated with five climate variables of temperature and precipitation. Allelic turnover was identified for SNPs associated with temperate seasonality and mean precipitation of the warmest quarter (39.2% and 36.9% deviance explained, respectively), suggesting that both temperature and precipitation are important factors in adaptation. SNPs within similarly function genes, according to gene ontology enrichment analysis, had analogous allelic turnover along climate gradients, while SNPs among temperature and precipitation variables had orthogonal patterns of adaptation. These contrasting patterns of adaptation provide evidence that there may be standing genomic variation adapted to changing climates, providing the substrate needed to promote adaptive management strategies to bolster forest resilience in the future.

INTRODUCTION

Climate change is a key pressure on ecosystem persistence and function (Urban, 2015; Brondizio et al., 2019). The shift in climate trends will have an impact on ecosystem structure, potentially making organisms more susceptible to the effects of extreme climate events (Pacifici et al., 2015; Harris et al., 2018). Precipitation patterns are changing in heterogenous ways, with some areas becoming wetter and others drier; and while global surface temperature is predicted to rise by 1–4 °C on average by the end of the current century, the level of temperature rise is also heterogeneous depending on various factors (e.g., latitude, elevation); in addition, the frequency of extreme events such as heatwaves, wildfires, floods and droughts have increased over recent decades in several regions of the world (IPCC, 2021). Because these changes are spatially assorted, predicting climate change impacts across affected landscapes and response patterns from organisms is often challenging.

Mediterranean-type climates (MTC) are defined by reliable precipitation and temperature regimes, with predictable summer periods of low rainfall and hot temperatures, and winter periods of high rainfall and

moderate temperatures. Changes in regions with MTC have already been observed with large ecosystem impacts. Ecological studies in the Mediterranean basin consistently identify more frequent drought periods, together with warmer temperatures, as main drivers for declines in oaks (*Quercus* spp.) (Corcobado et al., 2014; Gentilesca et al., 2017) and pines (*Pinus* spp.) (Camarero et al., 2018). In the south-west Western Australia biodiversity hotspot, the 2010-11 extreme drought and heatwave conditions resulted in large scale forest collapses in eucalypts (Matusick et al., 2013). While some variation in climatic factors exists in natural systems (Staudinger et al., 2013), the rapid and extreme shifts associated with anthropogenic climate change are challenging for most organisms to persist (Levin & Poe 2017; Carlo et al., 2018).

If new climatic scenarios are no longer suitable for species to maintain their normal ecology and physiology, they either develop new adaptations, shift their geographical range or in worst case scenarios, go extinct (Bellard et al., 2012; Soto-Correa et al., 2012). Species may persist through enhanced physiological tolerance, phenotypic plasticity and/or genetic adaptation (Anderson et al., 2011; Christmas et al., 2016). Maintenance of standing genetic variation (allelic variation at a locus held within existing population) is a key factor for adaptation to changing conditions in native habitats (Guzella et al., 2018, Chhatre et al., 2019) and for persistence through environmental stressors over generations (Sexton et al., 2011; Kremer et al., 2012). Genetic variation is critical for ecological adaptive capacity - the potential and ability to adjust to, and persist through, external factors - and consequently, the evolutionary potential of the species (Reed et al., 2011). Evolution to a specific environment through natural selection results in patterns of local adaptation, when a local population experiences higher fitness compared to non-local counterparts (Kawecki & Ebert, 2004).

Measuring local adaptation has benefited through recent improvements in DNA sequencing and statistical methodology, making it possible to investigate genetic divergence and the effects of environmental factors on the process of local genetic adaptation (Honjo & Kudoh, 2019; Gougherty et al., 2020). Environmental association analyses (EAA) have been gaining traction in the last decade (Ahrens et al., 2018), allowing identification of possible candidate genes for adaptation to the environment from tens of thousands of single-nucleotide polymorphisms (SNPs) sampled throughout the whole genome from samples collected from populations across environmental gradients. For example, EAAs have been used to explore adaptive genetic variation on diverse and widespread woody plant genera, like *Quercus* (Martins et al., 2018; Gugger et al., 2021), *Populus* (Ingvarsson & Bernhardsson, 2020; Gougherty et al., 2021) and *Corymbia* (Ahrens et al., 2019a). These studies have identified functional genes involved in adaptation to climatic factors that can be interpreted as divergent selection linked to population-specific environmental variables (i.e., local adaptation to climate). However, different climate factors identify different sets of adaptive candidates, and few studies have focused on how these sets of adaptive candidates sort across the landscape. If candidate SNPs independently sort across the landscape, then managing these species to maintain adaptive capacity to climate change may prove to be difficult.

Identifying the genetic basis of local adaptation and selective factors is still challenging, particularly for species with limited genomic resources and polygenic control of climate adaptations (Mayol et al., 2019; Capblancq et al., 2020). Non-synonymous mutations in gene regions result in amino acid changes, which often yields changes in gene functions (Kryazhimskiy & Plotkin, 2008). These changes in genes can be under selection among populations spread across that environment. Groups of genes found to be significantly associated with environment can be categorised into broader functional groups using gene ontology (GO) enrichment analysis (The Gene Ontology Consortium, 2019). GO terms have been used to predict polygenic adaptive biological processes and molecular functions associated with candidate SNPs in tree species (Jordan et al., 2017; Collevatti et al., 2019). However, few studies investigate how genes of similar function develop patterns of adaptation across complex landscapes. If genes with related functions are found to be adaptive, this might be indicative of additive genetic variation controlling adaptation to the environment.

This study investigated the putative patterns of local adaptation associated with climate gradients across complex landscapes. To test hypotheses associated with signals of adaptation, we focused on *Eucalyptus marginata* Donn ex. Sm. (jarrah) because of its high genetic diversity and low population differentiation (Wheeler

et al., 2003) and its ecological importance in the biodiverse hotspot of south-west Western Australia (SWWA). This region has prolonged periods of extensive drying, with an estimated reduction of 20% in rainfall, from the 1970s to the present (Water Corporation, 2020), documented impacts of drought and heatwave events (Matusick et al., 2013), and the future (2030) climate is projected to show increased frequency and intensity of extremes (BOM & CSIRO, 2020).

Furthermore, jarrah provenance trials have demonstrated genetic variation in functional traits associated to precipitation factors (O'Brien et al. 2007; Koch & Samsa 2007), indicating potential local adaptation to drought stress. Ecological studies have also confirmed that water availability is critical for jarrah seedling survival and persistence (Stoneman et al., 1994; McChesney et al., 1995; Standish et al., 2015). Considering these studies on jarrah, we hypothesize that (1) populations show strong genetic patterns of local adaptation to climate; (2) precipitation is a stronger determinant of genetic adaptation compared to temperature; (3) functionally related genes show similar signatures of adaptation; and (4) adaptive variants are independently sorting across the landscape. Lastly, we use this information to create predictive maps of adaptive variation across the landscape to facilitate informed strategies for forest management that incorporate response to future climate. We go on to assess how active management strategies, such as assisted gene migration (Hoffman et al., 2015; Prober et al., 2015; Aitken & Bemmels, 2016) may be employed to build adaptive capacity to climate change.

METHODS

Sample collection and DNA extraction

Leaf samples from a total of 280 individual mature trees from 28 natural jarrah populations across the geographic range of the species (Figure 1), including one outlier population (JIL), were collected during 2019 (Table 1). The sampling, which covered a total area of approximately 80,000 km^2, included independent (>50 km separation) and replicate (across similar climate of origin) populations over both temperature and precipitation gradients to ensure adequate partitioning of the adaptive and neutral genetic variation. For each population, mature leaves were collected from ten trees at least 100m apart from each other. Leaves were immediately stored in silica gel until freeze-dried using *FreeZone 6 Liter Benchtop Freeze Dryer*(Labconco Corporation, USA). Samples were stored in silica gel at room temperature until DNA extraction could be performed. For each sample, genomic DNA was extracted from 40mg of freeze-dried leaf material. Each leaf sample was independently ground into fine powder and a modified CTAB-DNA extraction protocol was employed (Doyle & Doyle, 1990), with 0.1M sodium sulphite (Byrne et al., 2001) and 2% w/v polyvinylpyrrolidone (MW 40,000) added to the extraction buffer. Quality of extracted DNA was estimated using gel electrophoresis and quantified using the *Qubit dsDNA BR* assay kit on a *Qubit* fluorometer (Invitrogen, Carlsbad, CA).

Genotyping by DArTseq Platform

Sequencing of the 280 jarrah individuals was undertaken using DArT-Seq^{TM} technology at *Diversity Arrays Technology Pty Ltd* (Canberra, Australia). This technology uses a double digestion complexity reduction method for next generation sequencing (Kilian et al., 2012). The reduction of the genome is accomplished by using a combination of PstI and HpaII enzymes in digestion/ligation reactions with different adapters corresponding to two different restriction-enzyme overhangs. The PstI-compatible adapter is designed to include flowcell attachment sequence, sequencing primer sequence and varying length barcode region. *Diversity Arrays Technology's*proprietary bioinformatics pipeline was used to demultiplex and align the raw *fastq* files. Identical sequences were then collapsed into "*fastqcall* files". These files were used in the secondary pipeline for *DArT P/L's* proprietary SNP calling algorithm (DArTsoft14). Minimum read depth for each individual was set to 6 and average read depth was 30.93 across all SNPs, guaranteeing call quality for all SNPs and individuals. For the SNP calling algorithm, only nucleotide substitutions were considered a SNP. Only one random SNP was retained on each 75 bp sequence to avoid linkage disequilibrium bias. The full data set was then filtered in R (R Core Development Team, 2020) using custom scripts. We applied a minor allele frequency (MAF) of 2%, which equates to a minor allele count of 11 calls, minimising inclusion of sequencing

errors. Missing data was set to 6% across individuals (minimum of 263 individuals scored for each SNP). These thresholds were chosen because this translates to, on average, an estimation of a population-level allele frequency from nine individuals, which is adequate for EAA type of method and identifying SNPs under selection (Ahrens et al., 2021a). Linkage disequilibrium (LD) was calculated within each of the chromosomes using the function *LD.Measures* in*LDcorSV* (Mangin et al., 2012). To guarantee adequate independence between SNPs and prevent potential linkage bias, the dataset was filtered by the within chromosome LD r^2 coefficient: if the r^2 value between two SNPs is >0.5, only one of the SNPs was randomly retained for analysis.

Environmental variables

Temperature and precipitation variables have been widely assessed as robust predictors for environmental adaptation in eucalypts for traits and genetic variants (Correia et al., 2018; Aspinwall et al., 2019; Pritzkow et al., 2020). We tested a total of five climate variables. Two of these represent extreme temperature and precipitation variables and we predicted they would drive patterns of adaptation: maximum temperature of the warmest month (T_{MAX}) and precipitation of the warmest quarter (P_{WQ}). Three other temperature and precipitation variables were selected as independent climatic factors (based on principal component analysis (PCA) and Pearson's correlation coefficients) and are known to be important for local adaptation in eucalypts (Queirós et al., 2020; Rocha et al., 2020): minimum temperature of the coldest month (T_{MIN}), mean annual precipitation (P_{MA}), and temperature seasonality (T_{SEAS}). Climatic data for all populations was downloaded from the 19 variables in the WorldClim v2 database (Fick & Hijmans, 2017) at a spatial resolution of 30 arcsec. Climate data for each population was extracted using the R package *raster* from the geo-located GPS coordinates of the sampled populations. PCA of environmental variables was performed with R package *ade4* and a Pearson's correlation coefficient matrix was calculated between all 19 climate variables using the*cor* function.

