MS. REBECCA JORDAN (Orcid ID : 0000-0002-4048-6792)

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Evidence of genomic adaptation to climate in *Eucalyptus microcarpa*: implications for adaptive potential to projected climate change

Rebecca Jordan [1], Ary A Hoffmann [1], Shannon K Dillon [2], Suzanne M Prober [3]

[1] Bio21 Institute, School of BioSciences, University of Melbourne, 30 Flemington Rd, Parkville Vic 3010, Australia
[2] CSIRO Agriculture, Clunies Ross Street, Black Mountain ACT 2601, Australia
[3] CSIRO Land and Water, 147 Underwood Ave, Floreat WA 6014, Australia

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Corresponding Author
Name: Rebecca Jordan
Address: Bio21 Institute, University of Melbourne, 30 Flemington Rd, Parkville Vic 3010, Australia
E-mail: Rebecca.Citroen.Jordan@gmail.com

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ABSTRACT

Understanding whether populations can adapt *in situ* or whether interventions are required is of key importance for biodiversity management under climate change. Landscape genomics is becoming an increasingly important and powerful tool for rapid assessments of climate adaptation, especially in long-lived species such as trees. We investigated climate adaptation in *Eucalyptus microcarpa* using the DArTseq genomic approach. A combination of F_{ST} outlier and environmental association analyses were performed using > 4,200 genome-wide single nucleotide polymorphisms (SNPs) from 26 populations spanning climate gradients in south-eastern Australia. Eighty-one SNPs were identified as putatively adaptive, based on significance in F_{ST} outlier tests and significant associations with one or more climate variables related to temperature (70 / 81), aridity (37 / 81) or precipitation (35 / 81). Adaptive SNPs were located on all 11 chromosomes, with no particular region associated with individual climate variables. Climate adaptation appeared to be characterized by subtle shifts in allele frequencies, with no consistent fixed differences identified. Based on these associations, we predict adaptation under projected changes in climate will include a suite of shifts in allele frequencies. Whether this can occur sufficiently rapidly through natural selection within populations, or would benefit from assisted gene migration, requires further evaluation. In some populations, the absence, or predicted increases to near fixation of particular adaptive alleles hint at potential limits to adaptive capacity. Together, these results reinforce the importance of standing genetic variation at the geographical level for maintaining species' evolutionary potential.

INTRODUCTION

Understanding patterns and drivers of local climate adaptation across plant species and the potential for future *in situ* adaptation is important in biodiversity management under climate change (Savolainen *et al.* 2013; Hoffmann *et al.* 2015). Varying selection pressures across a species distribution can result in genetic differences within a species and thus local adaptation (Kawecki & Ebert 2004). Local adaptation, i.e. the superior fitness of local genotypes in local environmental conditions, is well known in plant species (Savolainen *et al.* 2007; Leimu & Fischer 2008; Hereford 2009; Fournier-Level *et al.* 2011; Aitken & Bemmels 2015). However, climate change can uncouple this association, creating a mismatch between population climatic optima and current climate, especially in long-lived trees (Jump &

Peñuelas 2005; Aitken *et al.* 2008). Consequently, to persist under climate change, species must either migrate, alter their phenotype (plastic response) or adapt via genetic changes (evolutionary response) (Aitken *et al.* 2008; Hoffmann & Sgrò 2011).

Adaptation in trees likely arises primarily from standing variation (Alberto *et al.* 2013; Savolainen *et al.* 2013), facilitating more rapid adaptation than adaptation via new mutations (Barrett & Schluter 2008). Nevertheless, current rates of climate change may be faster than tree species' ability to adapt or migrate (Jump & Peñuelas 2005; Aitken *et al.* 2008). Additional challenges such as population fragmentation can reduce gene flow and sharing of adaptive alleles, increasing populations' risk of maladaptation when the environment changes. The position within a species distribution (warm vs cool, central vs peripheral), population size, degree of gene flow and variation in climate changes across the landscape may also affect the risk of a population being maladapted under climate change (Savolainen *et al.* 2007; Alberto *et al.* 2013; Aitken & Bemmels 2015). The ability for *in situ* adaptation is therefore expected to vary across a species' distribution.

Where current populations may be unable to adapt or migrate at a rate to match projected rates of climate change, restoration plantings may be necessary to increase connectivity and gene flow with pre-adapted populations or augment genetic resources through assisted migration, thereby facilitating *in situ* adaptation (Sgrò *et al.* 2011; Weeks *et al.* 2011; Aitken & Bemmels 2015). This needs to be balanced with considerations of potential negative impacts of moving genetic material such as outbreeding depression and adaptation to non-climatic factors such as soils (Aitken & Whitlock 2013). Alternative seed sourcing strategies to traditional local provenancing have been proposed for ecological restoration (Broadhurst *et al.* 2008; Breed *et al.* 2013; Prober *et al.* 2015). These aim to capture adaptive diversity and improve adaptive potential – the potential for an evolutionary adaptive response to, and persistence under changing conditions – of plantings under climate change. Determining where restoration plantings are required to augment genetic resources, how to source seed for such plantings, and assess the benefits versus potential risks of assisted migration, relies on understanding not only the distribution of climate adaptation across a species range, but also adaptive potential of current populations.

Landscape genomics has recently become an important tool for characterising adaptation and its environmental drivers (Stapley *et al.* 2010; Sork *et al.* 2013; Rellstab *et al.* 2015). It applies a 'bottom-up' approach, using genome-wide sequencing to identify genomic regions associated with environmental variables (Sork *et al.* 2013; Rellstab *et al.* 2015). Next-generation sequencing approaches are ideal for non-model organisms as they requires no *a priori* knowledge of traits or underlying candidate genes (Stapley *et al.* 2010). Landscape genomic studies can reveal potentially important environmental drivers of adaptation (e.g. Steane *et al.* 2014; De Kort *et al.* 2014) and the genetic architecture of climate adaptation, including potential genes and pathways involved in adaptation (e.g. Eckert *et al.* 2010b; Christmas *et al.* 2016; Rajora *et al.* 2016).

To date, landscape genomic studies investigating climate adaptation have focused primarily on characterising adaptive variation. However, landscape genomics also offers the ability to assess adaptive potential or vulnerability to climate change in a timely manner, avoiding lengthy common garden trials required for long-lived tree species (Sork *et al.* 2013). Such timely approaches could help identify populations vulnerable to climate change, where management interventions, such as restoration or assisted migration, may be necessary to ensure ongoing sustainability of current populations (Hoffmann *et al.* 2015). Few studies though have used adaptive genomic variants to directly infer future adaptive potential of populations under climate change (though see Rellstab *et al.* 2016).

This study uses landscape genomics to assess genomic signatures of adaptation to climate in the widespread Australian tree species *Eucalyptus microcarpa* (Maiden) Maiden (Grey box), and then applies these results to determine how they could be used to assess the potential for *in situ* adaptation to projected climate change. *Eucalyptus microcarpa* has been extensively cleared for agriculture across south-eastern Australia, resulting in a highly fragmented distribution and widespread use in restoration to mitigate these effects. Understanding the potential for *in situ* adaptation versus the need for introduction of pre-adapted genetic variation through improved connectivity or assisted migration will be essential for determining management actions to enhance long-term sustainability of populations of this species. To assess the potential resilience of *E. microcarpa* populations to climate change, we asked a series of questions. 1) Is there evidence of adaptation to climate and what are the key climatic drivers? 2) How is adaptive variation distributed across the genome, and what are the frequencies of adaptive variants and potential genes associated with climate? 3) What is the potential for *in situ* adaptation to future climate change within populations? 4) What are the implications for seed sourcing for ecological restoration under climate change?

MATERIALS & METHODS

Sampling & DArTseq

Eucalyptus microcarpa was sampled from 26 natural, remnant sites across the main species range in Victoria and New South Wales (NSW), Australia (Figure 1). Sampling sites were chosen to (i) capture the major gradients of mean annual temperature and annual precipitation whilst minimising correlations between these two key variables and between variables and spatial location, though some correlation, especially between temperature and latitude was unavoidable, and to (ii) represent larger populations to minimise the influence of fragmentation on allele frequencies. Following the description of *E. microcarpa* by Bean (2009), we sampled only central and southern NSW to avoid the closely related *E. woollsiana* and potential intergrades between the two species. Leaf material was collected from approximately 20 – 30 mature trees per site, > 40 m apart to avoid close relatives, and frozen immediately in a liquid nitrogen dewar, before later freeze-drying.

DNA was extracted from approximately 20 mg of freeze-dried leaf material using a modified CTAB method as per Jordan *et al.* (2016). Approximately 400 ng of DNA per sample was sent in two batches to Diversity Arrays Technology Pty Ltd, Canberra, for individual Eucalyptus DArTseq, a reduced-representation genomic approach (Sansaloni *et al.* 2011; see Supporting Information – Supplementary methods for further details).

Bioinformatics & SNP calling

Raw, single end 77 bp reads were split, where applicable, by individual barcode using Fastx Barcodesplitter (v 0.0.14; http://hannonlab.cshl.edu/fastx_toolkit/), allowing for a single mismatch. Barcodes, at the 5’ end, low quality 3’ end bases and potential Illumina adaptor sequences were removed with Trimmomatic (v 0.32; HEADCROP:8, TRAILING:3, SLIDINGWINDOW:4:15, ILLUMINACLIP:TruSeq2-SE.fa:0:30:7; Bolger *et al.* 2014) with a minimum overall read length of 36 bp and minimum average read quality of 20. Trimmed reads were aligned to the *Eucalyptus grandis* genome (v 1.1; Myburg *et al.* 2014) using BWA-MEM (Li 2013) with a seed length of 16 and mismatch penalty of 3. Only alignments with a minimum alignment score of 20 were output. For the 580 samples in the final analysis dataset, 93.5% (s.d. 2.4) of reads aligned to the *E. grandis* genome. Single nucleotide polymorphisms (SNPs) were called from aligned reads with GATK UnifiedGenotyper (v 3.3; DePristo *et al.* 2011) using a minimum base (and by default mapping) quality of 20, a minimum variant (SNP) confidence of 10 and a downsampling threshold of 18,000, to ensure all reads were maintained for variant calling.

Data filtering & quality control

Using an initial set of 614 samples, SNPs were filtered using VCFtools v 0.1.14 (Danecek *et al.* 2011) to a minimum genotype depth of 10 ×, minimum genotype quality Phred-score of 30 (99.9% genotype call accuracy, for given read data), a maximum mean locus depth of 100 × (to avoid 'SNPs' from incorrectly aligned paralogs and overrepresented organelle reads), a minor allele frequency of greater than 0.05 and less than 20% missing data across all sites. To reduce linkage, SNPs were pruned using the 'indep-pairwise' function in PLINK v 1.90b3p (Purcell *et al.* 2007), with a window of 50 SNPs, a step of 5 SNPs and an r^2 cut-off of 0.2. Only biallelic SNPs were retained. Samples with > 20% missing data, based on filtered, pruned SNPs were excluded (31 samples from 14 sites). An initial Principal Component Analysis (PCA) was performed using the 'adegenet' package v 2.0.1 (Jombart & Ahmed 2011) in R v 3.2.1 (R Core Team 2015) to identify potential outlier samples in the data, e.g. samples with high genetic divergences that might represent species misidentification. Three outlier samples (one from VGL and two from VMJ) were identified and excluded from further analysis (Supporting Information, Figure S1). A total of 580 samples were retained for analysis (Table 1).

Sample-level genotyping error was estimated for 153 replicate samples included in the two DArTseq runs using the filtered, linkage-pruned SNPs, though excluding 86 SNPs identified with potential run effects (see below). Percentage similarity was calculated between replicate pairs using a custom Python script (RepCheck4.py; available at https://github.com/rebecca-cj/GenomicAdaptation/), allowing for a half match between homozygotes and heterozygotes, and excluding genotypes comparisons with missing data for either replicate. Average genotyping error was 2.2%, or 0.4% when nine samples with a high error rate were excluded (< 90% similarity between replicates).

Reproducibility of SNPs within and between runs was assessed by calculating the, per SNP, average percentage genotype similarity between replicate samples, excluding nine replicates with high within-sample genotype error rates (see above). Calculations were performed using a custom Python script (RepCheck_similarity_by_locus.py; available at https://github.com/rebecca-cj/GenomicAdaptation/), allowing for a half match between homozygotes and heterozygotes. Overall SNP alternate allele frequencies were also calculated for the two DArTseq runs separately using the final analysis sample list of 580 samples (177 from run 1, 403 from run 2) using VCFtools. SNPs with an average similarity within or between runs of < 90%, or with a difference in overall alternate allele frequency between the two DArTseq runs of > 0.15 were excluded (86 SNPs).

After filtering and quality control, 4,218 SNPs for 580 samples from 26 sites were retained for analysis (Table 1).