Data analysis

To understand how genetic structure of jarrah populations might affect EAA, genetic structure was estimated by measure of genetic differentiation (F_{ST}) (Weir & Cockerham, 1984) using the *HierFSTAT* package (Goudet, 2005) in R. We also estimated individual ancestry coefficients for input for the EAA in LFMM. For this, we used the sparse nonnegative matrix factorization (SNMF) method in the R package *LEA* (Frichot & François, 2015). SNMF was run for each k -value between 1 and 10, with each$k$ -value ran 10 times (200 iterations each). The ideal$k$ -value was selected by visualising the cross entropies as defined in the SNMF manual (Frichot & François, 2015) and choosing the$k$ -value(s) with the lowest cross entropy score. For visualisation, a consensus for the optimum k -value across all 10 runs was estimated using the software CLUMPP (Jakobsson & Rosenberg, 2007), and the graphical parameters were drawn in the program DISTRUCT (Rosenberg, 2004).

Environmental Association Analysis

To elucidate the association between climate and genetic variation, three approaches were applied: a redundancy analysis (RDA), latent factor mixed models (LFMM) and BAYPASS. RDA is a multivariate method that assumes linear relationships from explanatory variables on response variables, thus allowing the estimation of genetic variance related to each distinct environmental factor simultaneously (Forester et al., 2018). RDA and LFMM require full data sets, therefore we imputed missing data as the most common allele in the locus from the optimal ancestral cluster (k) as defined in the SNMF output. The explanatory variables (i.e., climate) were then constrained by the dependent variables (i.e., individuals), using the *rda* function in the*VEGAN* package 2.5-1 in R (Oksanen et al., 2018). The*anova.cca* function was used to test for RDA significance using 999 permutations (randomised environmental variables). We did not explicitly control for population structure because RDA without explicit population structure inputs improves the output (Forester et al., 2018). We also used LFMM to test for climate associations (Frichot et al., 2013), which applies a univariate regression model to assess genotype-environment associations while using the optimal k -value estimated in SNMF to control for ancestral population structure. The analyses were independently

performed for each of the climate variables, consisting of 30,000 iterations each (15,000 discarded as initial burn-in). Median z -scores were combined from a total of 5 runs for each variable and recalibrated by computing the genomic inflation factor, λ, and then dividing the scores by λ. p -values were then adjusted manually to flatten the histogram (false discoveries were controlled with the Benjamin-Hochberg algorithm using q = 0.01), which ideally should display a peak close to zero. We used $\lambda = 0.45$ in the adjustment function to flatten the histogram and followed the steps and R script available from the LFMM manual. To account for multiple comparisons, we applied a false discovery rate (FDR) threshold of 0.05 to all runs. Lastly, we used a hierarchical clustering model implemented in BAYPASS (Gautier, 2015), based on the model from BayEnv (Coop et al., 2010). A population covariance matrix (Ω) was generated by running the core model. Each run had 100,000 iterations (50,000 discarded as initial burn-in), repeated five times and averaged. The covariance matrix was then used in the *AUX* covariate mode (100,000 iterations; 50,000 as burn-in), repeated five times and averaged for final results. Significant SNPs were identified if they had a Bayes Factor (BF) > 3 (Kass & Raftery, 1995). Like LFMM, BAYPASS is based on a mixed linear model to account for potentially confounding allele frequency variances due to population structure. However, the difference between the two approaches may provide a means of identifying any influence of population structure (Forester et al., 2018; Ahrens et al., 2021a).

Annotation and gene ontology analysis

To identify the potential role of significant SNPs in coding regions of genes, genomic annotation was run using the *blastn* function (Altschul et al., 1997) from BLAST (https://blast.ncbi.nlm.nih.gov/). The 75 bp sequences associated with each SNP were annotated against the*Eucalyptus grandis* genome (Myburg et al., 2014) and considered if their significance valuesmet two related thresholds: an E -value $< 1 \times 10^{-8}$ and a blast-score of at least 60.0. Chromosome number and location of significant SNPs were recorded, as well as specific gene functions. The annotated SNPs were used to predict broader biological functions using GO enrichment analysis through the web interface PlantRegMap (Tian et al., 2020). GO terms are organized within three categories: molecular function, cellular component and biological process. We explored the biological process aspect from the GO analysis, which refers to broad category of tasks that the genes or gene products are programmed to achieve. Each of the output GO terms delivered a set of genes that is associated with a specific biological process. We use this output to explore how functionally related SNPs, associated with the GO genes, might be additively correlated with environmental factors (e.g., abiotic stress response). For each climate variable, Fisher's exact test was used to test for significantly over-represented GO terms, with a threshold of*p-value* <0.01. GO terms with the highest number of SNPs and/or with pertinent biological processes associated with environmental response (e.g., response to heat, cold and drought) were recorded and considered for further landscape genomic analysis.

Landscape genomics

We used generalized dissimilarity modelling (GDM) to visualise the relationship between allele frequency and climate (Ferrier et al., 2007). GDM is a statistical method that predicts spatial patterns of allelic turnover across geographic regions due to climate by generating an I-spline turnover plot for each tested predictor and uses percent deviance explained as a measure of model fit. GDM analyses was run using the *gdm* package v 1.3.7 in R (Manion et al., 2018), considering a genotypic matrix (pairwise F_{ST}) and a pairwise climate matrix that includes geographic coordinates. GDM was applied on all the putatively adaptive SNPs identified by the EAA as significant. For each variable, the SNP with highest value of deviance explained was selected for plotting and mapping of predicted allelic turnover to test our landscape sorting of adaptive alleles hypothesis.

Following the GDM transformation of the climate variables for each SNP, we performed PCA on the extracted values using R to generate three principal components. The three PCs are then converted into a RGB raster grid (R = PC1, G = PC2 and B = PC3) using custom R rode. The RGB layers were displayed using QGIS V3.16 (QGI.S.org, 2021) overlaying the distribution of jarrah. The RGB colour palette assigned to each of the raster layers will display the allelic turnover in the geographic space, where similar colours correspond to similar predicted patterns of adaptive genetic variability. To test the hypothesis of additive variation, we ran

GDM analyses on groups of SNPs related to specific GO terms for each of the five climates and visualised how the allelic turnover within the GO term was related to that climate. To compare importance of GO terms, we added deviance of SNP groups together to create an 'additive score'. The *HierFSTAT* package (Goudet, 2005) in R was used to create population pairwise F_{ST}matrices with the SNPs from top GO terms for each climate variable. Overall, this model addresses genetic variation that is related to climate variables, discriminating this variation from geographic distances (Fitzpatrick & Keller, 2015). The GDM spline plots show the association between predicted ecological distances and genetic dissimilarities; the y -axis on the spline plots is therefore labelled as partial genetic distance, as it describes a portion of genetic distance, and the height of each spline indicates the magnitude of genomic turnover of a SNP along the climate gradient.

RESULTS

Sequencing and population structure

A total of 78,198 possible SNPs were generated and filtered down to 13,534 independent SNPs, with 8,824 SNPs mapped to the 11*Eucalyptus* chromosomes. The number of SNPs per chromosome varied from 599 to 1,083, with a mean of 802 SNPs per chromosome. Of the remaining SNPs, 477 fell on unspecified scaffolds and 4,233 on regions that could not be aligned to the *E. grandis* genome (unknown location). Population differentiation was low (globalF_{ST} = 0.04) and similar to that identified in a previous RFLP analysis of variation (F_{ST} = 0.034; Wheeler et al., 2003) with population pairwiseF_{ST} values ranging from 0.011 to 0.18. The cross-entropy analysis estimated that the optimal number of clusters (k -value) was 6 (Figure S1). SNMF analysis with six clusters revealed substantial admixture in populations. Five of the clusters could be geographically described (Figure 2, S2), one cluster was primarily located in the southern area, one in the central area and two in the northern area, where one cluster was dominant in populations along the coast. A fifth cluster occurred in the outlier population (JIL; blue colour), and the sixth cluster was present in four individuals (two individuals from BRA and BOO). The LES population displays mixed affinity, being similar to both southern (green) and northern (yellow and red) populations.

Environmental association analysis

All three EAA approaches found putatively adaptive SNPs for each of the five climate variables (Table S2, tabs 1-5). The RDA approach identified fewer candidate SNPs than BAYPASS and LFMM that identified similar numbers (Figure 3; Table S1). The proportion of overlapped SNPs is different for each variable (Figure 3). Overall, 2,336 unique SNPs were flagged to be associated with at least one of the tested climate variables across the three EAA approaches. RDA analysis (Figure S3) identified between 16 (T_{SEAS}) and 57 (T_{MAX}) SNPs significantly associated with each of the climate variables, for a total of 168 SNPs. All five climate variables were shown to be significantly associated with variation in the RDA (T_{SEAS}: F = 3.98, p = 0.001; T_{MAX}: F = 2.26, p = 0.001; T_{MIN}: F = 2.11, p = 0.001; P_{MA}: F = 1.80 p = 0.001; P_{WQ}: F = 1.49, p = 0.001). LFMM identified between 263 (P_{MA}) and 411 (T_{MAX}) SNPs with significant correlations, with a total of 1,753 candidate SNPs. BAYPASS identified between 284 (T_{MIN}) and 888 (T_{MAX}) SNPs with significant correlations, with a total of 2,327 candidate SNPs. Candidates found for all environmental variables from each EAA method were used in further analyses to predict the distribution of adaptive SNPs, specifically the ones occurring in genic regions (annotation) and have robust associations with climate (GDM).

Annotation and gene ontology

Full annotation results for SNPs associated with each variable are given in Supporting Information (Table S2, tabs 6–10). Of the 2,336 unique candidate SNPs associated with the climate variables, 1,440 SNPs were linked to functionally annotated genes (*Blast* -score > 60), which represents 10.6% of the total candidates set (13,534). T_{MAX} delivered the highest amount of linked functionally annotated genes (474), followed by P_{WQ}(312), T_{SEAS} (237), P_{MA} (214) and T_{MIN} (203). Most of these candidate SNPs were linked to functionally annotated genes (Table 2) . For example, JAR00198, associated with both T_{SEAS} and $T_{MIN,}$ was located in a trans-cinnamate 4-monooxygenase (TCMO) gene; JAR00662, associated with $T_{SEAS,}$ was found in a UPF0496 protein gene; two SNPS associated with $T_{MAX,}$ JAR00038 and JAR00207 were found

on transcription repressor MYB6 and transcription factor MYB44 genes respectively. For $P_{MA,}$ JAR02395 was located in a peroxidase 72 gene; and for P_{WQ}, JAR00273 was located in a 10 kDa chaperonin gene.

Gene ontology enrichment analysis explored how groups of annotated SNPs relate to similar functions (Table S2, tabs 11-15). Several enriched GO terms in the biological process category are highlighted (Table 3): a GO term associated with response to light stimulus (GO:0009416) was found with the SNPs related to T_{SEAS}. Genes associated with this GO term are linked to cellular response processes (in terms of components movement, enzyme production, and secretion and protein expression) from abiotic stimulus, specifically electromagnetic radiation and light. A GO term related to karrikin stimulus was found associated with T_{MIN} (GO:0080167). As for P_{MA} and P_{WQ}, GO terms with high counts of SNPs were found for each variable (GO:0044763 and GO:1901566, respectively) as well as a term related to UV response (GO:0009411) associated with P_{MA}.

Landscape Modelling

The SNPs associated with enriched GO terms (Table 3) were used in a GDM analysis to measure allelic turnover across climatic gradients (Figure 4). The patterns of allelic turnover varied by climatic variable: overall, GDM showed small to moderate response, in terms of deviance explained. The GO term associated with P_{WQ} explained more deviance on the SNPs group (n = 21 ; 21.22%, Figure 4e) than any other climate variable association using GO-groups of SNPs, followed by GO terms associated with T_{MIN} (n =15 ; 14.27%, Figure 4c). T_{SEAS}, T_{MAX} and P_{MA}showed a similar deviance for allelic turnover composition (<5% for each group of SNPs). A specific SNP associated with P_{WQ} , JAR00476, explained the highest deviance (35.5%) of all the GO terms groups of SNPs used for the GDM modelling. We also applied a GDM analysis to all individual SNPs associated with the 5 climatic variables (Figure S4), and the SNP that explained the highest deviance for each variable was selected to display spatial patterns of allelic turnover (Figure 5): T_{SEAS} – JAR00269 (39.2%); T_{MAX} – JAR11943 (25.5%); T_{MIN} – JAR01172 (16.8%); P_{MA} – JAR10596 (21.9%) and P_{WQ} – JAR06621 (36.9%). The SNP associated with T_{SEAS} (JAR00269) explained more deviance than any other in the whole dataset across the 5 climate variables, followed by a SNP associated with P_{WQ} (JAR06621). There is rapid turnover noticeable for the three temperature variables from the coastal to eastern populations in the north of the range, and more gradual turnover from the northern populations to the southern populations (Figure 5a, b, c). But even among the three temperature variables, there are major differences in adaptive patterns. For instance, while T_{SEAS} and T_{MAX} display a similar rapid turnover from the coastal to eastern populations in the north of the range, and fairly gradual turnover from the northern populations to the southern populations, T_{MIN} follows the same trend in the northern region, but a rapid turnover is present between the coastal and inland populations in the south region. In contrast, the precipitation variables showed rapid turnover in the southern or central parts of the distribution, and more gradual turnover in the northern distribution (Figure 5d, e). In southern areas, P_{WQ} shows a rapid turnover between coastal and inland southern populations, while P_{MA} shows a more gradual pattern in this region.

DISCUSSION

Our study identified putative patterns of climate adaptation in jarrah, with several strong associations between candidate SNPs and climatic gradients. The results provide support for our hypothesis of strong patterns of local adaptation to climate across the distribution of jarrah, although, contrary to our second hypothesis, we found adaptation to both temperature and precipitation variables rather than primarily with precipitation. As expected, annotation highlighted functional genes associated with biological processes, some of which relate to abiotic stress factors and provide good candidates for adaptations. Furthermore, the landscape genomics modelling assessed the magnitude of allelic turnover for candidate SNPs and highlighted temperature seasonality, mean maximum temperature of the warmest month and precipitation of the warmest quarter as explaining significantly more variation than other climate drivers. These patterns indicate that adaptive variants are independently sorting across the landscape, which is consistent with our fourth hypothesis. We discuss the mechanisms for adaptation to climate across complex landscape for forest trees, including a direct comparison with a co-dominant co-occurring foundation species, before providing

the scientific basis for implementation of management and conservation strategies to promote the resilience of foundation tree species.

Signatures of adaptation

These associations, indicating local adaptation, were found despite high levels of gene flow among populations across the distribution as it is common in eucalypt species (Supple et al., 2018; Murray et al., 2019; Ahrens et al., 2019a; Jones et al., 2002). Low differentiation among populations indicates that application of EAA in jarrah is appropriate to identify alleles putatively under selection. Overall, the distinct EAA approaches identified different sets of SNPs as potential candidates under selection for each climate variable, which is expected given the different statistical frameworks of the methods (Forester et al., 2018; Caye et al., 2019). One limitation of EEAs is the identification of SNPs that are found to be under selection but are in fact not (false positives). While false positives are an inherent limitation in EAA studies, it is considered that they are useful in consistently identifying adaptive SNPs, even if the adaptive coefficient is small (Ahrens et al., 2021a). We focus our interpretation on SNPs that are within gene space to lessen the impact of false positives, despite that candidate SNPs identified outside of gene space could also be true positives. For instance, SNPs could be in promoter regions, regions that are known to have high proportion of adaptive variants (Wittkopp & Kalay, 2012), SNPs could share a large haplotype with genes that are under selection (Todesco et al., 2020), or SNPs could be in linkage disequilibrium with adaptive SNPs. Future work should focus on improving the genomic resources of the species to elucidate these complex issues that are beyond the scope of this work.

Adaptation to temperature and precipitation

GDM analysis on all candidate SNPs found the highest deviance explained for a SNP associated with T_{SEAS} (39.2%), closely followed by P_{WQ} (36.9%), with overall results for all candidates showing low to moderate deviance across the 5 variables. Furthermore, P_{WQ} was linked to GO:1901566, with the highest number of associated SNPs (21) and also showed the highest deviance explained by the GDM analysis (Figure 4e). Overall, both temperature and precipitation variables are linked to adaptive genetic variants through the multi-EAA and annotation approach; although, GO and GDM analysis highlighted the specific precipitation variable (P_{WQ}) as a stronger adaptation driver.

The annotations of identified genes were made based on the reference genome of *Eucalyptus grandis* , a distant relative, so we provide a pertinent but cautious preliminary interpretation of functional results until a full jarrah (*E. marginata*) reference genome becomes available. Gene functions associated with the temperature and precipitation variables show biological functions associated with response and adaptation to these abiotic factors. For example, the KCS gene family (JAR02659), that was associated with T_{SEAS}, has been linked to cold and light responses (Joubes et al., 2008) in*Arabidopsis* , being involved in the biosynthesis of waxes that cover the leaves surface. Two SNPs (JAR13256 and JAR08936) are linked to the ABC transporter gene families, which have been shown to be associated with heat response and abiotic stress tolerance during seed germination (Zhang et al., 2012; Hwang et al., 2016). Similarly, for T_{MAX}, several SNPs were found to be linked to this same gene family (JAR07223, JAR03208, JAR09260, JAR00867, JAR09847, JAR03598 and JAR08936) as well as one for T_{MIN} (JAR01172). The HSF gene family was linked to a SNP (JAR02134) associated with both T_{SEAS} and T_{MAX}, and this gene family is identified as a strong thermotolerance regulator (Scharf et al., 1990) and has been widely reported in various plant species (Duan et al., 2019; Zhang et al., 2020a). The SNP JAR07972 associated with T_{MIN} was found in CBL genes that have been related to adaptation and tolerance to low temperatures, among other abiotic stresses (Ren et al., 2014; Su et al., 2020). The MYB transcription factors gene family has been extensively associated with abiotic tolerance, specifically cold and heat stress, but also dehydration (Mmadi et al., 2017; Liao et al., 2017). SNPs linked to this family were found across four climatic variables: T_{SEAS} (JAR07671 and JAR04859), T_{MAX} (JAR00038, JAR00207, JAR07671, JAR04859 and JAR07108), T_{MIN} (JAR08943) and P_{WQ} (JAR07671). In addition, the SNP associated with P_{WQ} (JAR13490) was found in the chromatin-remodelling factor PKL gene that has been consistently linked to multiple plant development processes, particularly to the abscisic acid (ABA) pathway regulation (Perruc et al., 2007). ABA is a phytohormone that is well known for controlling stomatal

closure (Rajab et al., 2019, Maheshwari et al., 2020), thus being crucial for efficient drought response (Yu et al., 2019, Zhang et al., 2020b). From P_{MA}, one SNP (JAR01263) was found associated with the methionine gamma-lyase (MGL) gene. MGL is an enzyme which activity is upregulated by osmotic stress caused by drought (Joshi & Jander, 2009) and it is considered to act as an osmolyte, protecting the plant tissues from dehydration (Gagne-Bourque et al., 2016). These are just a sample of the many compelling gene functions associated to both temperature and precipitation found across the five tested climatic variables, identifying these as potential drivers of local adaptation.

Functionally related genes have similar adaptive patterns

In our analysis of gene ontology enrichment, we focused on biological processes related with response to abiotic stress factors such as drought, cold and heat. Generally, we found that there was overrepresentation of important biological GO terms associated with adaptation in similar ways, consistent with expectations under our third hypothesis. For instance, a GO term associated with T_{SEAS}, GO:0009416, is related to light stimulus. Some of the SNPs associated with this GO term have been previously highlighted for the annotated functional results (e.g., JAR02659). A GO term related to karrikin stimulus was found associated with T_{MIN}(GO:0080167). Karrikins are a group of phytohormones that control several aspects of plant germination and growth and can be found in the smoke from wildfires (Nelson et al., 2012). This is especially important in SWWA where there is an ongoing shift to warmer and drier climatic conditions, and consequent increases in fire frequency in this fire prone environment (Dey et al., 2019, Kala et al., 2020). The term GO:1901566 was found for a high number of SNPs (21) that were associated with P_{WQ}. This term is related to organonitrogen compound biosynthetic process, a broad biological process that involves chemical reactions and pathways related to nitrogen metabolism. Organic nitrogen metabolism is a vital process for plant physiology and its regulation has been shown to be dependent on abiotic factors, such as temperature and water availability (Zielke et al., 2002; Gundale et al., 2012). GO term SNPs associated with T_{MIN} and P_{WQ} showed the highest deviance explained by the GDM analysis. High allelic turnover is observed for two SNPs in T_{MIN}, JAR03088 and JAR05151, and an even greater magnitude for two SNPs in P_{WQ}, JAR00476 and JAR11797. The SNP JAR00476 in particular explained more deviance than any other SNP linked to GO terms; and its functional annotation is associated with a MADS-box protein SOC1-like, involved in flowering regulation (Lee et al., 2000) and shown to be responsive to abiotic factors such as cold temperatures (Sheldon et al., 2006). Many plant functional traits are polygenic, involving complex interactions controlled by multiple genes, so it is also expected that patterns of climate adaptation are also the result of combined effects from several alleles of small-effect (Wadgymar et al., 2017). Indeed, climatic variables are expected to not be the main driver for variation in some candidate SNPs, as the genes associated can be pleiotropic and may be under selection from other biotic or abiotic factors. For example, although precipitation and temperature are consistently highlighted as key factors influencing plant' distribution and ecology, soil properties greatly affect these settings, as water availability depends on the interaction between climatic variables and soil characteristics (Piedallu et al., 2013). The identification and understanding of adaptive genetic variations might then be improved by including other relevant biotic factors such as soil characteristics. Nevertheless, by hierarchically categorising gene functions, we were able to find consistent adaptive patterns across the distribution, highlighting polygenic adaptations to climate variables in this species.

Adaptive variants are independently sorted

Across the species geographic distribution, climatic heterogeneity explains significant genomic variation. In particular, T_{SEAS} and P_{WQ} showed strong associations between environments and gene pools. The patterns of genomic turnover associated with the studied climatic variables are aligned with the climatic gradients of the region (Figure 5). These associations are indicative of the multidimensional patterns of adaptation resulting in orthogonal intraspecific selection among SNPs (White & Butlin, 2021). Here, we define dimensionality as the interaction between orthogonal climate variables to independently describe each habitat. Our dimensionality is driven by climate, and the independent sorting of putatively adaptive variants is indicative of this complex pattern. It has been modelled that local adaptation increases with dimensionality (MacPherson et al. 2015), and it likely leads to dimensionality of phenotypic traits (Kirkpatrick and Meyer

2004; McGuigan et al. 2005). Indeed, there is evidence of intraspecific variation among growth and morphological traits (e.g., height and diameter at breast height) locally adapted in jarrah, associated to climatic factors (O'Brien et al., 2007, 2010; Koch & Samsa, 2007).

In some ways, increased dimensionality is ubiquitous with increased habitat heterogeneity, and habitat heterogeneity has been shown to drive signatures of adaptation to temperature and precipitation in tree species (Shryock et al. 2020; von Takach et al., 2021; Walters et al., 2021). While these studies did not explore dimensionality explicitly, their results nevertheless show that tree species are able to independently adapt to multiple types of environments. While such patterns of differential adaptation makes management of the species more complex and nuanced in the future, our results provide a level of understanding that will allow for targeted responses to changing climatic conditions in different areas.