Population structure

To assess general population structure, an individual-level PCA was performed in adegenet, using all 580 samples and 4,218 filtered SNPs. Isolation-by-distance was tested via a Mantel test in the R package 'vegan' v 2.4–0 (Oksanen *et al.* 2016), with significance tested using 999 permutations. Pairwise F_{ST} values between the 26 sites were calculated in Arlequin. Pairwise geographic distances were calculated in the R package 'geosphere' v 1.5–1 (Hijmans 2015), using the 'distVincentyEllipsoid' option in the 'distm' function. Overall F_{ST} was calculated via AMOVA in Arlequin v 3.5.1.2 (Excoffier *et al.* 2005) with significance assessed using 1000 permutations.

Identification of putatively adaptive SNPs

Successful detection of SNPs under selection can be complicated by false positives. This may result from test models not accurately representing true population structure, or covariance between population structure and climate variables, making effects of drift and selection difficult to separate (De Mita *et al.* 2013; de Villemereuil *et al.* 2014; Lotterhos & Whitlock

2014, 2015; Rellstab *et al.* 2015). To address this issue and identify a robust set of putatively climate-adaptive SNPs, we applied a combined analysis approach that is increasingly being used to look for evidence of adaptation (e.g. Dillon *et al.* 2014; De Kort *et al.* 2014; Christmas *et al.* 2016). We used a set of analyses with different demographic assumptions, that may help reduce false positives (Manel *et al.* 2009; de Villemereuil *et al.* 2014; Rellstab *et al.* 2015) – four F_{ST} outlier tests (BayeScan, FDIST2, hierarchical FDIST2 and Bayenv $X^T X$) with an environmental association analysis (Bayenv2). Only SNPs under directional selection were retained due to high false positive rates in tests for balancing selection (Excoffier *et al.* 2009; Lotterhos & Whitlock 2014). As not all tests identify all SNPs under selection, especially weak selection (Lotterhos & Whitlock 2015), we considered SNPs identified in at least two of the four F_{ST} outlier tests performed and with a strong association with at least one climate variable to be putatively adaptive SNPs (similar to Christmas *et al.* (2016)). In this way we aimed to balance the exclusion of true positives against the inclusion of false positives whilst identifying SNPs robust across test methods and therefore likely to have good support for association with climate. All analyses are described briefly here with further details provided in Supporting Information – Supplementary methods. For all F_{ST} outlier and environmental association programs, a VCFtools PLINK output format was converted to the appropriate input format using PGDspider v 2.0.7.4 (Lischer & Excoffier 2012).

BayeScan v 2.0 (Foll & Gaggiotti 2008) and FDIST2 (Beaumont & Nichols 1996) F_{ST} outlier tests were chosen to represent simple island migration models, with BayeScan accounting for variation in sample size between populations (Foll & Gaggiotti 2008) whilst FDIST2 being better at detecting SNPs under weaker selection (De Mita *et al.* 2013). Both are fairly robust under isolation-by-distance scenarios, as found in *E. microcarpa* (see Results), especially when applying 'neutral parameterization' such as increasing prior odds in BayeScan (Beaumont & Nichols 1996; De Mita *et al.* 2013; Lotterhos & Whitlock 2014). Three independent runs of both BayeScan and FDIST2 (run in Lositan; Antao *et al.* 2008) were performed, with parameter details including false discovery rate (FDR) adjustments given in Supporting Information – Supplementary methods. SNPs were deemed outliers if the $\log_{10}$ Bayes Factor (BF) > 0.5 in all three runs of BayeScan, or if under directional selection (F_{ST} > mean F_{ST}) with $q <= 0.1$ in all three runs of FDIST2.

To account for more complex demographic histories, F_{ST} outliers tests were performed using hierarchical FDIST2 (h-FDIST2; Excoffier *et al.* 2009) and Bayenv $X^T X$, an F_{ST} analogue ($X^T X$; Günther & Coop 2013). Underlying population structure is accounted for by defining a hierarchical population structure (h-FDIST2) or a population-level covariance matrix that does not assume any particular demographic scenario ($X^T X$). In addition, $X^T X$ accounts for variation in sample size. Hierarchical FDIST2 was performed in Arlequin, as per details in Supporting Information – Supplementary methods, using three groups based on PCA results (Figure 2a): 1 = Central NSW, 2 = Southern NSW and Central Victoria and 3 = Western Victoria (refer to Figure 1). For $X^T X$, an input covariance matrix was created in Bayenv2.0 (Coop *et al.* 2010; Günther & Coop 2013) using 2,752 SNPs non-significant in all three other outlier tests ($q > 0.2$ in all three runs of BayeScan and FDIST2 and $p > 0.1$ in h-FDIST2; see Supporting Information – Supplementary methods for details). $X^T X$ was calculated over three independent runs in Bayenv2.0, using the average covariance matrix and 100,000 iterations. SNPs were deemed outliers if under directional selection (F_{ST} > mean F_{ST}) with $q <= 0.1$ from h-FDIST2 or if found in the top 5% of ranked values in all three $X^T X$ runs.

To complement the F_{ST} outlier tests, an environmental association analysis was performed in Bayenv2.0. This can be more sensitive to SNPs under weaker selection than F_{ST} outlier approaches (De Mita *et al.* 2013) though may miss associations that co-vary with population structure (Coop *et al.* 2010; Lotterhos & Whitlock 2015) or have a non-linear relationship with environment. As this study focuses on future adaptive potential of *E. microcarpa,* biologically-relevant climate variables that are projected to change in south-eastern Australia were chosen for analysis. South-eastern Australia is projected to become hotter and drier with an increased frequency of extreme events, and variable changes in precipitation patterns (Reisinger *et al.* 2014). Site-level data for temperature, precipitation, evaporation, moisture and aridity variables were downloaded from the Atlas of Living Australia (http://www.ala.org.au/; accessed 28th Apr 2016) and reduced to ten variables representing key climate variables of temperature (3), precipitation (5) and aridity (2) whilst reducing redundancy between similar variables and minimizing correlations (80% of pairwise correlations $r^2 < 0.6$; Table 2a; Supporting Information, Table S1-2; see Supporting Information – Supplementary methods). Several variables were retained despite high correlations to investigate associations with climate extremes versus climate means (e.g.

Driest and wettest period precipitation; Supporting Information, Table S2). All climate variables were standardised prior to association analysis.

A new covariance matrix for the environmental association analysis was created in Bayenv2.0, using 2,634 SNPs non-significant in all four F_{ST} outlier tests (BayeScan and FDIST2 $q > 0.2$ in all three runs, h-FDIST2 $p > 0.1$, $X^T X$ outside top 10% in all three runs; see Supporting Information – Supplementary methods). Three independent runs of the association analyses were performed in Bayenv2.0 on 'outlier' SNPs – those identified in at least two of the four outlier tests. Each run used the new covariance matrix, the 10 standardised climate variables and 100,000 iterations. The average BF across the three runs was calculated per SNP per climate variable. An average BF > 20 was considered a strong SNP-climate association (following Kass & Raftery 1995 as per the Bayenv2 manual).

Putatively adaptive SNPs for further investigation, hereafter referred to as 'adaptive SNPs', were those identified as an 'outlier' in at least two of the four outlier tests and with a strong association with at least one of the 10 climate variables tested.

A set of putatively 'neutral' SNPs was created, to provide a comparison of population structure with 'adaptive' SNPs. 'Neutral' SNPs were those not significant in all four outlier tests (BayeScan and FDIST2 $q > 0.2$ in all three runs, h-FDIST2 $p > 0.1$, $X^T X$ outside top 10% in all three runs) and annotated as synonymous or intergenic only based on the *E. grandis* genome v1.1 using a conservative window of 5,000 bp up and downstream (SNPs mapped to *E. grandis* gene annotations using SnpEff; Cingolani *et al.* 2012).

Characterisation of putatively adaptive SNPs

To visualise the distribution of SNPs across the genome, all SNPs, including 'outlier' and 'adaptive' SNPs were plotted onto the *E. grandis* v1.1 genome in R. To explore allele frequencies of adaptive SNPs, alternate allele frequencies per site were calculated using VCFtools. Linkage between 'adaptive' SNPs was calculated using the 'r' function in PLINK.

To compare population structure patterns with the full SNP dataset, PCAs using the putatively 'adaptive SNPs' and 'neutral' SNPs were performed in adegenet. To ensure differences between PCAs was not due to fewer SNPs in the adaptive and neutral subsets, PCAs were performed on 10 random sets of 81 and 418 SNPs drawn from the full dataset (with replacement between sets), respectively.

All SNPs were mapped to predicted *E. grandis* gene annotations within 2,000 bp (*E. grandis* genome v1.1) using SnpEff. The window size was based on average linkage disequilibrium in *E. grandis* dropping below $r^2 \approx 0.2$ after 2,000 bp (Silva-Junior & Grattapaglia 2015). Annotations of adaptive SNPs and further interpretation in this study assume sufficient similarity between *E. microcarpa* and *E. grandis* genomes and that predicted *E. grandis* Gene Ontology (GO) terms and *Arabidopsis thaliana* TAIR10 orthologues reflect possible gene functions.

To explore putative Gene Ontologies (GO) for adaptive SNPs, using predicted *E. grandis* gene GO terms, generic, non-species specific GO descriptions were retrieved using YeastMine (http://yeastmine.yeastgenome.org/yeastmine/bag.do, data update Oct-28-2016). Definitions were reduced to specific plant GOslim terms using ‘goatools’ map_to_slim.py script (https://github.com/tanghaibao/goatools, accessed 17th Nov 2016) and the Gene Ontology Consortium plant GOslim database (http://geneontology.org/page/download-ontology, accessed 17th Nov 2016). Enrichment of GO terms was tested in Gowinda v 1.12 (Kofler & Schlötterer 2012; see Supporting Information – Supplementary methods).

Potential for adaptation to future climates

Moving beyond characterisation of adaptive variation, we explored how genomic adaptation information could be used to infer the potential for *in situ* adaptation of populations under climate change. We compared current allele frequencies of adaptive SNPs to theoretical expected allele frequency changes if populations are to match projected climate change. This assumed adjustments in population-level allele frequencies toward those frequencies found in populations currently experiencing the projected climate, as per the linear SNP-climate associations identified. The linear model used in this study does not predict what population allele frequencies *will be*, but rather quantifies the theoretical change in allele frequency ‘required’ under projected climate change and uses this result to consider how feasible *in situ* adaptation may be. Whilst linear models are a simplification of a complex system and many other loci and processes are likely to be involved in adaptation, the primary aim of this study was to gain some initial insight into the pattern and magnitude of allele frequency change that may be associated with projected climate change and how molecular information could be used to infer adaptive potential to projected future climates.

This analysis focused on mean annual temperature, summer and winter precipitation as they had the highest numbers of adaptive SNPs for the two major variables of projected change (temperature and precipitation). Projected future climates for 2070 at each site were drawn from a recent Australia-wide study by Briscoe *et al.* (2016) using data from the Australian Water Availability Project (AWAP; Raupach *et al.* 2009, 2012; see Supporting Information – Supplementary methods for brief description). All models tested used a Representative Concentration Pathway (RCP) of 8.5 which reflects a ‘business-as-usual’ scenario, commensurate with the current trajectory of climate change. Here we present results for ACCESS 1.0 (Bi *et al.* 2013), the top model for Australia based on performance against historical climate data (Watterson *et al.* 2013). As future projections can vary between climate models, we explored two other high-performing models for southern and eastern Australia (Bureau of Meteorology and CSIRO 2015) from those available in Briscoe *et al.* (2016) – HadGEM2-ES (Jones *et al.* 2011) and GFDL-CM3 (Griffies *et al.* 2011). Overall patterns of expected allele frequency changes given projected climate change were similar between the three climate models, with variation in absolute changes reflecting variation in projected climates. As the general patterns are the focus of this study, results for the ACCESS 1.0 model (RCP 8.5) are presented here. Results for the other two models are provided in Supporting Information (see Supporting Information – Supplementary methods). For sites in this study, the ACCESS 1.0 model projected an increase in mean annual temperature (+2.5 °C ± (s.d.) 0.4 by 2070) and a greater increase in winter precipitation compared to summer precipitation though there was high variation in precipitation projections between sites (+6.9% ± 8.6 and +1.4% ± 7.1 for winter and summer precipitation 2070 respectively; Supporting Information, Figure S2).

To determine the expected change in allele frequencies associated with projected climate changes, a simple linear regression of allele frequency versus climate was first performed using all 26 populations and ‘current’ climate data. This linear model reflects the linear association used to identify these ‘adaptive’ SNPs. For each SNP, the allele associated with an increase in the climate variable was used in the linear model. Projected change in climate at each site was calculated as the difference between the projected ‘future’ climate and ‘current’ climate data. The Expected Allele Frequency Change, i.e. that frequency change theoretically expected at a site to match projected future climates, was then calculated using the slope of the linear model (*Slope*) and projected site-level change in climate ($Change_{SITE}$), such that:

$$\text{Expected Allele Frequency Change} = Slope * Change_{SITE}$$

This approach was modeled on that of Rellstab *et al.* (2016), though it used the slope of the linear model only, rather than the full linear model. In this way, we aimed to capture natural variation around the mean SNP-climate association represented by the linear model, recognising the imperfect nature of the model. Note that this model considers only the theoretical change expected within a population, not if or how such a change may occur (see Discussion).