Landscape adaptations of forests

Comparative analysis can provide broader patterns for forest management, where concurrent genetic and spatial patterns of local adaptation within co-occurring tree species provides strong evidence for environmental fitness and evolution (Bragg et al., 2015). Our analysis here identified SNPs associated with both temperature and precipitation in jarrah; while a similar study on a co-occurring species, marri (*Corymbia calophylla*), found SNPs associated with temperature to explain more deviance than precipitation (Ahrens et al., 2019a), thereby suggesting that temperature is a stronger driver of local adaptation for marri. It is interesting that there were similarities in functional genes associated with several adaptive variants between jarrah and marri (e.g., ABC transporters and CBL gene families). Comparison of SNPs across both species identified a set of 26 SNPs that were also found to be associated with at least one of the five variables analysed (Table S3). Most of these shared SNPs are associated with either T_{MAX} (16) or P_{WQ} (12) in jarrah; while for marri, the majority of the shared SNPs are associated with T_{MAX} (24), which is consistent with adaptation to both temperature and precipitation in jarrah and with temperature in marri. This comparison adds weight to our finding that the hypothesis of precipitation as a more important driver than temperature in jarrah is not supported

as our evidence indicates that both temperature and precipitation are important climate factors for adaptation in jarrah.

Management perspectives

Our analysis of standing genetic variation across the distribution of jarrah found putative links between potentially adaptive loci and crucial abiotic factors in stress response, which provide a source of adaptation to climate change. The evidence that genetic variants are involved with climate adaptation occurred as either association with specific gene functions or abiotic responses. Our analysis here, and that of the co-dominant species marri, are also consistent with results from recent genomic studies on other eucalypt species in other regions of Australia (Steane et al., 2017; Jordan et al., 2017, 2020), providing evidence of adaptation to climate in natural populations and stressing the role of temperature (particularly T_{SEAS} and T_{MAX}) and precipitation (P_{WQ}) variables. The presence of climate adaptation provides a basis for implementation of assisted gene migration for forest management strategies (Aitken & Bemmels, 2016) and climate adjusted provenance in restoration practices (Prober et al., 2015). As a foundation tree, jarrah is a vital component in the ecosystem and has a significant role in regulating local hydrological systems and carbon storage (CCWA, 2013; Bradshaw, 2015). Additionally, it offers abundant habitats for a wide variety of groups, from vascular flora and lichens to terrestrial vertebrates and birds (Whitford & Williams, 2002; Whitford et al., 2015), as well as unique food sources for fauna, especially birds (Wrigley, 2012; Lee et al., 2013). Since the middle of the 19th century, it has been a major component of timber production from SWWA forests (CCWA, 2013; Davison, 2015). The Forest Management Plan 2014–2023 (CCWA, 2013) for SWWA forests has provision for implementation of assisted gene migration in management strategies for response to climate change. Our findings of standing variation harbouring putative adaptations to climate associated with temperature and precipitation factors provides an evidence base for design and implementation of such strategies. In addition, phenotypic approaches on other eucalypt species have also highlighted the role of local climate in

the development of adaptive traits (Costa e Silva et al., 2019; Ahrens et al., 2019b, Ahrens et al., 2021b). Expanding this work to a phenotypic approach in jarrah for identifying patterns of plasticity and adaptation associated with climate would contribute to further understanding the association of genomic and phenotypic diversity across environmental gradients. While it appears that genetic variants associated with similarly functioning genes are adapting to the environment in similar ways, we found that putative adaptations among climate variables are sorted through the landscape in contrasting ways. This makes implementation of assisted gene migration strategies more complex and targeted in particular areas of the distribution. In fact, our findings illustrate that perhaps we could consider germplasm from multiple sources to bolster the adaptability in adaptively depauperate populations and, by design, we would implicitly choose variation from functionally related genes, potentially increasing the diversity, adaptability, and new combinations of genetic variations.

ACKNOWLEDGEMENTS

The authors thank Bronwyn Macdonald for assistance with laboratory work.

FUNDING

Funding support was provided by the Australian Research Council (LP150100936), Western Australia Department of Biodiversity, Conservation and Attractions, and Eucalypt Australia.

AVAILABILITY OF DATA AND MATERIALS

The datasets supporting the conclusions of this article are available in the Murdoch University

institutional repository, [DOI will be provided upon acceptance].

AUTHOR CONTRIBUTIONS

JC Filipe, CW Ahrens, PD Rymer, M Byrne and G Hardy designed the research. JC Filipe and R Mazanec collected and prepared all samples for sequencing. JC Filipe and CW Ahrens analysed and interpreted the data. JC Filipe wrote the manuscript with intellectual contribution from all authors.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

COMPETING INTERESTS

The authors declare that they have no competing interests.

ADDITIONAL FILES

Additional file 1 – Supplementary figures and tables. (docx)

Figure S1 – Cross entropy scores for all Ks in the SNMF analysis of *Eucalyptus marginata*

Figure S2 – Individual assignment of ancestral genetic clusters in *Eucalyptus marginata* using SNMF (K=6)
Table S1 – Number of candidate SNPs identified as having significant correlations with climate variables by RDA, LFMM
Figure S3 – Identification of SNPs under putative selection using redundancy analysis (RDA) in *Eucalyptus marginata*
Figure S4 – Geographic generalized dissimilarity modelling (GDM) in *Eucalyptus marginata* showing allelic turnover from
Table S3 – SNPs identified in *Eucalyptus marginata* shared with those identified in a with previous study on *Corymbia ca*

Additional file 2 – Supplementary tables. (xlsx)

Table S2 – Candidate SNPs from environmental association analysis (tabs 1-5); full annotation results for

all candidate SNPs (tabs 6-10); and top GO terms (tabs 11-15) for each climate variable.

FIGURES AND TABLES

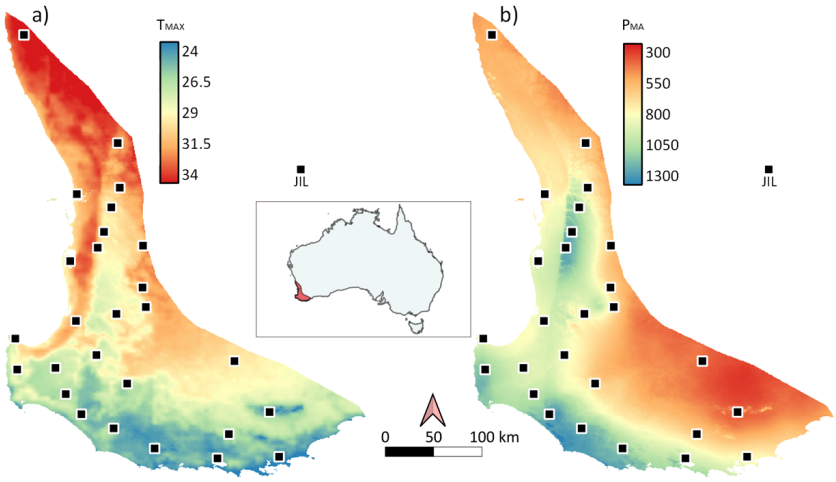

Figure 1 – Sampling locations of *Eucalyptus marginata*in south-west Western Australia (black squares). Two climate gradients are shown for the species distribution area: a) maximum temperature of the warmest month, (°C; T_{MAX}) and b) mean annual precipitation (mm; P_{MA}). Bio-climatic layers from worldclim.org (Fick & Hijmans, 2017). Insert shows distribution of*Eucalyptus marginata* in Australia.

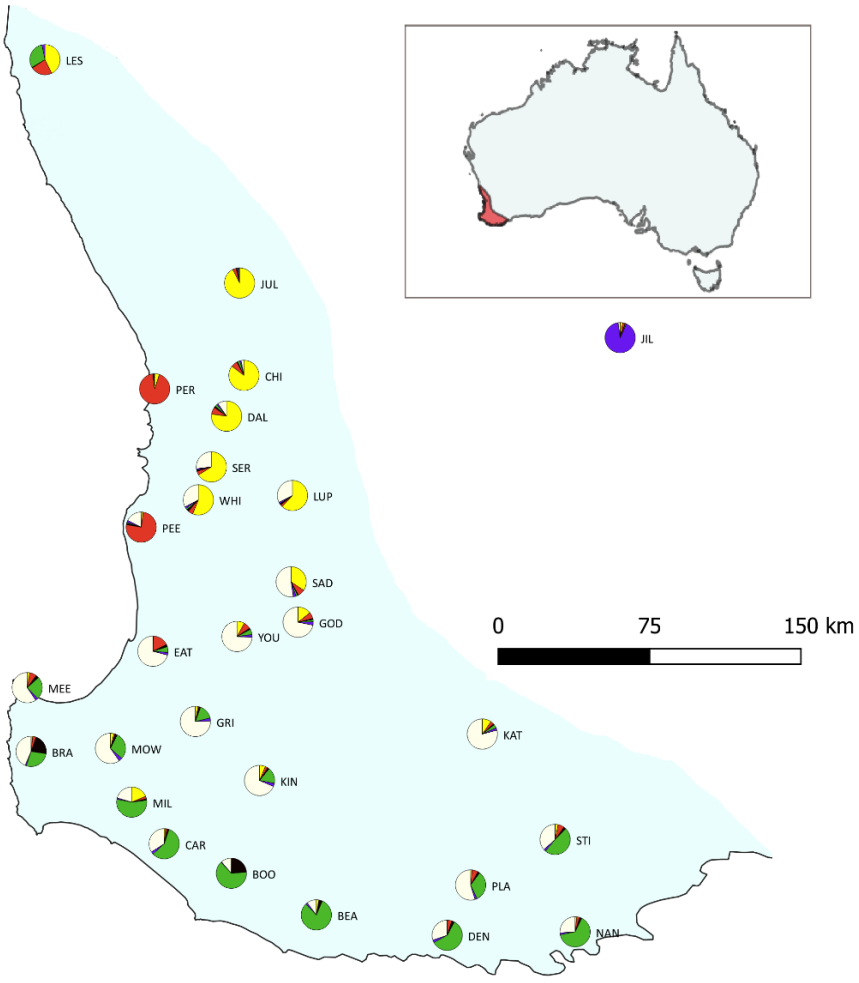

Figure 2 – Distribution of sampled *Eucalyptus marginata*displaying population structure probabilities for $K = 6$ genetic clusters, depicted as pie charts. Refer to Table 1 for more details on each population.

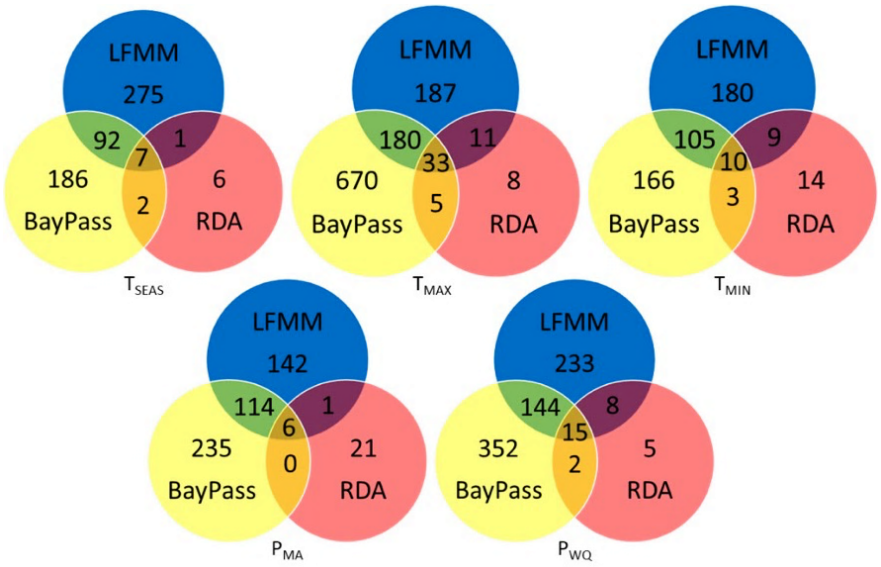

Figure 3 – Summary of environmental association analysis in*Eucalyptus marginata* . Venn diagrams show the intersections between the three approaches of environmental association analyses (RDA, red; LFMM, blue; BAYPASS, yellow) considering candidate SNPs associated with each of the climate variables: T_{SEAS} = temperature seasonality; T_{MAX} = mean maximum temperature of the warmest month; T_{MIN} = mean minimum temperature of the coldest month; P_{MA} = mean annual precipitation; P_{WQ} = mean precipitation of the warmest quarter.

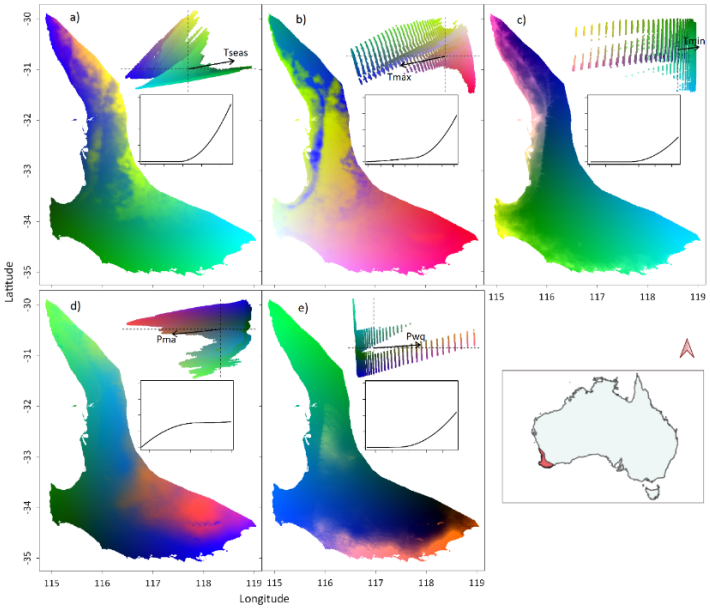

Figure 5 – Predicted spatial variation of allelic turnover based on the outputs from GDM of the top SNP for each climate variable for *Eucalyptus marginata.* a) T_{SEAS} – JAR00269; b) T_{MAX} – JAR11943; c) T_{MIN} – JAR01172; d) P_{MA} – JAR10596 and e) P_{WQ}– JAR06621. Principal component analysis (PCA plots in the top right corner of each map) was applied to reduce transformed climatic variables and assign RGB colours.

Similarity of colours within each map frame indicates similarity in predicted patterns of genetic composition. Insets are spline plots of partial genetic distance (y-axis) by climatic distance (x-axis) for the individual SNP (dimensions of the plot are the same as in Figure 4).

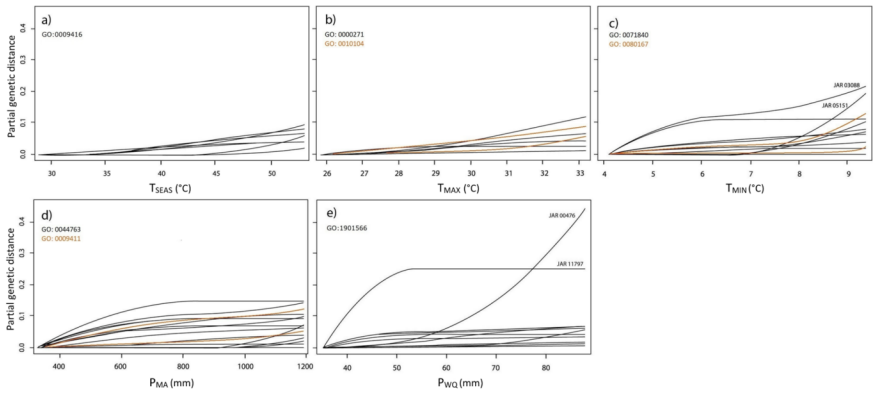

Figure 4 – Geographic generalized dissimilarity modelling (GDM) in *Eucalyptus marginata* showing SNPs allelic turnover for gene functions (GO terms) across each environmental variable. T_{SEAS} = temperature seasonality; T_{MAX} = mean maximum temperature of the warmest month; T_{MIN} = mean minimum temperature of the coldest month; P_{MA} = mean annual precipitation; and P_{WQ} = mean precipitation of the warmest quarter. GO terms with different SNP sets in the same plot are represented with different colours (black or orange).

Table 1 – Locations and climatic variables for the 28 sampled populations of *Eucalyptus marginata* in SWWA. Lat = latitude; Long = longitude; T_{SEAS} = temperature seasonality; T_{MAX} = mean maximum temperature of the warmest month; T_{MIN} = mean minimum temperature of the coldest month; P_{MA} = mean annual precipitation; and P_{WQ}= mean precipitation of the warmest quarter. Temperature (T) and precipitation (P) variables are expressed in degrees Celsius (°C) and millimetres (mm) respectively.

Population	Code	Lat	Long	T_{SEAS}	T_{MAX}	T_{MIN}	P_{MA}	P_{WQ}
Mt Lesueur	LES	-30.1644	115.1991	41.1	32.2	8.2	578	35
Julimar	JUL	-31.3491	116.2470	49.0	33.1	6.1	635	44
Jilakin Rock*	JIL	-31.6647	118.3261	52.8	33.2	5.0	326	46
Chidlow	CHI	-31.8622	116.2266	47.4	32.3	6.1	876	54
Perry Lakes	PER	-31.9436	115.7838	37.6	30.4	9.4	765	38
Dale	DAL	-32.1017	116.1900	45.9	31.5	6.2	1053	58
Serpentine	SER	-32.3451	116.072	43.9	30.6	6.4	1151	57
Lupton	LUP	-32.5292	116.5003	48.3	31.4	4.3	705	45
Whittaker	WHI	-32.5499	116.0100	43.1	29.9	5.8	1190	62
Peel	PEE	-32.6920	115.7103	37.5	30.4	8.3	888	42
Saddleback	SAD	-32.9967	116.535	46.1	30.8	4.3	681	44
Godfrey	GOD	-33.2142	116.5712	45.0	30.2	4.1	661	45
Yourdaming	YOU	-33.3035	116.2407	43.9	30.4	4.1	851	46
Eaton	EAT	-33.3177	115.7482	39.2	30.5	6.7	853	47
Meelup	MEE	-33.5939	115.088	30.1	27.4	9.1	839	43
Grimwade	GRI	-33.7612	115.9988	40.2	29.6	5.3	881	53

Population	Code	Lat	Long	T_{SEAS}	T_{MAX}	T_{MIN}	P_{MA}	P_{WQ}
Katanning	KAT	-33.8294	117.5731	41.9	29.2	5.2	457	50
Bramley	BRA	-33.9035	115.0871	28.8	26.1	8.8	1072	54
Mowen	MOW	-33.9133	115.5434	34.7	27.8	6.9	965	54
Nannup	NAN	-33.9852	115.7778	36.1	28.3	6.6	928	56
Kingston	KIN	-34.0825	116.3374	38.8	28	5.1	785	61
Milylannup	MIL	-34.1928	115.6654	32.3	26.6	7.4	1027	64
Stirling Range	STI	-34.3850	117.9927	35.4	26.9	5.8	493	67
Carey	CAR	-34.4257	115.8223	30.6	26	7.6	1112	72
Boorara	BOO	-34.6126	116.2060	31.4	25.9	6.9	1126	79
Plantagenet	PLA	-34.6402	117.4987	33.7	26.7	6.5	738	79
Beadmore rd	BEA	-34.8171	116.4834	31.3	25.8	7.0	1088	83
Denmark	DEN	-34.9535	117.3805	30.3	25.8	7.6	976	88

*Outlier population

Climate	**SNP**	**RDA (*p*-value)**	**LFMM (*p*-value)**	**BAYPASS (BF)**	**chr**	**Blast e-val**	**Gene ann**
T_{SEAS}	**JAR00166**	-	0.00034	4.708	un	1.0E-28	Mitochondr
	JAR00198	-	0.00064	6.788	10	1.0E-28	Trans-cinna
	JAR00273	-	3.11E-05	21.663	11	1.0E-28	Mitochondr
	JAR00499	-	0.00071	-	8	1.0E-28	Probable L
	JAR00662	-	4.78E-06	-	6	1.0E-28	UPF0496 p
T_{MAX}	JAR00038	-	-	3.270	6	8.0E-29	Transcripti
	JAR00207	-	-	3.054	6	8.0E-29	Transcripti
	JAR00209	-	-	9.466	11	8.0E-29	AT-hook m
	JAR00214	-	-	11.262	6	8.0E-29	Protein ind
	JAR00262	-	-	6.154	4	8.0E-29	Uncharacte
T_{MIN}	JAR00013	-	-	9.801	10	8.0E-29	Mitochondr
	JAR00166	-	-	18.303	un	1E-28	Mitochondr
	JAR00198	-	0.00026	6.788	10	1E-28	Trans-cinna
	JAR00273	-	1.32E-06	-	11	1E-28	Mitochondr
	JAR00620	0.242	-	-	11	8.0E-29	Uncharacte
P_{MA}	JAR00027	-	0.00014	10.316	7	1.0E-28	Mitochondr
	JAR00500	-	-	6.788	4	1.0E-28	Putative yi
	JAR01426	-	0.0004	-	11	1.0E-28	Tyrosine de
	JAR01512	-	0.0001	-	5	1.0E-28	Uncharacte
	JAR02395	-	0.00092	-	9	1.0E-28	Peroxidase
P_{WQ}	JAR00214	0.454	0.00053	-	6	8E-29	Protein ind
	JAR00273	-	-	11.889	11	8E-29	10 kDa cha
	JAR00499	-	0.00021	-	8	1E-28	Probable L
	JAR00690	-	0.00031	6.266	1	8E-29	Zinc finger
	JAR01091	-	5.34E-07	4.388	7	1E-28	LOB doma

Table 2 – Gene annotation showing the top five SNPs (*Blast* score = 125) for *Eucalyptus marginata* , with NCBI blast e-value score, relative ranks based on levels of significance for each EAA and chromosome number (chr) from *Eucalyptus grandis*genomic mapping for each environmental variable. T_{SEAS} = temperature seasonality; T_{MAX} = mean maximum temperature of the warmest month; T_{MIN} = mean minimum temperature of the coldest month; P_{MA} = mean annual precipitation; and P_{WQ} = mean precipitation of the warmest quarter). SNPs that were simultaneously found associated with GO terms (table 3) are in bold.

Climate	**GO id**	**GO term**	**GDM % deviance explained**	***p*-value**	**SNPs**	**Count**
T_{SEAS}	GO:0009314	Response to light stimulus	3.66	0.00261	JAR02551, JAR04603, JAR00284, JAR02659, JAR06621, JAR04257, JAR07363, JAR00198, JAR01133, JAR07395	10
T_{MAX}	GO:0000271	Polysaccharide biosynthetic process	3.81	0.0067	JAR05227, JAR06489, JAR06314, JAR08046, JAR12549, JAR11847, JAR08134, JAR12439	8
	GO:0010104	Regulation of ethylene-activated signalling pathway		0.0097	JAR09402, JAR12137	2
T_{MIN}	GO:0071840	Cellular component organization or biogenesis	14.27	0.00859	JAR05151, JAR02381, JAR00166, JAR02528, JAR04603, JAR03088, JAR05858, JAR01284, JAR06869, JAR05668, JAR04700, JAR07286	12
	GO:0080167	Response to karrikin		0.00391	JAR06869, JAR00198, JAR03623	3

Climate	GO id	GO term	GDM % deviance explained	p-value	SNPs	Count
P_{MA}	GO:0044763	Single-organism cellular process	4.62	0.0061	JAR07368, JAR04995, JAR05607, JAR07695, JAR05954, JAR12984, JAR11156, JAR00284, JAR11454, JAR08984, JAR13223, JAR06091, JAR07363, JAR08184, JAR12280	15
	GO:0009411	Response to UV		0.0059	JAR00284, JAR07363	2
P_{WQ}	GO:1901566	Organonitrogen compound biosynthetic process	21.22	0.0064	JAR12369, JAR00189, JAR00543, JAR11122, JAR05879, JAR12666, JAR02347, JAR11253, JAR11737, JAR06747, JAR07829, JAR06097, JAR10308, JAR12789, JAR12316, JAR00476, JAR13196, JAR11797, JAR11414, JAR11170, JAR10452	21

Table 3 – Overrepresented gene ontology (GO) terms for SNPs identified in *Eucalyptus marginata* for each environmental variable. T_{SEAS} = temperature seasonality; T_{MAX} = mean maximum temperature of the warmest month; T_{MIN} = mean minimum temperature of the coldest month; P_{MA} = mean annual precipitation; and P_{WQ}= mean precipitation of the warmest quarter by count of SNPs and/or relevant biological functio

REFERENCES

Ahrens, C. W., Rymer, P. D., Stow, A., Bragg, J. G., Dillon, S. K., Umbers, K. D. L., & Dudaniec, R. Y.