We derived two measures to indicate potential limits to *in situ* adaptation from current standing genetic variation: (1) the proportion of adaptive SNPs currently fixed, based on the population sample, and therefore unable to contribute to adaptation of a local population through allele frequency changes alone, and (2) the proportion of adaptive SNPs that, under expected allele frequency changes, would lead to fixation or to an allele frequency currently not seen within the sampled distribution. These measures were calculated per site for each of the three climate variables.

To understand how far away seed would need to be collected to match projected climate change, the minimum distance to a sampled site with the projected future climate was calculated for mean annual temperature, summer and winter precipitation.

RESULTS

Population structure

Results of the Mantel test (Mantel $r = 0.738$, $p = 0.001$) and ordination of the 4,218 filtered SNPs indicated a clinal population structure, the latter reflecting *E. microcarpa*'s geographic distribution (Figure 2a). These support previous findings in this species (Jordan *et al.* 2016). The first axis (PC1) represented a strong latitudinal cline from central NSW to central

Victoria, with the second axis (PC2) showing an east-west separation of western Victorian sites from more eastern central Victoria and southern NSW sites.

Almost no population structure was seen when using the 418 putatively ‘neutral’ SNPs, except for a weak latitudinal cline on PC1 (Figure 2b). This was not purely due to fewer SNPs being analysed, as more structure was evident in a random subset of 418 SNP from the full dataset (Supporting Information, Figure S3).

Overall F_{ST} was low ($F_{ST} = 0.010$, $p < 0.001$) suggesting little genetic differentiation among populations across the sampled distribution. Together with low variation explained by the first two axes of the full PCA (1.52% in total), a significant relationship between genetic and geographic distance and minimal population structure for putatively ‘neutral’ SNPs, these results suggest low population structure in *E. microcarpa*, likely due to historic widespread gene flow and isolation-by-distance across its range.

Identification of putatively adaptive SNPs

In total, 135 SNPs were identified as directional outliers in at least two of the four outlier tests (Figure 3). BayeScan, FDIST2 (Lositan), h-FDIST2 (Arlequin), and Bayenv $X^T X$ identified 39, 223, 65 and 173 significant directional outlier SNPs respectively. Thirty-four SNPs were identified in all four programs, 21 in three programs and 80 in two programs, with remaining SNPs unique to h-FDIST2 (8), FDIST2 (89) or Bayenv2 $X^T X$ (44). No unique outlier SNPs were identified by BayeScan.

Eighty-one of the 135 putative outlier SNPs had a strong environmental association with at least one of the 10 climate variables tested (Table 2a; see Table 1 for climate gradient ranges). The three temperature-related variables had the highest number of associations (70 SNPs in total), especially annual mean temperature (62 SNPs) and maximum temperature of the warmest period (56 SNPs). Fewer SNPs were associated with precipitation (35 SNPs) or aridity (37 SNPs) variables. For precipitation, most SNPs were associated with winter or summer precipitation, with few associations with annual precipitation or peak dry or wet conditions (precipitation driest / wettest period). Many SNPs were associated with more than one climate category – 33 / 81 SNPs were associated with two climate categories and 14 / 81 were associated with all three categories (Table 2b).

Characterisation of adaptive SNPs

The 81 adaptive SNPs, including associations with different climate categories, were distributed across the entire *E. grandis* genome (Figure 4). There was little linkage between all 81 adaptive SNPs (average r^2 = 0.006, s.d. = 0.008) nor between adaptive SNPs associated with individual climate variables (data not shown).

Ordination of the 81 adaptive SNPs showed a general latitudinal cline (Figure 2c) similar to the full dataset (Figure 2a). However, there was less distinction between western Victoria, central Victoria, and southern NSW sites on the first two PCs of the adaptive SNPs ordination. Lower climate variation east-west in Victoria, compared to north-south variation for the climate variables used to identify adaptive SNPs may explain the lack of distinction between western and central Victorian sites. Despite the reduced structure, putatively adaptive SNPs showed a stronger population structure pattern than expected by chance (Supporting Information, Figure S4) and much stronger pattern than putatively 'neutral' SNPs (Figure 2b). The first two axes of the adaptive SNP ordination explained more variance than the full SNP ordination, though this likely reflects adaptive SNPs being identified based on their greater than expected differentiation across the distribution.

Of the 81 adaptive SNPs identified, 60 were located within 2,000 bp of at least one putative *E. grandis* gene (Supporting Information, Table S3). Only two SNPs were within 2000 bp of the same *E. grandis* gene (1:15999719 and 1:15999761, upstream of gene Eucgr.A01027; Supporting Information, Table S3). Based on mapping to the *E. grandis* genome v1.1, 'adaptive' SNPs were located in a range of genomic regions including genic, regulatory and intergenic (Supporting Information, Table S3). No predicted *E. grandis* genes were found within 2,000 bp for adaptive SNPs associated with maximum absolute mean maximum temperature nor precipitation in the wettest period.

As linkage decays rapidly in eucalypts (Silva-Junior & Grattapaglia 2015), only the 37 adaptive SNPs located within a putative *E. grandis* genic region or 5'/3' UTRs, were considered further (Table 3). Plant GOslim gene ontology terms for predicted *E. grandis* genes associated with the 37 adaptive SNPs suggest climate adaptation may involve a range of biological processes and molecular functions including protein modification, biosynthesis and metabolism, transport and stress responses (Supporting Information, Table S4). Similarly, the best *Arabidopsis thaliana* TAIR10 gene orthologue for predicted *E. grandis* genes suggest adaptive SNPs may be associated with growth, especially development, or stress responses, as well as transcription regulation (Table 3). No GO terms were found to be overrepresented in either the 37 'adaptive' SNPs located in putative *E. grandis* genic regions, nor the full set of 81 'adaptive' SNPs.

Expected future allele frequency changes – potential for adaptation to future climates

Current population-level allele frequencies for adaptive SNPs varied widely between populations and between SNPs (Figure 5; Supporting Information, Figure S5). Results here focus on adaptive SNPs associated with mean annual temperature (MAT) and summer and winter precipitation, variables chosen to represent two key projected climate changes in south-eastern Australia (see Materials and Methods).

For most adaptive SNPs, warm- or wet-associated alleles were generally found in mid- to low-frequencies within populations (Figure 5). However, all populations, including cool-climate Victorian populations, had several MAT-associated adaptive SNPs where the 'warm allele' was already at high frequencies (Figure 5a, b). Similarly, all populations had some summer or winter precipitation-associated adaptive SNPs for which the 'wet allele' was at high frequencies (Figure 5c–f).

All populations had at least one adaptive SNP that was fixed within the population sample (Figure 6a, c, e). However, the fixed adaptive SNP(s) varied between populations, with no consistent pattern of fixation across populations, e.g. no adaptive SNPs were fixed for the 'warm-allele' in warmer populations and the 'cool-allele' in cooler populations, and all SNPs were found in intermediate frequencies in other populations (Supporting Information, Figure S5). Further, the predominant adaptation pattern was associated with shifts in allele frequency across climate gradients, not presence-absence changes of alleles of adaptive SNPs.

Expected allele frequency changes associated with projected climate change varied widely between climate variables and adaptive SNPs, assuming linear shifts in allele frequency toward frequencies found in populations currently experiencing the projected climate. Results of the ACCESS 1.0 model (RCP 8.5) for 2070 are presented here (Figure 5), with results for the two alternative models tested, showing similar patterns, given in Supporting Information

(Table S5 and Figures S6–9). There was greater variability in expected allele frequency changes, given current allele frequency, for MAT-associated adaptive SNPs than for summer or winter precipitation-associated adaptive SNPs (Figure 5). Alleles already at high frequencies had lower expected changes to match projected climates, though this is likely due to only small changes possible before the adaptive SNP becomes fixed.

Absolute expected allele frequency changes varied between climate variables (and climate models, see Supporting Information – Supplementary methods, Table S5), reflecting differences in projected climate changes. Note, all sites had projected increases in MAT whilst both increases and decreases in summer and winter precipitation were projected across sites. Therefore, for adaptive SNPs associated with summer or winter precipitation, the specific allele under selection within an adaptive SNP can change from the ‘dry-associated’ to ‘wet-associated’ allele, depending on the projected direction of precipitation change.

Under projected 2070 climates (ACCESS 1.0, RCP 8.5), expected changes in allele frequency (Figure 5), and the proportion of adaptive SNPs expected to change to fixation or to frequencies higher than seen in the sampled distribution (Figure 6b, d, f) were greater for MAT-associated adaptive SNPs than SNPs associated with summer or winter precipitation. Absolute expected allele frequency changes in MAT-associated adaptive SNPs were up to 0.33 compared to 0.05 and 0.12 for summer and winter precipitation-associated SNPs, respectively (Figure 5, Supporting Information, Table S5). For MAT-associated adaptive SNPs, fixation of the ‘warm allele’, or changes to higher frequencies than currently found was expected more often in warmer sites than cooler sites (up to 42% and 53% of MAT-associated adaptive SNPs respectively; Figure 6b). In contrast, expected allele frequency changes for summer or winter precipitation-associated adaptive SNPs rarely resulted in fixation for either the ‘dry’ or ‘wet allele’, or frequencies not currently found in the sampled distribution (Figure 6d, f). The greater expected changes seen in MAT-associated adaptive SNPs is in part due to the linear model underlying the projections having a steeper slope for current allele frequencies versus current MAT (0.07 / °C ± (s.d.) 0.02, $n = 62$ SNPs) than for current allele frequencies versus current summer or winter precipitation (0.02 / 10 mm ± 0.01 for both summer, $n = 23$, and winter precipitation, $n = 13$).

A similar proportion of climate-associated adaptive SNPs were currently fixed within populations for MAT, summer and winter precipitation (up to 19%, 22% and 23% respectively; Figure 6a, c, e). This may reflect true fixation within the wider population or low allele frequencies not captured in the population sampling. Most populations had at least one adaptive SNP currently fixed for each of the three climate variables (Figure 6a, c, e), though this could be fixation of either the ‘warm’ or ‘cool’ allele (MAT), or the ‘dry’ or ‘wet allele’ (precipitation).

The minimum distance to a site matching the projected 2070 climates (ACCESS 1.0, RCP 8.5) varied greatly between sites and between MAT and summer and winter precipitation (Figure 7), with no consistent distance across sites or geographic regions. In general, greater distances were required to match projected future MAT (170 km to > 500 km) than summer or winter precipitation (< 200 km for 24 / 26 sites). None of the 18 warmer NSW sites had a site match within the sampled *E. microcarpa* distribution for projected MAT (Figure 7a). In contrast, only 2 sites for summer precipitation and only 1 site for winter precipitation had projected climates beyond the sampled range of this study (Figure 7b, c). Due to both projected increases and decreases in precipitation, the climatic direction required to match future precipitation varied between sites.

DISCUSSION

This study revealed evidence of climate adaptation in *Eucalyptus microcarpa* through a combined F_{ST} outlier and environmental association analysis approach on 4,218 genome-wide SNPs. These data allowed us to characterise climate adaptation including potential climatic drivers and possible gene functions. Moving beyond characterisation of adaptation, we applied these results to infer population-level allele frequency changes that may theoretically be expected under projected climate change in the absence of migration, assuming linear shifts in allele frequency based on current SNP-climate relationships, and the implications of this for the capacity of *E. microcarpa* populations to adapt to climate change.

Climatic drivers of adaptation

Consistent with our results for *E. microcarpa*, temperature, precipitation and water availability are commonly associated with adaptive clines in trees (Eckert *et al.* 2010a; Steane *et al.* 2014; De Kort *et al.* 2014; Evans *et al.* 2014; McKown *et al.* 2014). Temperature is known to be an important driver of growth and phenology in trees, including *E. microcarpa* (Hudson *et al.* 2011; Rawal *et al.* 2015a). Given the high confidence of projected temperature changes (Reisinger *et al.* 2014), temperature is likely to remain a key driver of adaptation in *E. microcarpa* into the future.

Given the number of SNP associations, summer and winter precipitation appear to be more important than annual precipitation for *E. microcarpa*. Flowering onset in eucalypts can be influenced by monthly precipitation, including in combination with temperature (Keatley *et al.* 2002). Timing of precipitation may therefore have a greater influence on periodic events such as flowering or germination and establishment, highlighting that broader annual variables may not always be the most appropriate for assessing adaptation.

Whilst these results indicate some important climate drivers for *E. microcarpa*, they are not exhaustive. The subset of the genome surveyed in this study, length of climate gradients, strength of selection and collinearity with population structure, as well as effect size of adaptive SNPs, may influence the number of associations identified (De Mita *et al.* 2013; Lotterhos & Whitlock 2015; Rellstab *et al.* 2015). Furthermore, whilst temperature, aridity and summer / winter precipitation may be important, other climatic and environmental factors warrant future consideration. For example, many Australian plants including eucalypts, show adaptation to soils (Wardell-Johnson *et al.* 1997) and therefore adaptation to soil, in conjunction with climate, may have important implications for restoration seed sourcing.