(2018). The search for loci under selection: Trends, biases and progress. Molecular Ecology, 27, 1342–1356. https ://doi.org/10.1111/mec.14549

Ahrens, C.W., Byrne, M., & Rymer, P.D. (2019a). Standing genomic variation within coding and regulatory regions contributes to the adaptive capacity to climate in a foundation tree species. Molecular Ecology, 28, 2502–16. https://doi.org/10.1111/mec.15092

Ahrens, C.W., Andrew, M.E., Mazanec, R.A., Ruthrof, K.X., Challis, A., Hardy, G., Byrne, M., Tissue, D.T., & Rymer, P.D. (2019b). Plant functional traits differ in adaptability and are predicted to be differentially affected by climate change. Ecology and Evolution, 10, 232–248. https://doi.org/10.1002/ece3.5890

Ahrens, C.W., Jordan, R., Bragg, J., Harrison, P.A., Hopley, T., Bothwell, H., Murray, K., Steane, D.A., Whale, J.W., Byrne, M., Andrew, R., & Rymer, P.D. (2021a). Regarding the F-word: the effects of data filtering on inferred genotype-environment associations. Molecular Ecology Resources, https://doi.org/10.1111/1755-0998.13351

Ahrens, C.W., Rymer, P.D. & Tissue, D.T. (2021b). Intra-specific trait variation remains hidden in the environment. New Phytologist, 229: 1183-1185. https://doi.org/10.1111/nph.16959

Aitken, S.N., & Bemmels, J.B. (2016). Time to get moving: assisted gene flow of forest trees. Evolutionary Applications, 9, 271–290. https://doi.org/10.1111/eva.12293

Altschul, S.F., et al., (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research, 25, 3389-3402. https://doi.org/10.1093/nar/25.17.3389

Anderson, J.T., Willis, J.H., & Mitchell-Olds, T. (2011). Evolutionary genetics of plant adaptation. Trends in Genetics, 27, 258–266. https:// doi.org/10.1016/j.tig.2011.04.001

Aspinwall, M.J., Pfautsch, S., Tjoelker, M.G., Vårhammar, A., Possell, M., Tissue, D.T., Drake, J.E., Reich, P.B., Atkin, O.K., Rymer, P.D., Dennison, S., & Van Sluyter, S.C. (2019). Range size and growth temperature influence *Eucalyptus* species responses to an experimental heatwave. Global Change Biology, 25, 1665–1684. https://doi.org/10.1111/gcb.14590

BOM and CSIRO. State of the climate. 2020. Available at: http://www.bom.gov.au/state-of-the climate/documents/State-of-the-Climate-2020. pdf [Accessed 08 May 2021]

Bradshaw, F.J. (2015). Reference material for jarrah forest silviculture. Forest Management Series FEM061. Department of Parks and Wildlife, Perth, pp. 141.

Bragg, J. G., Supple, M. A., Andrew, R. L., & Borevitz, J. O. (2015). Genomic variation across landscapes: Insights and applications. New Phytologist, 207, 953–967. https://doi.org/10.1111/nph.13410

Brondizio, E.S., Settele, J., Díaz, S., & Ngo, H.T. (2019). Global assessment report on biodiversity and ecosystem services of the intergovernmental science-policy platform on biodiversity and ecosystem services. IPBES secretariat, Bonn, Germany. https://doi.org/10.5281/zenodo.3553579

Byrne, M., Macdonald, B., & Francki, M. (2001). Incorporation of sodium sulfite into extraction protocol minimizes degradation of *Acacia*DNA. BioTechniques, 30, 742–4–748. https://doi.org/10.2144/01304bm06

Camarero, J.J., Alvarez-Taboada, F., Hevia, A., & Castedo-Dorado, F. (2018). Radial growth and wood density reflect the impacts and susceptibility to defoliation by gypsy moth and climate in radiata pine. Frontiers in Plant Science, 9, 1582. https://doi.org/10.3389/fpls.2018.01582

Capblancq, T., Fitzpatrick, M.C., Bay, R.A., Exposito-Alonso, M., & Keller, S.R. (2020). Genomic prediction of (mal)adaptation across current and future climatic landscapes. Annual Review of Ecology, Evolution, and Systematics, 51, 245–69. https://doi.org/10.1146/annurev-ecolsys-020720-042553

Carlo, M.A., Riddell, E.A., Levy, O., & Sears, M.W. (2018). Recurrent sublethal warming reduces embryonic survival, inhibits juvenile growth, and alters species distribution projections under climate change. Ecology Letters, 21, 104–116. https://doi.org/10.1111/ele.12877

Caye, K., Jumentier, B., Lepeule, J., & François, O. (2019). LFMM 2: Fast and accurate inference of gene-environment associations in genome-wide studies. Molecular Biology and Evolution, 36, 852 860. https://doi.org/10.1093/molbev/msz008

CCWA. (2013). Forest Management Plan 2014–2023. Conservation Commission of Western Australia (CCWA), Perth.

Chhatre, V.E., Fetter, K.C., Gougherty, A..V, Fitzpatrick, M.C., Soolanayakanahally, RY., et al., (2019). Climatic niche predicts the landscape structure of locally adaptive standing genetic variation. BioRxiv 817411. https:// doi.org/10.1101/817411

Christmas, M.J., Breed, M.F., & Lowe, A.J. (2016). Constraints to and conservation implications for climate change adaptation in plants. Conservation Genetics, 17, 305–20. https://doi.org/10.1007/s10592-015-0782-5.

Collevatti, R.G., Novaes, E., Silva-Junior, O.B., Vieira, L.D., Lima-Ribeiro, M.S., & Grattapaglia, D. (2019). A genome-wide scan shows evidence for local adaptation in a widespread keystone neotropical forest tree. Heredity, 123, 117–137. https://doi.org/10.1038/s41437-019-0188-0

Coop, G., Witonsky, D., Di Rienzo, A., & Pritchard, J. K. (2010). Using environmental correlations to identify loci underlying local adaptation. Genetics, 185, 1411–1423. https://doi.org/10.1534/genetics.110.114819

Corcobado, T., Cubera, E., Juarez, E., Moreno, G., & Solla, A. (2014). Drought events determine performance of *Quercus ilex* seedlings and increase their susceptibility to *Phytophthora cinnamomi* . Agricultural and Forest Meteorology, 192, 1–8. https://doi.org/10.1016/j.agrformet.2014.02.007

Correia, B., Hancock, R.D., Amaral, J., Gomez-Cadenas, A., Valledor, L., Pinto, G. (2018). Combined drought and heat activates protective responses in *Eucalyptus globulus* that are not activated when subjected to drought or heat stress alone. Frontiers in Plant Science, 9, 819. https://doi.org/10.3389/fpls.2018.00819

Costa e Silva, J., Potts, B., Harrison, P.A., & Bailey, T. (2019). Temperature and rainfall are separate agents of selection shaping population differentiation in a forest tree. Forests, 10, 1145. https://doi.org/10.3390/f10121145

Davison, E.M. (2015). How *Phytophthora cinnamomi* became associated with the death of *Eucalyptus marginata* – the early investigations into jarrah dieback. Australasian Plant Pathology, 44, 263-271. https://doi.org/10.1007/s13313-015-0356-5

Davison, E.M. (2018). Relative importance of site, weather and phytophthora cinnamomi in the decline and death of *Eucalyptus marginata* – Jarrah dieback investigations in the 1970s to 1990s. Australasian Plant Pathology, 47, 245–57. https://doi.org/10.1007/s13313-018-0558-8.

Dey, R., Lewis, S.C., Arblaster, J.M., & Abram NJ (2019). A review of past and projected changes in Australia's rainfall. Wiley Interdiscip Rev: Clim Change, 10, 577. https://doi/org/10.1002/wcc.577

Doyle JJ and Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus, 12, 13–15.

Duan, S., Liu, B., Zhang, Y., Li, G., & Guo, X. (2019). Genome-wide identification and abiotic stress-responsive pattern of heat shock transcription factor family in *Triticum aestivum* L. BMC Genomics, 20, 1–20. https://doi.org/10.1186/s12864-019-5617-1

Ferrier, S., Manion, G., Elith, J., & Richardson, K. (2007). Using generalized dissimilarity modelling to analyze and predict patterns of beta diversity in regional biodiversity assessment. Diversity and Distributions,

13, 252–264. https://doi.org/10.1111/j.1472-4642.2007.00341

Fick, S.E., & Hijmans, R.J. (2017). Worldclim 2: New 1-km spatial resolution climate surfaces for global land areas. International Journal of Climatology, 37, 4302–4315. https://doi.org/10.1002/joc.5086

Fitzpatrick, M.C., & Keller, S.R. (2015). Ecological genomics meets community-level modelling of biodiversity: Mapping the genomic landscape of current and future environmental adaptation. Ecology Letters, 18, 1–16. https ://doi.org/10.1111/ele.12376

Forester, B.R., Lasky, J.R., Wagner, H.H., & Urban D.L. (2018). Comparing methods for detecting multilocus adaptation with multivariate genotype–environment associations. Molecular Ecology, 27, 2215–33. https://doi.org/10.1111/mec.14584.

Frichot, E., Schoville, S.D., Bouchard, G., & Francois, O. (2013). Testing for associations between loci and environmental gradients using latent factor mixed models. Molecular Biology and Evolution, 30, 1687– 1699. https://doi.org/10.1093/molbev/mst063

Frichot, E., & Francois, O. (2015). LEA: an R package for landscape and ecological association studies. Methods in Ecology and Evolution, 6, 925– 929. https://doi.org/10.1111/2041-210X.12382

Gagne-Bourque, F., Bertrand, A., Claessens, A., Aliferis, K. A., & Jabaji, S. (2016). Alleviation of drought stress and metabolic changes in timothy (*Phleum pratense* L.) colonized with *Bacillus subtilis* B26. Frontiers in Plant Science, 7, 584. https://doi.org/10.3389/fpls.2016.00584

Gautier, M. (2015). Genome-wide scan for adaptive divergence and association with population specific covariates. Genetics, 201, 1555–1579. https://doi.org/10.1534/genetics.115.181453

Gentilesca, T., Camarero, J.J., Colangelo, M., Nole, A. & Ripullone, F. (2017). Drought-induced oak decline in the western Mediterranean region: an overview on current evidences, mechanisms and management options to improve forest resilience. iForest - Biogeosciences and Forestry, 10, 796–806. https://doi.org/10.3832/ifor2317-010

Goudet, J. (2005). Hierfstat, a package for R to compute and test variance components and F -statistics. Molecular Ecology Notes, 5, 184–186. https://doi.org/10.1111/j.1471-8286.2004.00828.