Genomics of climate adaptation

In line with findings from other tree species, climate adaptation in *E. microcarpa* appears to be a genome-wide phenomenon (e.g. Yeaman *et al.* 2016; Rajora *et al.* 2016; Sork *et al.* 2016; Steane *et al.* 2017), polygenic (Kremer *et al.* 2014; Yeaman *et al.* 2016), and related to allele frequency shifts rather than binary changes in the SNP allele (Hornoy *et al.* 2015; Lind *et al.* 2016). These results may also suggest possible redundancy in climate adaptation responses. Alternative genes and pathways may enable similar adaptive, physiological responses to climate. However limits on adaptive responses, especially for genes and pathways not identified here, may still exist. Adaptive SNPs identified here differed from those found in three other eucalypt species, which themselves had minimal shared adaptive SNPs (Steane *et al.* 2017). This potentially reflects different adaptive mechanisms between species, or differences in analytical approach between studies. Whilst climate associations in this study spanned the genome, sampling across only a subset of the genome and alignment to a related species limit further conclusions regarding adaptive genomic regions. Future analysis using a greater number and density of SNPs across the genome could help identify potentially adaptive regions or islands of divergence (e.g. Holliday *et al.* 2016), important for adaptive potential within populations of *E. microcarpa*.

Landscape genomics is revealing a diverse array of genes and pathways associated with climate adaptation in trees (Eckert *et al.* 2010a; McKown *et al.* 2014; Sork *et al.* 2016). *Arabidopsis thaliana* (TAIR10) orthologues of predicted *E. grandis* genes suggest a similarly diverse set of genes may be associated with climate adaptive SNPs in *E. microcarpa*, including TAIR10 orthologues involved in growth (e.g. TET5; Wang *et al.* 2015), starch metabolism (e.g. Starch synthase 2, SS2), photosynthesis (e.g. LHCA5; Jensen *et al.* 2007) and stress responses (e.g. MYB78; Yanhui *et al.* 2006). A role for gene regulation, not simply gene function, in climate adaptation was suggested by the identification of multiple TAIR10 transcription factor orthologues e.g. NF-YA1 (At5g12840), important in abiotic stress responses (Ding *et al.* 2013; Zhao *et al.* 2016), and WRKY7 (At4g24240) identified as a 'multiple stress regulator gene', regulating multiple stress pathways in plants (Kant *et al.* 2008). Several genes had TAIR10 orthologues matching results of other tree studies include NAC007, GUS1 and GH9B18, previously associated with wood growth in *E. nitens* (Thavamanikumar *et al.* 2014) and two leucine-rich repeat receptor-like kinases (LRR-RLK), TMKL1 and RKL1, with TMKL1 identified as an outlier in *Pinus taeda* (Eckert *et al.* 2013) and LRR-RLKs generally overrepresented in selection outliers in *Populus trichocarpa* (Evans *et al.* 2014).

The influence of temperature on flowering in *E. microcarpa* (Rawal *et al.* 2015b) corroborated a range of temperature-associated genes with TAIR10 orthologues related to reproductive development. SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 2 (SPL2), NET2B, GH9B18 and CHX2 TAIR10 orthologues have been associated with floral tissue, including pollen, and floral organ development (Sze *et al.* 2004; Wuest *et al.* 2010;

Chen *et al.* 2010; Wang *et al.* 2016). EDA8, involved in endosperm development (Pagnussat *et al.* 2005) was associated with an elevational gradient in *Arabidopsis halleri* ssp. *gemmifera* (Kubota *et al.* 2015), consistent with temperature associations in this study. These suggest a role for flowering traits in future climate adaptation in *E. microcarpa*.

Despite limited gene information in this study, relying on TAIR10 orthologues for predicted genes of a related species, the results of this study and other studies suggest that adaptation (i) involves a wide range of genes spanning the genome, (ii) is likely to be polygenic with potentially multiple adaptive mechanisms including regulation of expression, and (iii) arises from shifts in current allele frequencies. Consequently, maintaining standing variation and adaptive diversity generally may be more important for supporting adaptation within populations than attempting to capture particular sets of 'adaptive genes'.

Potential for *in situ* adaptation

Moving beyond characterisation of adaptive variation, we attempted to directly assess genomic adaptation potential in *E. microcarpa* populations. Our approach calculated the expected change in allele frequency 'required' to match projected future climates, assuming adaptation via linear shifts in allele frequency toward frequencies seen in populations currently experiencing projected climates. Whilst this is a simplification of a complex process, our aim was to gain insight into the magnitude of genomic changes that may be expected to occur under climate change and use this information to infer the potential for *in situ* adaptation versus the need for gene flow or management interventions such as assisted migration.

In comparison to the study of oaks our approach was based on (Rellstab *et al.* 2016), similar expected allele frequency changes for projected mean annual temperature were found; 0.1 – 0.3 for *E. microcarpa* compared to mean changes of 0.09 – 0.3 for oak species. Though not directly comparable due to different variables, expected frequency changes associated with water-related changes were lower in *E. microcarpa* (less than 0.07) than oak species (0.13 – 0.32). Differences may reflect variation in selection pressure, projected climate changes or differences in frequency change calculations. Further studies employing similar approaches will enhance estimates of potential expected allele frequency changes under climate change.

Greater expected allele frequency changes for adaptive SNPs associated with mean annual temperature compared to summer or winter precipitation likely reflects both a steeper association between allele frequency and temperature and a larger relative projected change in temperature compared to precipitation across the sampled distribution. Such results highlight the importance of considering the strength of adaptation, i.e. the degree of allele frequency change across the climate range, and the magnitude of projected changes when considering adaptive potential within populations. For *E. microcarpa*, these results suggest greater genomic changes may be required in populations to adapt to temperature changes, compared to changes in precipitation. How this may impact *in situ* adaptation will be dependent on how rapidly population allele frequencies can change as well as the influence of other factors such as gene flow and plasticity.

Expected allele frequency changes could provide an indication of the ability to adapt *in situ*, if rates of allele frequencies change are known. Selection over a single generation or season can alter allele frequencies by up to 0.1 in invertebrates (Pespeni *et al.* 2013; Bergland *et al.* 2014; Egan *et al.* 2015). In trees, frequency changes of 0.1 – 0.2 per decade were found in *Fagus sylvatica* (Jump *et al.* 2006), with tree mortality potentially able to increase rates of evolution within populations (Kuparinen *et al.* 2010). These data suggest expected allele frequency changes < 0.1 may be able to match projected climate change, whilst changes greater than 0.1 – 0.2 may result in a lag between allele frequency and climate adaptation. If so, of the climate variables examined in this study, populations of *E. microcarpa* appear to be at greater risk of maladaptation to temperature (expected changes of up to 0.3) with a possibility for adaptation to summer and winter precipitation (expected changes < 0.1).

However, other factors such as allele fixation, balancing selection, pleiotropic interactions or fitness costs may also influence changes in allele frequencies (Mitchell-Olds *et al.* 2007; Hoffmann & Sgrò 2011). Furthermore, as rates of climate change increase, the expected allele frequency change to match new climates may also increase, potentially further limiting the ability of *in situ* adaptation. The potential rate of increase of adaptive alleles and their maximum frequency under selection needs to be determined, along with the impacts of allele frequency change on fitness and thus demography. Such data will help clarify rates of *in situ* climate change adaptation in different locations.

Restrictions on allele frequency changes within a population may not limit adaptation if other factors are involved. Gene flow can facilitate climate adaptation within populations by introducing ‘pre-adapted’ genetic diversity (Sgrò *et al.* 2011; Kremer *et al.* 2012), increasing rates of alleles frequency shifts and countering fixed SNPs. For *E. microcarpa*, where fragmentation or long distances to areas matching projected future climates may impede natural gene flow, assisted migration may enhance genetic diversity for adaptation (Weeks *et al.* 2011; Aitken & Bemmels 2015). Beyond allelic changes, epigenetic and expression changes as well as phenotypic plasticity can provide alternatives for continued adaptation (Franks & Hoffmann 2012; Nicotra *et al.* 2015; Huang *et al.* 2015; Kenkel & Matz 2016). Therefore whilst shifts in allelic frequencies are likely to be important for adaptation, other processes can contribute to the adaptive potential of populations.

Implications for restoration under climate change

The results of this study highlight the importance of maintaining genetic diversity generally in restoration. A single geographic distance, and even single climatic direction for seed sourcing isn’t appropriate due to both variation in projected climate change between sites and variation in the strength of different climate gradients across the landscape. This is especially true for precipitation where the projected direction of change, and therefore the climatic direction in which to capture ‘pre-adapted’ genetic variation, varied from site to site. These results support past findings of low correlations between local adaptation and geographic distance (Leimu & Fischer 2008) and support more recent seed sourcing guidelines suggesting the use of mixed populations guided by climate rather than geographic distance (Prober *et al.* 2015).

For some regions of *E. microcarpa*, especially the warmer, northern end of the distribution, there may be no ‘future climate site’ from which to source seed. In these situations, adaptation through genetic changes or alternatives such as plasticity and expression changes, will be essential for current populations or new restoration plantings of *E. microcarpa*. Maintaining high genetic diversity will therefore be important for assisting these populations to adapt.

CONCLUSION

There is now strong evidence of adaptation to climate in widespread tree species (Savolainen *et al.* 2007; Alberto *et al.* 2013; Aitken & Bemmels 2015). Results from this study are in line with an emerging suite of landscape genomic analyses suggesting that adaptation is likely to be polygenic and is likely to arise from standing variation. Taking these results one step further, this study showed that allele frequency shifts expected to match projected climate change vary between adaptive SNPs and between sites. Consequently, the capacity to adapt *in situ*, may also vary between locations. It also demonstrated the potential use of genomic data to understand patterns of genomic change associated with climate change and how this may assess the ability of populations to adapt *in situ*.

A critical next question is how fast allele frequencies can change within a population. Data here indicate the potential magnitude of change that may be needed, however additional work is needed to determine the potential for *in situ* adaptation based on standing variation, the role of gene flow or assisted migration in facilitating allele frequency change, and the potential fitness effects associated with allelic changes. This is especially true in fragmented environments, where restricted gene flow and population size may reduce the capacity for habitat remnants to evolve at speeds required to match current climate change (Aitken *et al.* 2008; Alberto *et al.* 2013). Such knowledge will improve the ability to assess future adaptive potential and identify vulnerable populations requiring management intervention.

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DATA ACCESSIBILITY

Sequencing read data is archived in the NCBI Sequence Read Archive (SRA) – BioProject PRJNA396661. A VCF file of final samples and genotypes used in analysis, and associated metadata, is available from Dryad, doi:10.5061/dryad.tj1ss. Scripts available at https://github.com/rebecca-cj/GenomicAdaptation/ .

AUTHOR CONTRIBUTIONS

All authors contributed to the development of research question and design. RJ performed the fieldwork and analysis and prepared the manuscript. AAH, SKD and SMP assisted with analysis and editing of the manuscript

Table 1 Site information including climate data for 26 *Eucalyptus microcarpa* sites sampled. Climate data from Atlas of Living Australia (http://www.ala.org.au). See Table 2 for full variable names and Supporting Information, Table S1 for climate variable definitions.

				Aridity Index (ratio) [1]		Precipitation (mm)					Temperature (ºC)		
Site	*n*	Latitude (°)	Longitude (°)	Mean annual	Max. month	Annual	Winter	Summer	Driest period	Wettest period	Annual mean	month abs. mean	Warmest period max.
Central NSW													
NDU	25	-32.33	148.51	0.42	0.91	583	132	165	8	17	17.1	44	32.6
NAL	21	-32.38	147.48	0.33	0.71	500	103	150	6	14	17.8	46	33.9
NBG	29	-32.88	148.07	0.41	0.84	536	118	147	7	13	17.3	45	33.1
NCD	20	-32.91	147.07	0.32	0.7	465	96	123	7	13	17.6	45	33.6
EWN	9	-33.07	148.24	0.47	0.98	586	134	158	8	14	16.7	44	32.7
NBY	24	-33.33	148.25	0.52	1.12	594	144	165	9	15	16.5	43	32.8
NHI	22	-33.84	147.22	0.43	1.01	463	114	124	7	12	16.5	45	32.9
NGR	23	-34.00	148.12	0.58	1.31	561	153	144	8	15	16.1	43	32.7
NCR	26	-34.09	148.52	0.61	1.38	572	159	154	8	15	15.6	43	32.3
NNA	20	-34.75	146.50	0.4	0.98	428	114	94	6	10	16.5	47	32.9
Southern NSW													
NBJ	21	-35.05	147.04	0.49	1.19	484	131	109	7	12	16.4	45	33
NTA	18	-35.25	147.68	0.74	1.95	662	185	131	9	18	15.2	43	31.7
NTR	25	-35.31	147.09	0.62	1.55	556	167	123	7	15	15.5	44	31.9
NCB	27	-35.50	144.96	0.35	0.88	376	102	82	5	10	15.9	47	32
NFN	16	-35.68	145.55	0.43	1	450	115	98	6	12	16.1	46	32.3

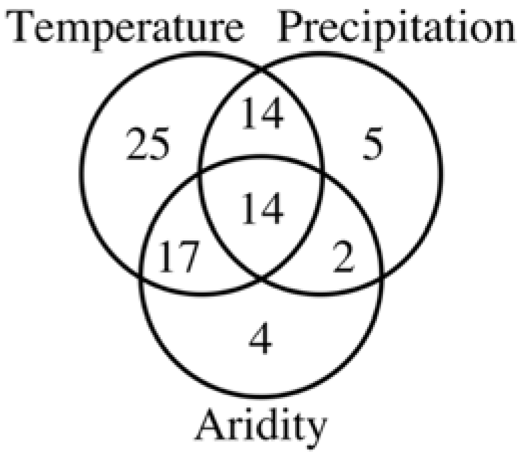
NMV	21	-35.70	144.91	0.39	0.98	373	109	85	5	10	15.6	46	31.5
NCN	24	-35.74	146.34	0.52	1.28	463	135	107	5	13	15.9	46	32.6
Central Victoria													
VTT	23	-36.14	144.22	0.45	1.09	396	116	79	5	10	15.3	45	31.1
VDB	33	-36.40	145.68	0.72	1.8	555	174	108	7	16	15.2	44	30.5
VRH	17	-36.58	145.95	0.91	2.3	682	223	120	8	21	14.9	44	30.9
VMC	27	-36.77	144.11	0.61	1.55	472	146	86	5	12	14.5	45	30
VGL	25	-37.10	143.53	0.74	1.83	529	176	103	6	14	13.7	43	28.8
VWG	28	-37.67	144.36	0.72	1.54	571	140	145	8	16	13.6	44	27.5
Western Victoria													
VMJ	10	-36.28	143.14	0.48	1.21	402	124	76	5	11	15	44	30.8
VBU	26	-36.82	142.18	0.55	1.4	446	154	79	4	13	14.5	45	30
VMA	20	-36.77	141.82	0.61	1.54	468	182	84	4	15	14.2	45	29.6
Avg	22.3		**Min**	0.32	0.7	373	96	76	4	10	13.6	43	27.5
SD	5.4		**Max**	0.91	2.3	682	223	165	9	21	17.8	47	33.9
			Range	0.59	1.6	309	127	89	5	11	4.2	5	6.4

[1] Ratio precipitation to potential evaporation (pan, free-water surface)

Table 2 Climatic variables used in environmental association analysis of putatively adaptive SNPs in *Eucalyptus microcarpa*, including a) number of SNPs strongly associated which each variable and b) Venn diagram of outlier SNPs associated with at least one climate variables within the three broad categories. BF = Bayes Factor.

a) Climate variable [1]	Number of associated SNPs (BF > 20)
Aridity Index (ratio) [2]	
Mean annual aridity index	30
Maximum month aridity index	36
Precipitation (mm)	
Annual precipitation (Bio12)	2
Average winter (Jun, Jul, Aug) precipitation	13
Average summer (Dec, Jan, Feb) precipitation	23
Precipitation of driest period (Bio14)	9
Precipitation of wettest period (Bio13)	1
Temperature (°C)	
Annual mean temperature (Bio01)	62
Max month absolute mean max temperature	1
Max temperature of warmest period (Bio05)	56

b)



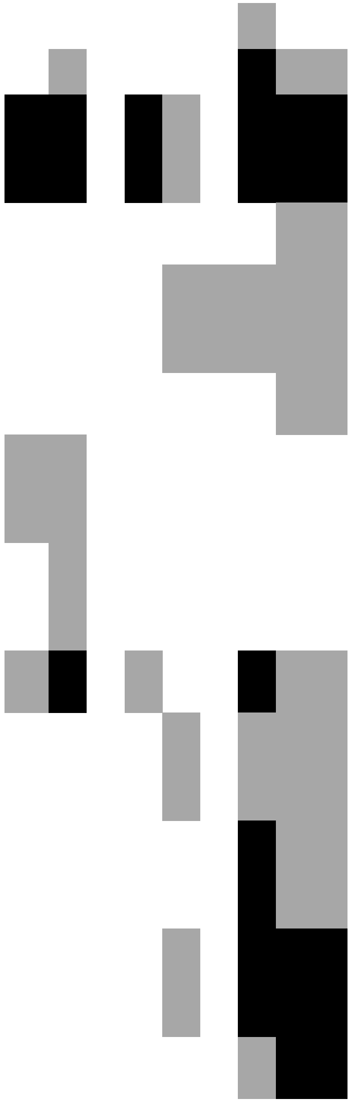
[1] From Atlas of Living Australia (http://www.ala.org.au). See Supporting Information, Table S1 for variable definitions

[2] Ratio precipitation to potential evaporation (pan, free-water surface)

Table 3 Gene information for 37 adaptive *Eucalyptus microcarpa* SNPs located in putative *E. grandis* genic or 5'/3' UTR regions. Includes F_{ST} outlier tests for which SNP was significant and climate associations. Gene information, including 'Best' TAIR orthologue, from *E. grandis* v1.1 genome annotation.

Adaptive SNP (Chr:Pos)	*Eucalyptus grandis* gene information (+/- 2000 bp)		'Best' TAIR10 gene orthologue			F_{ST} Outlier Tests [1]	Environmental Association [2]							
	Name	Gene Effect	Name	Symbol	Definition		Ann. Aridity	Max. Aridity	Ann. Prec.	Winter Prec.	Summer Prec.	Driest Prec.	MAT	Warmest Max. Temp.
1:5286743	Eucgr.A00381	synonymous	AT2G23540.1		GDSL-like Lipase/Acylhydrolase superfamily protein	LX								▒
1:31064541	Eucgr.A02032	3' UTR	AT1G31300.1		TRAM, LAG1 and CLN8 (TLC) lipid-sensing domain containing protein	BALX	■	■		■			▒	▒
1:34586557	Eucgr.A02381	intron	AT5G12840.1	ATHAP2A, EMB2220, HAP2A, NF-YA1	nuclear factor Y, subunit A1	LX								▒
1:39155813	Eucgr.A02875	missense	AT5G51600.1	ATMAP65-3, MAP65-3, PLE	Microtubule associated protein (MAP65/ASE1) family protein	BALX					▒		▒	▒
1:39264069	Eucgr.A02887	5' UTR	AT1G12260.1	ANAC007, EMB2749, NAC007, VND4	NAC 007	ALX					▒	▒	■	■
1:39740158	Eucgr.A02930	3' UTR	AT4G09320.1	NDPK1	Nucleoside diphosphate kinase family protein	BALX					■	■	▒	▒

2:1101427	Eucgr.B00047	synonymous	AT1G72220.1		RING/U-box superfamily protein	BALX
2:56849625	Eucgr.B03174	intron	AT5G61250.1	AtGUS1, GUS1	glucuronidase 1	BALX
2:59710840	Eucgr.B03500	missense	AT5G43270.1	SPL2	squamosa promoter binding protein-like 2	BALX
2:60747034	Eucgr.B03637	missense	AT1G79400.1	ATCHX2, CHX2	cation/H+ exchanger 2	AL
2:63702271	Eucgr.B03985	missense	AT5G49620.1	AtMYB78, MYB78	myb domain protein 78	LX
3:16106241	Eucgr.C01016	missense	AT5G17050.1	UGT78D2	UDP-glucosyl transferase 78D2	LX
3:76309309	Eucgr.C04152	missense	AT5G55740.1	CRR21	Tetratricopeptide repeat (TPR)-like superfamily protein	LX
4:37193624	Eucgr.D02329	3' UTR; missense	AT3G61570.1	GC3, GDAP1	GRIP-related ARF-binding domain-containing protein 1	LX
4:40809575	Eucgr.D02626	intron	AT1G32050.1		SCAMP family protein	LX
6:2898330	Eucgr.F00187	synonymous	AT4G24240.1	ATWRKY7, WRKY7	WRKY DNA-binding protein 7	BALX
6:3363400	Eucgr.F00228	intron; synonymous	AT1G45474.1	LHCA5	photosystem I light harvesting complex gene 5	LX
6:5418198	Eucgr.F00428	missense	AT5G65520.1		Tetratricopeptide repeat (TPR)-like superfamily protein	LX
6:29233064	Eucgr.F02153	synonymous	AT1G09720.1	NET2B	Kinase interacting (KIP1-like) family	LX



					protein								▨	■	■
6:35104920	Eucgr.F02553	intron	AT5G11420.1		Protein of unknown function, DUF642	BALX	▨	■							
6:41009171	Eucgr.F03165	synonymous	AT1G17540.1		Protein kinase protein with adenine nucleotide alpha hydrolases-like domain	BALX							■	▨	▨
6:41245283	Eucgr.F03190	intron	AT1G17210.1	ATILP1, ILP1	IAP-like protein 1	LX	■	■		▨					
7:31827179	Eucgr.G01728	synonymous	AT4G39010.1	AtGH9B18, GH9B18	glycosyl hydrolase 9B18	ALX	▨	▨					■	■	■
8:7499743	Eucgr.H00539	intron	AT1G48480.1	RKL1	receptor-like kinase 1	BALX					▨		■	■	■
8:9754130	Eucgr.H00759	intron	AT5G48630.2		Cyclin family protein	BALX							■	■	■
8:10228147	Eucgr.H00812	missense	AT3G24660.1	TMKL1	transmembrane kinase-like 1	LX			▨			▨			
8:58344239	Eucgr.H04049	synonymous	AT1G53025.1		Ubiquitin-conjugating enzyme family protein	LX							■	■	■
9:6475303	Eucgr.I00343	synonymous	AT2G22090.2	UBA1A	RNA-binding (RRM/RBD/RNP motifs) family protein	ALX					■	■	▨		
9:20676952	Eucgr.I00992	missense	AT4G00310.1	EDA8, MEE46	Putative membrane lipoprotein	BALX		▨			▨		■	■	■
9:27865319	Eucgr.I01789	intron	AT3G01510.1	LSF1	like SEX4 1	LX	■	■		▨			▨		
9:29125069	Eucgr.I01918	intron	AT1G80170.1		Pectin lyase-like superfamily protein	LX							▨	▨	▨
10:808833	Eucgr.J00053	synonymous	AT5G43240.1		Protein of unknown function (DUF674)	BALX					■	▨	■	■	■

10:12150982	Eucgr.J01104	missense	AT4G23410.1	TET5	tetraspanin5	BALX					▒	▒	■	■
10:16128763	Eucgr.J01349	5' UTR; synonymous	AT3G01180.1	AtSS2, SS2	starch synthase 2	BALX	▒	▒			▒		■	■
10:29282238	Eucgr.J02333	synonymous	AT3G27150.1		Galactose oxidase/kelch repeat superfamily protein	LX	▒	▒					■	■
11:28408708	Eucgr.K02126	intron	AT4G13020.1	MHK	Protein kinase superfamily protein	LX	■	■	▒	▒				
11:29546106	Eucgr.K02229	missense	AT1G73830.1	BEE3	BR enhanced expression 3	LX		▒					■	▒

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[1] B = BayeScan (logBF > 0.5), A = Arlequin (Hierarchical FDIST2; $q <= 0.1$), L = Lositan (FDIST2; $q <= 0.1$), X = Bayenv $X^T X$ (top 5%)

[2] Black shading = very strong association (BF > 150), grey shading = strong association (20 < BF < 150). Ann. Aridity = Mean annual aridity index (ratio precipitation to potential evaporation), Max. Aridity = Maximum month aridity index (ratio precipitation to potential evaporation), Ann. Prec. = Annual precipitation (mm), Winter Prec. = Winter precipitation (mm), Summer prec. = Summer precipitation (mm), Driest Prec. = Driest period precipitation (mm), MAT = Mean annual temperature (ºC), Warmest Max. Temp. = Warmest period maximum temperature (ºC). See Supporting Information, Table S1 for climate variable definitions.