Gougherty, A.V., Keller, S.R., Chhatre, V.E., & Fitzpatrick, M.C. (2020). Future climate change promotes novel gene-climate associations in balsam poplar (*Populus balsamifera* L.), a forest tree species. BioRxiv, 961060. https://doi.org/10.1101/2020.02.28.961060

Gougherty, A. V., Keller, S. R., & Fitzpatrick, M. C. (2021). Maladaptation, migration and extirpation fuel climate change risk in a forest tree species. Nature Climate Change, 11, 166–171. https://doi.org/10.1038/s41558-020-00968-6

Gugger, P.F., Fitz-Gibbon, S.T., Albarran-Lara, A., Wright, J.W. & Sork, V.L. (2021). Landscape genomics of *Quercus lobata* reveals genes involved in local climate adaptation at multiple spatial scales. Molecular Ecology, 30, 406–423. https://doi.org/10.1111/mec.15731

Gundale, M.J., Nilsson, M., Bansal, S., & Jaderlund, A. (2012). The interactive effects of temperature and light on biological nitrogen fixation in boreal forests. New Phytologist, 194, 453–463. https://doi.org/10.1111/j.1469-8137.2012.04071.x

Guzella, T.S., Dey, S., Chelo, I.M., Pino-Querido, A., Pereira, V.F., Proulx, S.R., et al., (2018). Slower environmental change hinders adaptation from standing genetic variation. PLoS Genetics, 14, e1007731. https://doi.org/10.1371/journal. pen.1007731

Harris, R.M.B., Beaumont, L.J., Vance, T.R., Tozer, C.R., Remenyi, T., Perkins-Kirkpatrick, S.E., et al. (2018). Biological responses to the press and pulse of climate trends and extreme events. Nature Climate Change, 8, 579–587. https://doi.org/10.1038/s41558-018-0187-9

Hoffmann, A., Griffin, P., Dillon, S., Catullo, R., Rane, R., Byrne, M., & Sgro, C. (2015). A framework for incorporating evolutionary genomics into biodiversity conservation and management. Climate Change Responses, 2, 1. https://doi.org/10.1186/s4066 5-014-0009-x

Honjo, M.N., & Kudoh, H. (2019). *Arabidopsis halleri* : a perennial model system for studying population differentiation and local adaptation. AoB PLANTS, 11, 1–13. https://doi.org/10.1093/aobpla/plz076.

Hwang, J.U., Song, W.Y., Hong, D., Ko, D., Yamaoka, Y., Jang, S., & Lee, Y. (2016). Plant ABC transporters enable many unique aspects of a terrestrial plant's lifestyle. Molecular Plant, 9, 338–355. https://doi.org/10.1016/j.molp.2016.02.003

Ingvarsson, P. K., & Bernhardsson, C. (2020). Genome-wide signatures of environmental adaptation in European aspen (*Populus tremula*) under current and future climate conditions. Evolutionary Applications, 13, 132–142. https://doi.org/10.1111/eva.12792

IPCC, 2021: Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Masson-Delmotte, V., P. Zhai, A. Pirani, S. L. Connors, C. Pean, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M. I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J. B. R. Matthews, T. K. Maycock, T. Waterfield, O. Yelekci, R. Yu and B. Zhou (eds.)]. Cambridge University Press. In Press.

Jakobsson, M., & Rosenberg, N. A. (2007). CLUMPP: A cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics, 23, 1801–1806. https://doi.org/10.1093/bioin forma tics/btm233

Jones, R. C., Steane, D. A., Potts, B. M., & Vaillancourt, R. E. (2002). Microsatellite and morphological analysis of *Eucalyptus globulus* populations. Canadian Journal of Forest Research, 32, 59–66. https://doi.org/10.1139/x01-172

Jordan, R., Hoffmann, A.A., Dillon, S.K., & Prober, S.M. (2017). Evidence of genomic adaptation to climate in *Eucalyptus microcarpa* : Implications for adaptive potential to projected climate change. Molecular Ecology, 26, 6002–6020. https://doi.org/10.1111/mec.14341

Jordan, R., Prober, S.M., Hoffmann, A.A., & Dillon, S.K. (2020). Combined analyses of phenotype, genotype and climate implicate local adaptation as a driver of diversity in *Eucalyptus microcarpa*(grey box). Forests, 11, 495. https://doi.org/10.3390/F11050495.

Joshi, V. & Jander, G. (2009). *Arabidopsis* methionine gamma-lyase is regulated according to isoleucine biosynthesis needs but plays a subordinate role to threonine deaminase. Plant Physiology, 151, 367 378. https://doi.org/10.1104/pp.109.138651

Joubes, J., Rafaele, S., Bourdenx, B., Garcia, C., Laroche-Traineau, J., Moreau, P., et al., (2008). The VLCFA elongase gene family in*Arabidopsis thaliana* : phylogenetic analysis, 3D modelling and expression profiling. Plant Molecular Biology, 67, 547–566. https://doi.org/10.1007/s11103-008-9339-z

Kala, J., Tenna A.S., Rudloff, D., Andrys, J., Rieke, O., & Lyons, T.J. (2020). Evaluation of the weather research and forecasting model in simulating fire weather for the South-west of Western Australia." International Journal of Wildland Fire, 29, 779–92. https://doi.org/10.1071/WF19111.

Kass, R. E., & Raftery, A. E. (1995). Bayes factors. Journal of the American Statistical Association, 90, 773–795. https://doi.org/10.1080/01621 459.1995.10476572

Kawecki, T. J., & Ebert, D. (2004). Conceptual issues in local adaptation. Ecology Letters, 7, 1225–1241. https ://doi.org/10.1111/j.1461-0248.2004.00684.x

Kilian, A., Wenzl, P., Huttner, E., Carling, J., Xia, L., Blois, H., ... Uszynski, G. (2012). Diversity arrays technology: A generic genome profiling technology on open platforms. In F. Pompanon & A. Bonin (Eds.),

Data production and analysis in population genomics: Methods and protocols (pp. 67–89). Totowa, NJ: Humana Press.

Kirkpatrick, M., &Meyer, K. (2004). Direct estimation of genetic principal components: simplified analysis of complex phenotypes. Genetics, 168, 2295–2306. https://doi.org/10.1534/genetics.104.029181.

Koch, J.M., & Samsa, G.P. (2007). Restoring Jarrah Forest in southwestern Australia after bauxite mining. Restoration Ecology, 15, S17–S25. https://doi.org/10.1111/j.1526-100X.2007.00289.x

Kremer, A., Ronce, O., Robledo-Arnuncio, J.J., Guillaume, F., Bohrer, G., Nathan, R., Bridle, J.R, Gomulkiewicz, R., Klein, E.K., Ritland. K., Kuparinen, A., Gerber, S., & Schueler, S. (2012). Long-distance gene flow and adaptation of forest trees to rapid climate change. Ecology Letters, 15, 378–392. https://doi.org/10.1111/j.1461-0248.2012.01746.x

Kryazhimskiy, S., & Plotkin, J. B. (2008). The population genetics of dN/dS. PLoS Genetics, 4, e1000304. https://doi.org/10.1371/journ al.pgen.1000304

Lee, H., Suh, S.S., Park, E., Cho, E., Ahn, J. H., Kim, S.G., et al., (2000). The AGAMOUS-LIKE 20 MADS domain protein integrates floral inductive pathways in *Arabidopsis* . Genes and Development, 14, 2366–2376.

Lee, J., Finn, H., & Calver, M. (2013). Feeding activity of threatened black cockatoos in mine-site rehabilitation in the jarrah forest of south-western Australia. Australian Journal of Zoology ,61, 119 131. https://doi.org/10.1071/ZO12101

Levin, P., & Poe, M. (2017). Conservation for the Anthropocene Ocean: interdisciplinary science in support of nature and people. Cambridge: Academic Press, 530.

Liao, C., Zheng, Y., & Guo, Y. (2017). MYB30 transcription factor regulates oxidative and heat stress responses through ANNEXIN-mediated cytosolic calcium signalling in *Arabidopsis* . New Phytologist, 216, 163–177. https://doi.org/10.1111/nph.14679

MacPherson, A., Hohenlohe P.A., & Nuismer, S.L. (2015). Trait dimensionality explains widespread variation in local adaptation. Proceedings of the Royal Society B: Biological Sciences, 282, 1802. https://doi.org/10.1098/rspb.2014.1570.

Maheshwari, P., Assmann, S.M., & Albert, R. (2020). A guard cell abscisic acid (ABA) network model that captures the stomatal resting state. Frontiers in Physiology, 11, 11-927 https://doi.org/10.3389/fphys.2020.00927.

Mangin, B., Siberchicot, A., Nicolas, S., Doligez, A., This, P., & Cierco-Ayrolles, C. (2012). Novel measures of linkage disequilibrium that correct the bias due to population structure and relatedness. Heredity, 108, 285–291. https://doi.org/10.1038/hdy.2011.73

Manion, G., Lisk, M., Ferrier, S., Nieto-Lugilde, D., Mokany, K., & Fitzpatrick, M. C. (2018). gdm: Generalized dissimilarity modeling. R package version 1.3.7. Retrieved from https://cran.rproject.org/web/packages/gdm/index.html

Martins, K., Gugger, P. F., Llanderal-Mendoza, J., Gonzalez-Rodrigruez, A., Fitz-Gibbon, S. T., Zhao, J., Rodriguez-Correa, H., Oyama, K. & Sork, V. L. (2018). Landscape genomics provides evidence of climate-associated genetic variation in Mexican populations of*Quercus rugosa* . Evolutionary Applications, 11, 1842–1858. https://doi.org/10.1111/eva.12684

Matusick, G., Ruthrof, K., Brouwers, N.C., Dell, B., & Hardy, G.S.J. (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. https://doi.org/10.1007/s10342-013-0690-5

Mayol, M. (2019). A multiscale approach to detect selection in non-model tree species: widespread adaptation despite population decline in *Taxus baccata* L. – Evolutionary Applications, 13, 143–160. https://doi.org/10.1111/eva.12838

McChesney, C.J., Koch, J.M., & Bell, D.T. (1995). Jarrah forest restoration in Western Australia: Canopy and topographic effects. Restoration Ecology, 3, 105–110. https://doi.org/10.1111/j.1526100X.1995.tb00083.x

McGuigan, K., Chenoweth, S.F., & Blows, M.W. (2005). Phenotypic divergence along lines of genetic variance. American Naturalist, 165, 32–43. https://doi.org/10.1086/426600.

Mmadi, M.A., Dossa, K., Wang, L., Zhou, R., Wang, Y.,Cisse, N., Sy, M.O., & Zhang, X. (2017). Functional characterization of the versatile MYB gene family uncovered their important roles in plant development and responses to drought and waterlogging in sesame. Genes, 8, 362. https://doi.org/10.3390/genes8120362

Murray, K.D., Janes, J.K., Jones, A., Bothwell, H.M., Andrew, R.L., & Borevitz, J.O. (2019). Landscape drivers of genomic diversity and divergence in woodland *Eucalyptus* . Molecular Ecology, 28, 5232 5247. https://doi.org/10.1111/mec.15287

Myburg, A.A., Grattapaglia, D., Tuskan, G.A., Hellsten, U., Hayes, R.D., Grimwood, J., Schmutz, J., et al., (2014). The genome *of Eucalyptus grandis* . Nature, 510, 356–362. https://doi.org/10.1038/nature13308

Nelson, D.C., Flematti, G.R., Ghisalberti, E.L., Dixon, K.W., & Smith, S.M. (2012). Regulation of seed germination and seedling growth by chemical signals from burning vegetation. Annu Rev Plant Biol., 63, 107–30. https://doi.org /10.1101/gad.813600.

O'Brien, E.K., Mazanec, R.A., & Krauss, S.L (2007). Provenance variation of ecologically important traits of forest trees: implications for restoration. Journal of Applied Ecology, 44, 583–93. https://doi.org/10.1111/j.1365-2664.2007.01313.x.