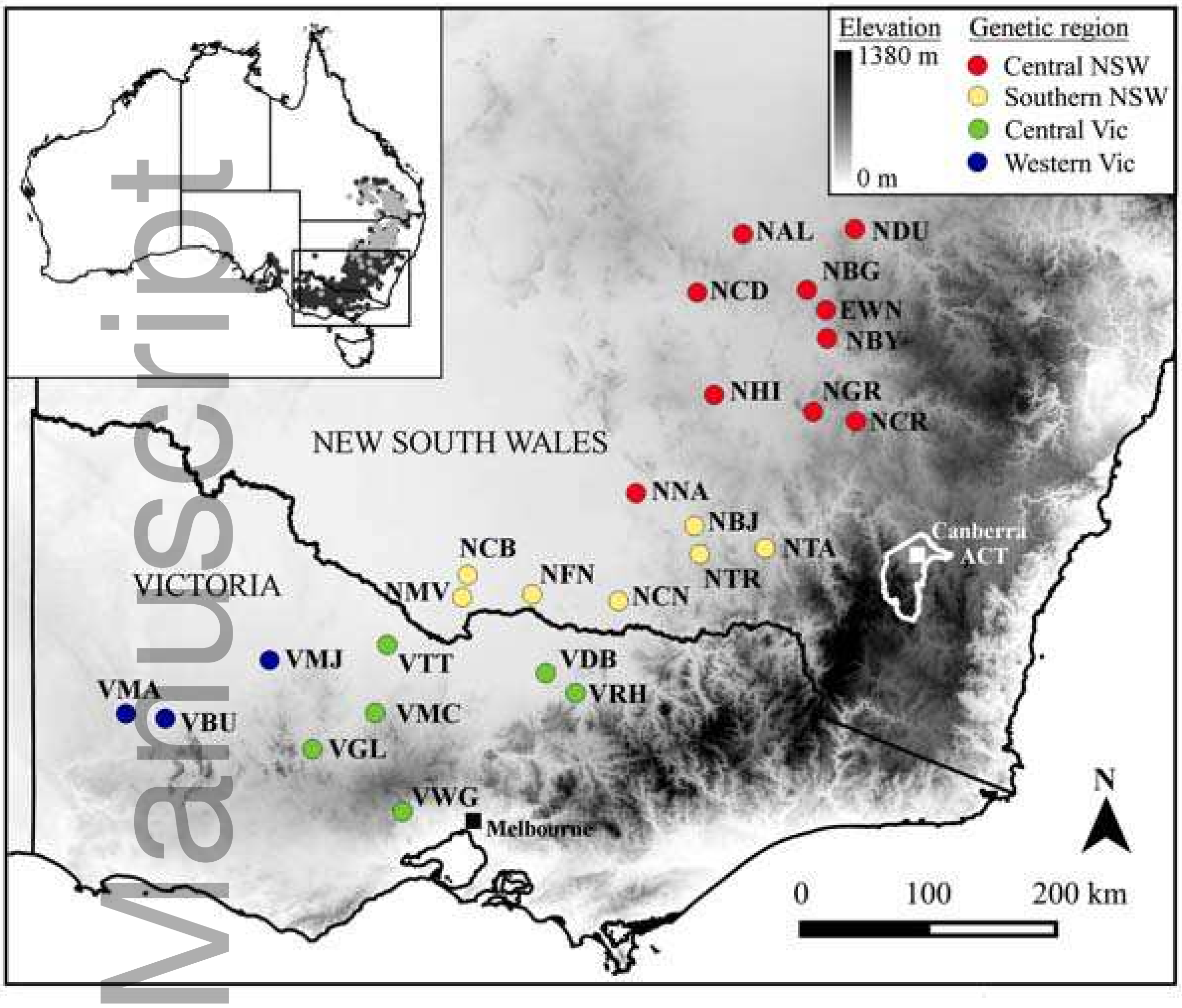


Figure 1 Map of 26 *Eucalyptus microcarpa* sampling sites across south-eastern Australia. Colours indicate genetic regions based on PCA results (see Figure 2a). Inset: Map of Australia showing distribution of *E. microcarpa*. Box indicates enlarged region. Grey dots indicate recorded occurrences of *E. microcarpa* (dark) and *E. woollsiana* (light) respectively, providing an indication of the species' distribution (data from Atlas of Living Australia; http://www.ala.org.au).

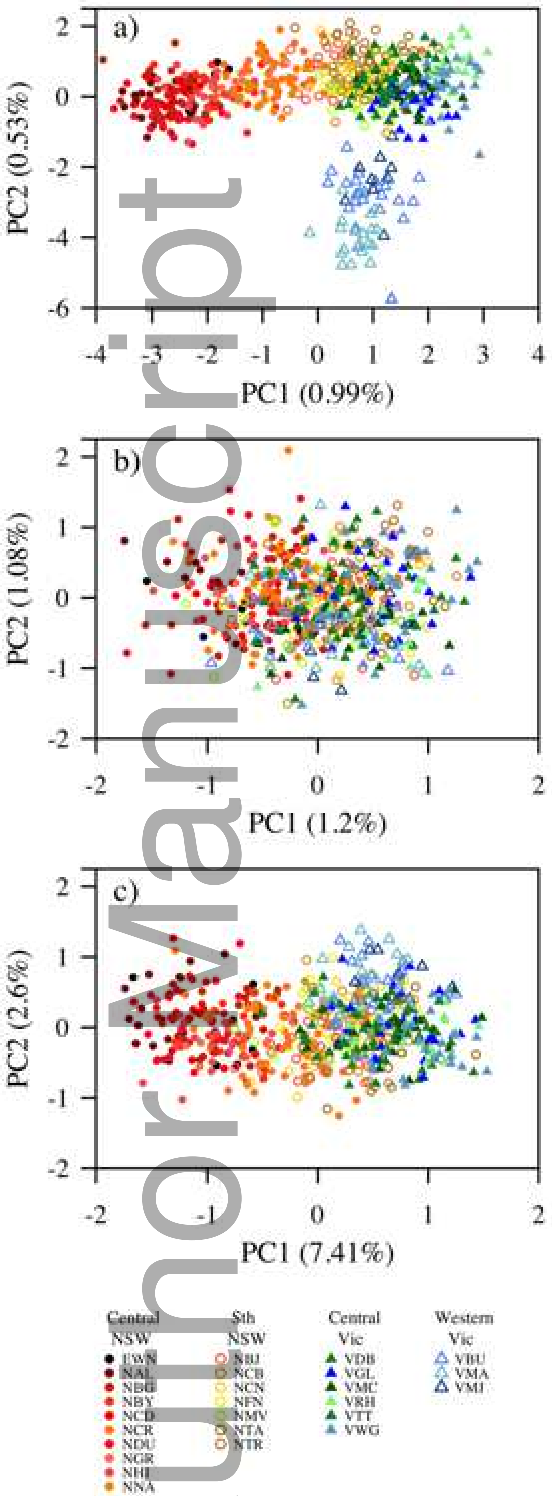


Figure 2 Principal component analysis (PCA) of 580 *Eucalyptus microcarpa* individuals from 26 sites using a) all 4,218 SNPs, b) 418 putatively neutral SNPs and c) 81 putatively adaptive SNPs. Numbers in brackets = percentage variance explained by axis. Solid circles = central

NSW, open circles = southern NSW, solid triangles = central Victoria and open triangles = western Victoria (refer to Figure 1).

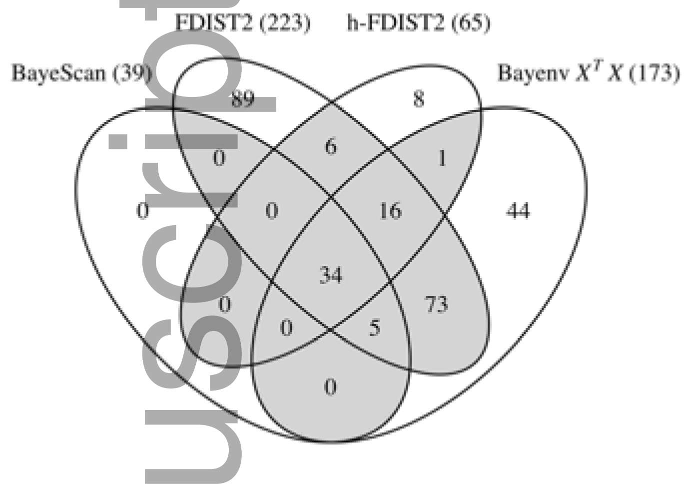


Figure 3 Number of *Eucalyptus microcarpa* SNPs identified as being under directional selection in four F_{ST} outlier style tests (number identified per test in brackets). BayeScan – logBF > 0.5, FDIST2 (Lositan) and hierarchical FDIST2 (h-FDIST2, Arlequin) – q <= 0.1 and Bayenv $X^T X$ – top 5%. Shaded regions indicate SNPs considered ‘outliers’ in this study (outliers in > 2 tests).

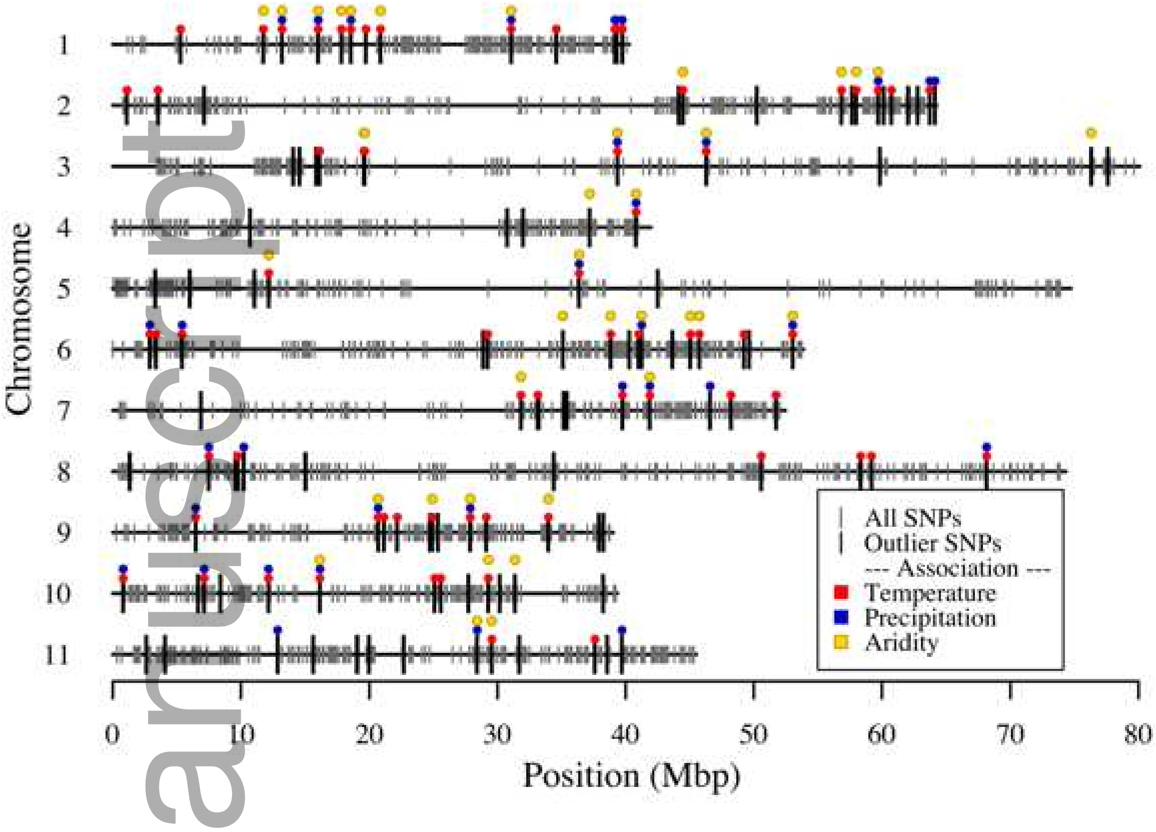


Figure 4 Physical genome map of all 4,218 *Eucalyptus microcarpa* SNPs used in this study on *E. grandis* genome (v 1.1), as well as outliers (identified in > 2 tests) and putatively adaptive SNPs (identified as outlier in > 2 tests plus strong climate association).

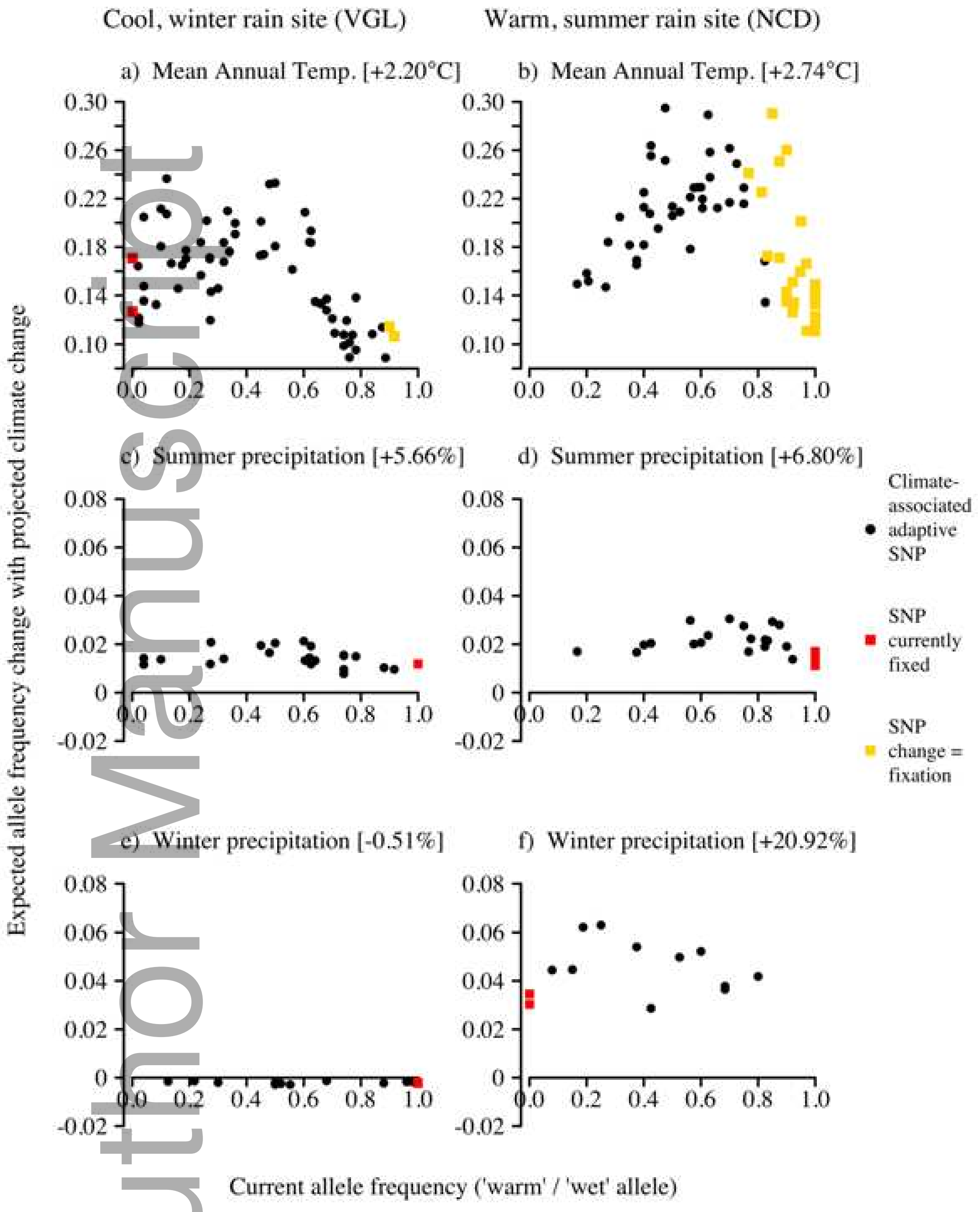


Figure 5 Current versus expected allele frequency changes for individual adaptive SNPs under projected 2070 climates (ACCESS 1.0, RCP 8.5) in two example *Eucalyptus microcarpa* populations; a ‘cool’, ‘winter rain’ site (a, c, e; VGL) and a ‘warm’, ‘summer rain’ site (b, d, f; NCD). Change to 2070 climate in square brackets. For each adaptive SNP, that allele associated with climate increase is plotted. a, b) mean annual temperature, c, d) summer

precipitation, and e, f) winter precipitation. For precipitation, positive frequency change = increase of ‘wet allele’, negative change = increase of ‘dry allele’. Note different scale on y-axes of c-f compared to a-b.

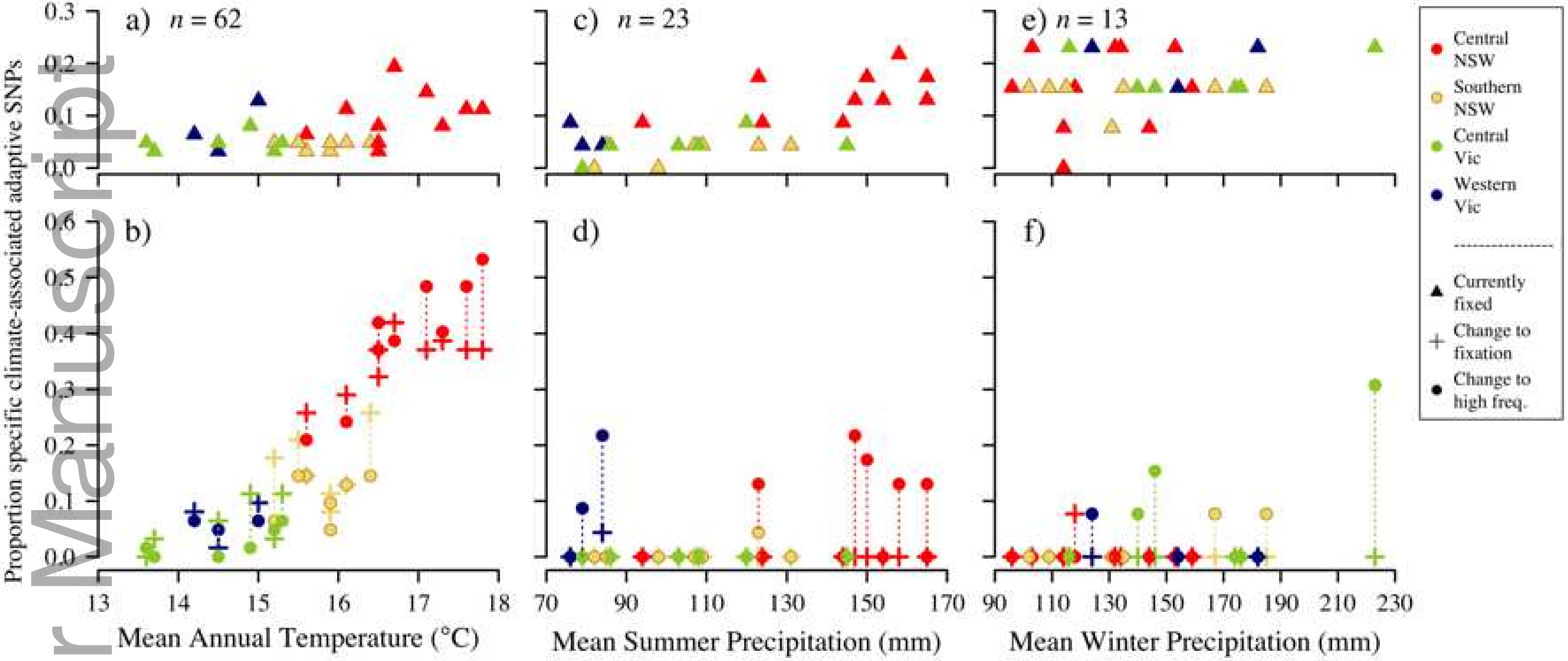


Figure 6 Proportion of climate-associated adaptive SNPs in 26 sampled *Eucalyptus microcarpa* sites that are currently fixed (a, c, e) or for which expected allele frequency changes under projected 2070 climates (ACCESS 1.0, RCP 8.5) result in fixation or allele frequencies beyond the range seen in the sampled distribution (b, d, f). Note x-axis represents current site climate. Dotted lines (b, d, f) connect points from the same site.

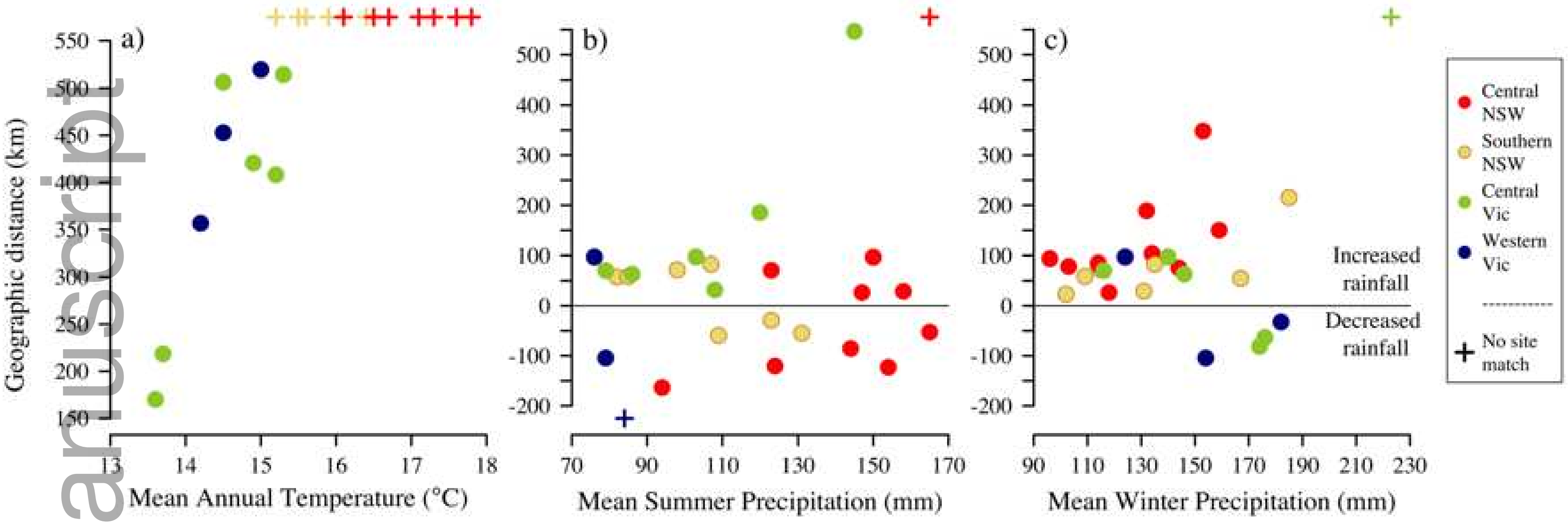


Figure 7 Minimum geographic distance to a sampled site with the corresponding 2070 projected (a) mean annual temperature, (b) summer precipitation, or (c) winter precipitation (ACCESS 1.0, RCP 8.5) for 26 sampled sites of *Eucalyptus microcarpa*. Positive distances indicate distances to sites with higher temperature or precipitation (matching 2070 projections of the sampled site). Negative distances indicate distances to sites with lower precipitation (no projected decreases in temperature). Plus signs (+) indicate projections outside sampling range, with position above or below y-axis limits indicating projected increase or decrease in climate. Note different y-axis for (a) temperature versus summer (b) and winter (c) precipitation.

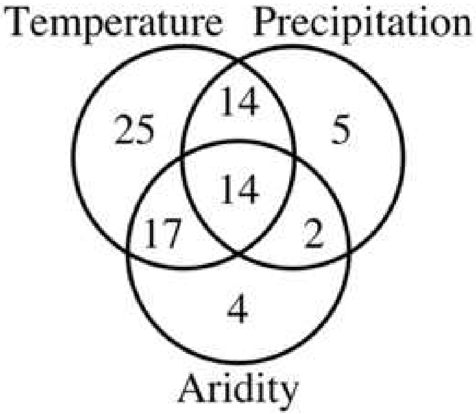
Table 1 Site information including climate data for 26 Eucalyptus microcarpa sites sampled. Climate data from Atlas of Living Australia (http://www.ala.org.au). See Table 2 for full variable names and Supporting Information, Table S1 for climate variable definitions.

				Aridity Index (ratio) [1]		Precipitation (mm)					Temperature (ºC)		
Site	n	Latitude (°)	Longitude (°)	Mean annual	Max. month	Annual	Winter	Summer	Driest period	Wettest period	Annual mean	month abs. mean	Warmest period max.
Central NSW													
NDU	25	-32.33	148.51	0.42	0.91	583	132	165	8	17	17.1	44	32.6
NAL	21	-32.38	147.48	0.33	0.71	500	103	150	6	14	17.8	46	33.9
NBG	29	-32.88	148.07	0.41	0.84	536	118	147	7	13	17.3	45	33.1
NCD	20	-32.91	147.07	0.32	0.7	465	96	123	7	13	17.6	45	33.6
EWN	9	-33.07	148.24	0.47	0.98	586	134	158	8	14	16.7	44	32.7
NBY	24	-33.33	148.25	0.52	1.12	594	144	165	9	15	16.5	43	32.8
NHI	22	-33.84	147.22	0.43	1.01	463	114	124	7	12	16.5	45	32.9
NGR	23	-34.00	148.12	0.58	1.31	561	153	144	8	15	16.1	43	32.7
NCR	26	-34.09	148.52	0.61	1.38	572	159	154	8	15	15.6	43	32.3
NNA	20	-34.75	146.50	0.4	0.98	428	114	94	6	10	16.5	47	32.9
Southern NSW													
NBJ	21	-35.05	147.04	0.49	1.19	484	131	109	7	12	16.4	45	33
NTA	18	-35.25	147.68	0.74	1.95	662	185	131	9	18	15.2	43	31.7
NTR	25	-35.31	147.09	0.62	1.55	556	167	123	7	15	15.5	44	31.9
NCB	27	-35.50	144.96	0.35	0.88	376	102	82	5	10	15.9	47	32
NFN	16	-35.68	145.55	0.43	1	450	115	98	6	12	16.1	46	32.3
NMV	21	-35.70	144.91	0.39	0.98	373	109	85	5	10	15.6	46	31.5
NCN	24	-35.74	146.34	0.52	1.28	463	135	107	5	13	15.9	46	32.6