O'Brien, E.K., & Krauss, S.L. (2010). Testing the home-site advantage in forest trees on disturbed and undisturbed sites. Restoration Ecology, 18, 359–372. https://doi.org/10.1111/j.1526100X.2008.00453.x

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., ... Wagner, H. (2018). vegan: Community Ecology Package. R package version 2.5-1. Retrieved from https://cran.rproject.org/web/packa ges/vegan/index.html

Pacifici, M., Foden, W., Visconti, P., et al., (2015). Assessing species vulnerability to climate change. Nature Climate Change, 5, 215–224. https://doi.org/10.1038/nclimate2448

Perruc, E., Kinoshita, N., & Lopez-Molina, L. (2007). The role of chromatin-remodeling factor PKL in balancing osmotic stress responses during *Arabidopsis* seed germination. The Plant Journal, 52, 927-936. https://doi.org/10.1111/j.1365-313X.2007.03288.x

Piedallu, C., Gegout, J.C., Perez, V., & Lebourgeois, F. (2013) Soil water balance performs better than climatic water variables in tree species distribution modelling. Global Ecology and Biogeography, 22, 470–482. https://doi.org/10.1111/geb.12012

Pritzkow, C., Szota, C., Williamson, V., & Arndt, S.K. (2020). Phenotypic plasticity of drought tolerance traits in a widespread eucalypt (*Eucalyptus obliqua*). Forests, 11, 1371.doi:https://doi.org/10.3390/f11121371

Prober, S.M., Byrne, M., McLean, E.H., Steane, D.A., Potts, B.M., Vaillancourt, R.E., & Stock, W.D. (2015). Climate-adjusted provenancing: a strategy for climate-resilient ecological restoration. Frontiers in Ecology and Evolution, 3, 1–5. https://doi.org/10.3389/fevo.2015.00065.

QGIS.org. (2021). QGIS Geographic Information System. QGIS Association. http://www.qgis.org

Queiros, L., Deus, E., Silva, J.S., Vicente, J., Ortiz, L., Fernandes, P.M., & Castro-Diez, P. (2020). Assessing the drivers and the recruitment potential of *Eucalyptus globulus* in the Iberian Peninsula. Forest Ecology and Management, 466, 118147. https://doi.org/10.1016/j.foreco.2020.118147

R Core Team. (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Rajab, H., Khan, M.S., Malagoli, M., Hell, R., & Wirtz, M. (2019). Sulfate-induced stomata closure requires the canonical aba signal transduction machinery. Plants, 8, 21. https://doi.org/10.3390/plants8010021

Reed, T.E., Schindler, D.E., & Waples, R.S. (2011). Interacting effects of phenotypic plasticity and evolution on population persistence in a changing climate. Conservation Biology, 25, 56–63. https://doi.org/10.1111/j.1523-1739.2010.01552.x

Ren, L., Sun, J., Chen, S., Gao, J., Dong, B., Liu, Y., & Jiang, J. (2014). A transcriptomic analysis of *Chrysanthemum nankingense*provides insights into the basis of low temperature tolerance. BMC Genomics, 15, 844. https ://doi.org/10.1186/1471-2164-15-844

Rocha, S.M.G., Vidaurre, G.B., Pezzopane, J.E.M., Almeida, M.N.F., Carneiro, R.L., Campoe, O.C., Scolforo, H.F., Alvares, C.A., Neves, J.C.L., Xavier, A.C., & Figura, M.A. (2020). Influence of climatic variations on production, biomass and density of wood in*Eucalyptus* clones of different species. Forest Ecology and Management, 473, 118290. https://doi.org/10.1016/j.foreco.2020.118290

Rosenberg, N. A. (2004). distruct: A program for the graphical display of population structure. Molecular Ecology Notes, 4, 137–138. https :// doi.org/10.1046/j.1471-8286.2003.00566.x

Scharf, K. D., Rose, S., Zott, W., Schofl, F., & Nover, L. (1990). Three tomato genes code for heat stress transcription factors with a region of remarkable homology to the DNA binding domain of the yeast HSF. EMBO J., 13, 4495-501. https://doi.org/10.1002/j.14602075.1990.tb07900.x

Sexton, J.P., Strauss, S.Y., & Rice, K.J. (2011). Gene flow increases fitness at the warm edge of a species' range. Proceedings of the National Academy of Sciences, 108, 11704–11709. https://doi.org/10.1073/pnas.1100404108

Sheldon, C.C., Finnegan, E.J., Dennis, E.S., & Peacock, W.J. (2006). Quantitative effects of vernalization on FLC and SOC1 expression. The Plant Journal, 45, 871–883. https://doi.org/10.1111/j.1365 313X.2006.02652.x

Shryock, D. F., Washburn, L. K., DeFalco, L. A., & Esque, T. C. (2020). Harnessing landscape genomics to identify future climate resilient genotypes in a desert annual. Molecular Ecology, 792, 698-717. https://doi.org/10.1111/mec.15672

Soto-Correa, J.C., Saenz-Romero, C., Lindig-Cisneros, R., & de la Berrera, E. (2012). The neotropical shrub *Lupinus elegans* , from temperate forests, may not adapt to climate change. Plant Biology, 15, 607-610. https://doi.org/10.1111/j.1438-8677.2012.00716.x

Standish, R.J., Daws, M.I., Gove, A.D., Didham, R.K., Grigg, A.H., Koch, J.M., & Hobbs, R.J. (2015). Long term data suggest jarrah-forest establishment at restored mine sites is resistant to climate variability. Journal of Ecology, 103, 78–89. https://doi.org/10.1111/1365-2745.12301

Staudinger, M.D., Carter, S.L., Cross, M.S., Dubois, NS., Duffy, J. E., Enquist, C., ... Turne, W. (2013). Biodiversity in a changing climate: A synthesis of current and projected trends in the US. Frontiers in Ecology and the Environment, 11, 465–473. https://doi.org/10.1890/120272=

Steane, D. A., McLean, E. H., Potts, B. M., Prober, S. M., Stock, W. D., Stylianou, V. M., ... Byrne, M. (2017). Evidence for adaptation and acclimation in a widespread eucalypt of semi-arid Australia. Biological

Journal of the Linnean Society, 121, 484–500. https://doi.org/10.1093/biolinnean/blw051

Stoneman, G.L., Turner, N.C., & Dell, B. (1994). Leaf growth, photosynthesis and tissue water relations of greenhouse-grown*Eucalyptus marginata* seedlings in response to water deficits. Tree Physiology, 14, 633-646. http://dx.doi.org/10.1093/treephys/14.6.633

Su, W., Huang, L., Ling, H., et al., (2020). Sugarcane calcineurin B-like (*CBL*) genes play important but versatile roles in regulation of responses to biotic and abiotic stresses. Scientific Reports, 10, 167. https://doi.org/10.1038/s41598-019-57058-7

Supple, MA., Bragg, J.G., Broadhurst, L.M., Nicotra, A.B., Byrne, M., Andrew, R.L., & Borevitz, J.O. (2018). Landscape genomic prediction for restoration of a *Eucalyptus* foundation species under climate change. eLife, 7, e31835. https://doi.org/10.7554/eLife.31835

The Gene Ontology Consortium. (2019). The Gene Ontology Resource: 20 years and still GOing strong. Nucleic Acids Research, 47, D330–D338. https://doi.org/10.1093/nar/gky1055

Tian, F., Yang, D.C., Meng, Y.Q., Jin, J., & Gao. G. (2020). PlantRegMap: charting functional regulatory maps in plants. Nucleic Acids Research, 48, D1104–13. https://doi.org/10.1093/nar/gkz1020.

Todesco, M., Owens, G.L., Bercovich, N., Legare, J.S., Soudi, S., Burge, D.O., Huang, K., Ostevik, K.L., Drummond, E.B.M., Imerovski, I., Lande, K., Pascual-Robles, M.A., Nanavati, M., Jahani,M., Cheung, W., Staton, S.E., Munos, S., Nielsen, R., Donovan, L.A., Rieseberg, L.H. (2020). Massive haplotypes underlie ecotypic differentiation in sunflowers. Nature, 584, 602-607. https://doi.org/10.1038/s41586-020-2467-6

Urban, M. (2015). Accelerating extinction risk from climate change. Science, 348, 571–573. https://doi.org/10.1126/science.aaa4984

von Takach, B., Ahrens, C.W., Lindenmayer, D.B. and Banks, S.C. (2021), Scale-dependent signatures of local adaptation in a foundation tree species. Molecular Ecology, 30, 2248-2261. https://doi.org/10.1111/mec.15894

Wadgymar, S.M., Lowry, D.B., Gould, B.A., Byron, C.N., Mactavish, R.M., & Anderson, J.T. (2017). Identifying targets and agents of selection: innovative methods to evaluate the processes that contribute to local adaptation. Methods in Ecology and Evolution, 8, 738–749. https://doi.org/10.1111/2041-210X.12777

Walters, S.J., Robinson, T.P., Byrne, M., Wardell-Johnson, G.W., & Nevill, P. (2021). Association of putatively adaptive genetic variation with climatic variables differs between a parasite and its host. Evolutionary Applications, 14, 1732– 1746. https://doi.org/10.1111/eva.13234

Water Corporation. (2020) Streamfow. https://pw-cdn.watercorporatio n.com.au/Our-water/Rainfall-and-dams/Streamfow. Accessed 11/05/2021

Weir, B.S., & Cockerham, C.C. (1984). Estimating F -statistics for the analysis of population structure. Evolution, 38, 1358–1370,

Wheeler, M.A., Byrne, M., & McComb, J.A. (2003). Little genetic differentiation within the dominant forest tree, *Eucalyptus marginata* (Myrtaceae) of south-western Australia. Silvae Genetica, 52, 254-259. http://researchrepository.murdoch.edu.au/id/eprint/16460

White, N.J. & Butlin, R.K. (2021). Multidimensional divergent selection, local adaptation, and speciation. Evolution. https://doi.org/10.1111/evo.14312

Whitford, K.R., & Williams, M.R. (2002) . Hollows in jarrah and marri trees, II: selecting trees to retain for hollow dependent fauna. Forest Ecology and Management, 160, 2151–2232. https://doi.org/10.1016/S0378-1127(01)00447-9

Whitford, K.R., Wiseman, D., McCaw, W.L., & Bradshaw, F.J. (2015). Characteristics of nest trees and nest hollows used by the forest red-tailed black cockatoo (*Calyptorhynchus banksii naso*) in south-

west Western Australia: comments on Johnstone et al., (2013). Pacific Conservation Biology, 21,133 145. https://doi.org/10.1071/PC14911

Wittkopp, P. J., & Kalay, G. (2012). Cis-regulatory elements: Molecular mechanisms and evolutionary processes underlying divergence. Nature Reviews Genetics, 13, 59–69. https://doi.org/10.1038/nrg3095

Wrigley, J., & Fagg, M. (2012). Eucalypts: A Celebration. Allen and Unwin, Crowsnest, New South Wales, p. 60. ISBN: 9781743310809

Yu, W., Zhao, R., Wang, L., Zhang, S., Li, R., Sheng, J., & Shen, L. (2019). ABA signalling rather than ABA metabolism is involved in trehalose-induced drought tolerance in tomato plants. Planta, 250, 643-655. https://doi.org/10.1007/s00425-019-03195-2

Zhang, H., Li, G., Fu, C., Duan, S., Hu, D., & Gu, X. (2020a). Genome-wide identification, transcriptome analysis and alternative splicing events of Hsf family genes in maize. Scientific Reports, 10, 1–11. https://doi.org/10.1038/s41598-020-65068-z.

Zhang, H., Liu, D., Yang, B., Liu, W.Z., Mu, B., Song, H., Chen, B., et al., (2020b). *Arabidopsis* cpk6 positively regulates ABA signalling and drought tolerance through phosphorylating ABA-responsive element binding factors." Journal of Experimental Botany, 71, 188–203. https://doi.org/10.1093/jxb/erz432.

Zhang, X., Li, J., Liu, A., Zou, J., Zhou, X., Xiang, J., & Chen, X. (2012). Expression profile in rice panicle: Insights into heat response mechanism at reproductive stage. PLoS ONE, 7, e49652. https://doi.org/10.1371/journal.pone.0049652

Zielke, M., Ekker, A.S., Olsen, R.A., Spjelkavik, S., & Solheim, B. (2002). The influence of abiotic factors on biological nitrogen fixation in different types of vegetation in the High Arctic, Svalbard. Arctic, Antarctic, and Alpine Research, 34, 293-299. https://doi.org/10.1080/15230430.2002.12003497