Central Victoria													
VTT	23	-36.14	144.22	0.45	1.09	396	116	79	5	10	15.3	45	31.1
VDB	33	-36.40	145.68	0.72	1.8	555	174	108	7	16	15.2	44	30.5
VRH	17	-36.58	145.95	0.91	2.3	682	223	120	8	21	14.9	44	30.9
VMC	27	-36.77	144.11	0.61	1.55	472	146	86	5	12	14.5	45	30
VGL	25	-37.10	143.53	0.74	1.83	529	176	103	6	14	13.7	43	28.8
VWG	28	-37.67	144.36	0.72	1.54	571	140	145	8	16	13.6	44	27.5
Western Victoria													
VMJ	10	-36.28	143.14	0.48	1.21	402	124	76	5	11	15	44	30.8
VBU	26	-36.82	142.18	0.55	1.4	446	154	79	4	13	14.5	45	30
VMA	20	-36.77	141.82	0.61	1.54	468	182	84	4	15	14.2	45	29.6
Avg	22.3		**Min**	0.32	0.7	373	96	76	4	10	13.6	43	27.5
SD	5.4		**Max**	0.91	2.3	682	223	165	9	21	17.8	47	33.9
			Range	0.59	1.6	309	127	89	5	11	4.2	5	6.4

[1] Ratio precipitation to potential evaporation (pan, free-water surface)

Table 2 Climatic variables used in environmental association analysis of putatively adaptive SNPs in Eucalyptus microcarpa, including a) number of SNPs strongly associated which each variable and b) Venn diagram of outlier SNPs associated with at least one climate variables within the three broad categories. BF = Bayes Factor.

a) Climate variable [1]	Number of associated SNPs (BF > 20)	**b)**
Aridity Index (ratio) [2]		
Mean annual aridity index	30	
Maximum month aridity index	36	
Precipitation (mm)		
Annual precipitation (Bio12)	2	
Average winter (Jun, Jul, Aug) precipitation	13	
Average summer (Dec, Jan, Feb) precipitation	23	
Precipitation of driest period (Bio14)	9	
Precipitation of wettest period (Bio13)	1	
Temperature (°C)		
Annual mean temperature (Bio01)	62	
Max month absolute mean max temperature	1	
Max temperature of warmest period (Bio05)	56	

[1] From Atlas of Living Australia (http://www.ala.org.au). See Supporting Information, Table S1 for variable definitions

[2] Ratio precipitation to potential evaporation (pan, free-water surface)

Table 3 Gene information for 37 adaptive Eucalyptus microcarpa SNPs located in putative E. grandis genic or 5'/3' UTR regions. Includes F_{ST} outlier tests for which SNP was significant and climate associations. Gene information, including ‘Best’ TAIR orthologue, from E. grandis v1.1 genome annotation.

Adaptive SNP (Chr:Pos)	Eucalyptus grandis gene information (+/- 2000 bp)		‘Best’ TAIR10 gene orthologue			F_{ST} Outlier Tests [1]	Environmental Association [2]							
	Name	Gene Effect	Name	Symbol	Definition		Ann. Aridity	Max. Aridity	Ann. Prec.	Winter Prec.	Summer Prec.	Driest Prec.	MAT	Warmest Max. Temp.
1:5286743	Eucgr.A00381	synonymous	AT2G23540.1		GDSL-like Lipase/Acylhydrolase superfamily protein	LX								▨
1:31064541	Eucgr.A02032	3' UTR	AT1G31300.1		TRAM, LAG1 and CLN8 (TLC) lipid-sensing domain containing protein	BALX	■	■		■			▨	▨
1:34586557	Eucgr.A02381	intron	AT5G12840.1	ATHAP2A, EMB2220, HAP2A, NF-YA1	nuclear factor Y, subunit A1	LX								▨
1:39155813	Eucgr.A02875	missense	AT5G51600.1	ATMAP65-3, MAP65-3, PLE	Microtubule associated protein (MAP65/ASE1) family protein	BALX					▨		▨	▨
1:39264069	Eucgr.A02887	5' UTR	AT1G12260.1	ANAC007, EMB2749, NAC007, VND4	NAC 007	ALX					▨	▨	■	■
1:39740158	Eucgr.A02930	3' UTR	AT4G09320.1	NDPK1	Nucleoside diphosphate kinase family protein	BALX					■	■	▨	▨
2:1101427	Eucgr.B00047	synonymous	AT1G72220.1		RING/U-box superfamily protein	BALX							▨	

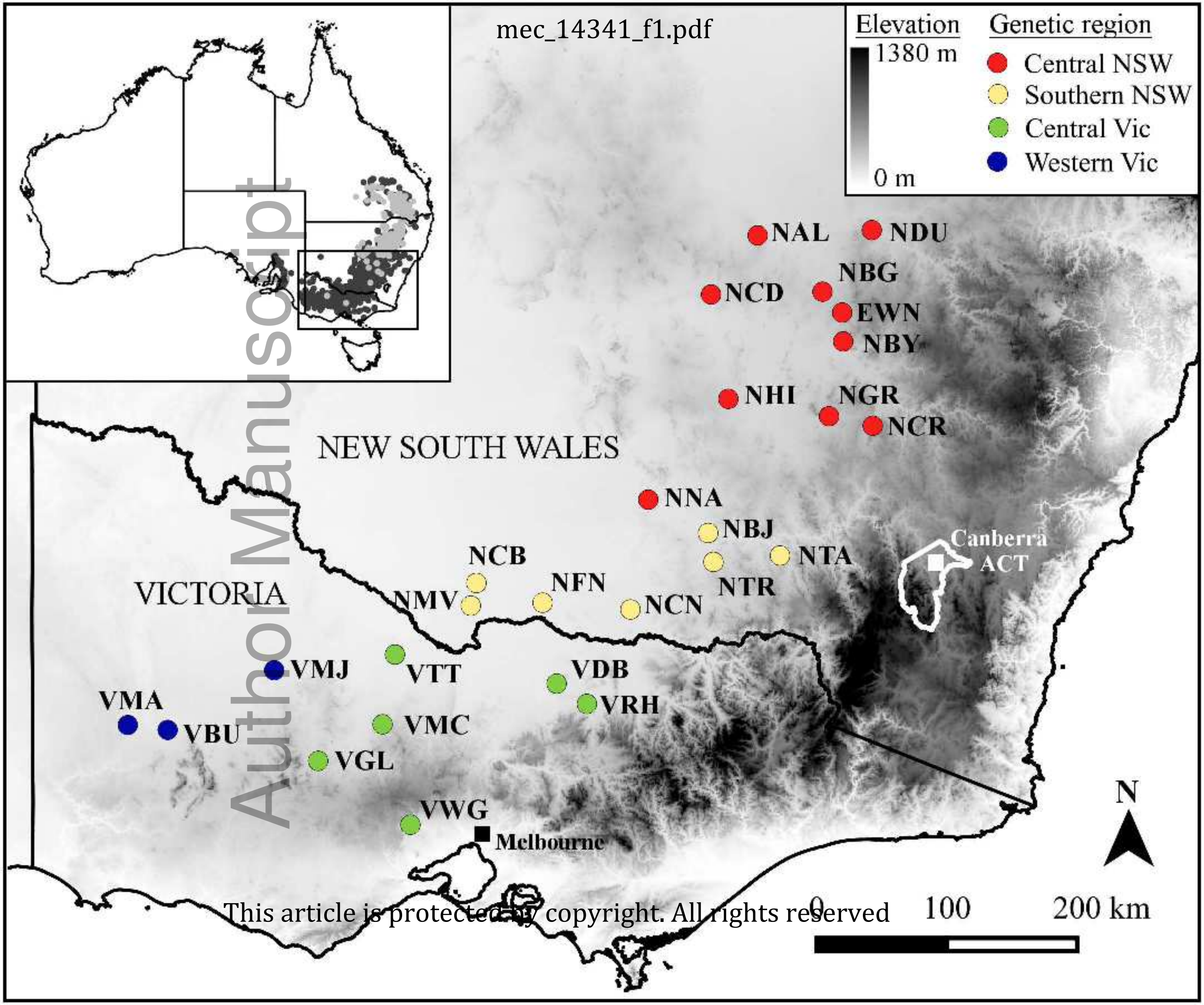
2:56849625	Eucgr.B03174	intron	AT5G61250.1	AtGUS1, GUS1	glucuronidase 1	BALX		▨					■	▨	▨
2:59710840	Eucgr.B03500	missense	AT5G43270.1	SPL2	squamosa promoter binding protein-like 2	BALX	■	■		■	▨		■	■	■
2:60747034	Eucgr.B03637	missense	AT1G79400.1	ATCHX2, CHX2	cation/H+ exchanger 2	AL								▨	▨
2:63702271	Eucgr.B03985	missense	AT5G49620.1	AtMYB78, MYB78	myb domain protein 78	LX					▨	▨	▨	▨	▨
3:16106241	Eucgr.C01016	missense	AT5G17050.1	UGT78D2	UDP-glucosyl transferase 78D2	LX								▨	▨
3:76309309	Eucgr.C04152	missense	AT5G55740.1	CRR21	Tetratricopeptide repeat (TPR)-like superfamily protein	LX	▨	▨							
4:37193624	Eucgr.D02329	3' UTR; missense	AT3G61570.1	GC3, GDAP1	GRIP-related ARF-binding domain-containing protein 1	LX		▨							
4:40809575	Eucgr.D02626	intron	AT1G32050.1		SCAMP family protein	LX	▨	■		▨			■	▨	▨
6:2898330	Eucgr.F00187	synonymous	AT4G24240.1	ATWRKY7, WRKY7	WRKY DNA-binding protein 7	BALX					▨		▨	▨	▨
6:3363400	Eucgr.F00228	intron; synonymous	AT1G45474.1	LHCA5	photosystem I light harvesting complex gene 5	LX							■	▨	▨
6:5418198	Eucgr.F00428	missense	AT5G65520.1		Tetratricopeptide repeat (TPR)-like superfamily protein	LX					▨		■	■	■
6:29233064	Eucgr.F02153	synonymous	AT1G09720.1	NET2B	Kinase interacting (KIP1-like) family protein	LX							▨	■	■
6:35104920	Eucgr.F02553	intron	AT5G11420.1		Protein of unknown function, DUF642	BALX	▨	■							

6:41009171	Eucgr.F03165	synonymous	AT1G17540.1		Protein kinase protein with adenine nucleotide alpha hydrolases-like domain	BALX							■	▨	▨
6:41245283	Eucgr.F03190	intron	AT1G17210.1	ATILP1, ILP1	IAP-like protein 1	LX	■	■		▨					
7:31827179	Eucgr.G01728	synonymous	AT4G39010.1	AtGH9B18, GH9B18	glycosyl hydrolase 9B18	ALX	▨	▨					■	■	■
8:7499743	Eucgr.H00539	intron	AT1G48480.1	RKL1	receptor-like kinase 1	BALX					▨		■	■	■
8:9754130	Eucgr.H00759	intron	AT5G48630.2		Cyclin family protein	BALX							■	■	■
8:10228147	Eucgr.H00812	missense	AT3G24660.1	TMKL1	transmembrane kinase-like 1	LX			▨			▨			
8:58344239	Eucgr.H04049	synonymous	AT1G53025.1		Ubiquitin-conjugating enzyme family protein	LX							■	■	■
9:6475303	Eucgr.I00343	synonymous	AT2G22090.2	UBA1A	RNA-binding (RRM/RBD/RNP motifs) family protein	ALX					■	■	▨		
9:20676952	Eucgr.I00992	missense	AT4G00310.1	EDA8, MEE46	Putative membrane lipoprotein	BALX		▨			▨		■	■	■
9:27865319	Eucgr.I01789	intron	AT3G01510.1	LSF1	like SEX4 1	LX	■	■		▨			▨		
9:29125069	Eucgr.I01918	intron	AT1G80170.1		Pectin lyase-like superfamily protein	LX							▨	▨	▨
10:808833	Eucgr.J00053	synonymous	AT5G43240.1		Protein of unknown function (DUF674)	BALX					■	▨	■	■	■
10:12150982	Eucgr.J01104	missense	AT4G23410.1	TET5	tetraspanin5	BALX					▨	▨	■	■	■
10:16128763	Eucgr.J01349	5' UTR; synonymous	AT3G01180.1	AtSS2, SS2	starch synthase 2	BALX	▨	▨			▨		■	■	■

10:29282238	Eucgr.J02333	synonymous	AT3G27150.1		Galactose oxidase/kelch repeat superfamily protein	LX	▨	▨					■	■	■
11:28408708	Eucgr.K02126	intron	AT4G13020.1	MHK	Protein kinase superfamily protein	LX	■	■	▨	▨					
11:29546106	Eucgr.K02229	missense	AT1G73830.1	BEE3	BR enhanced expression 3	LX		▨					■	▨	▨

[1] B = BayeScan (logBF > 0.5), A = Arlequin (Hierarchical FDIST2; q <= 0.1), L = Lositan (FDIST2; q <= 0.1), X = Bayenv $X^T X$ (top 5%)

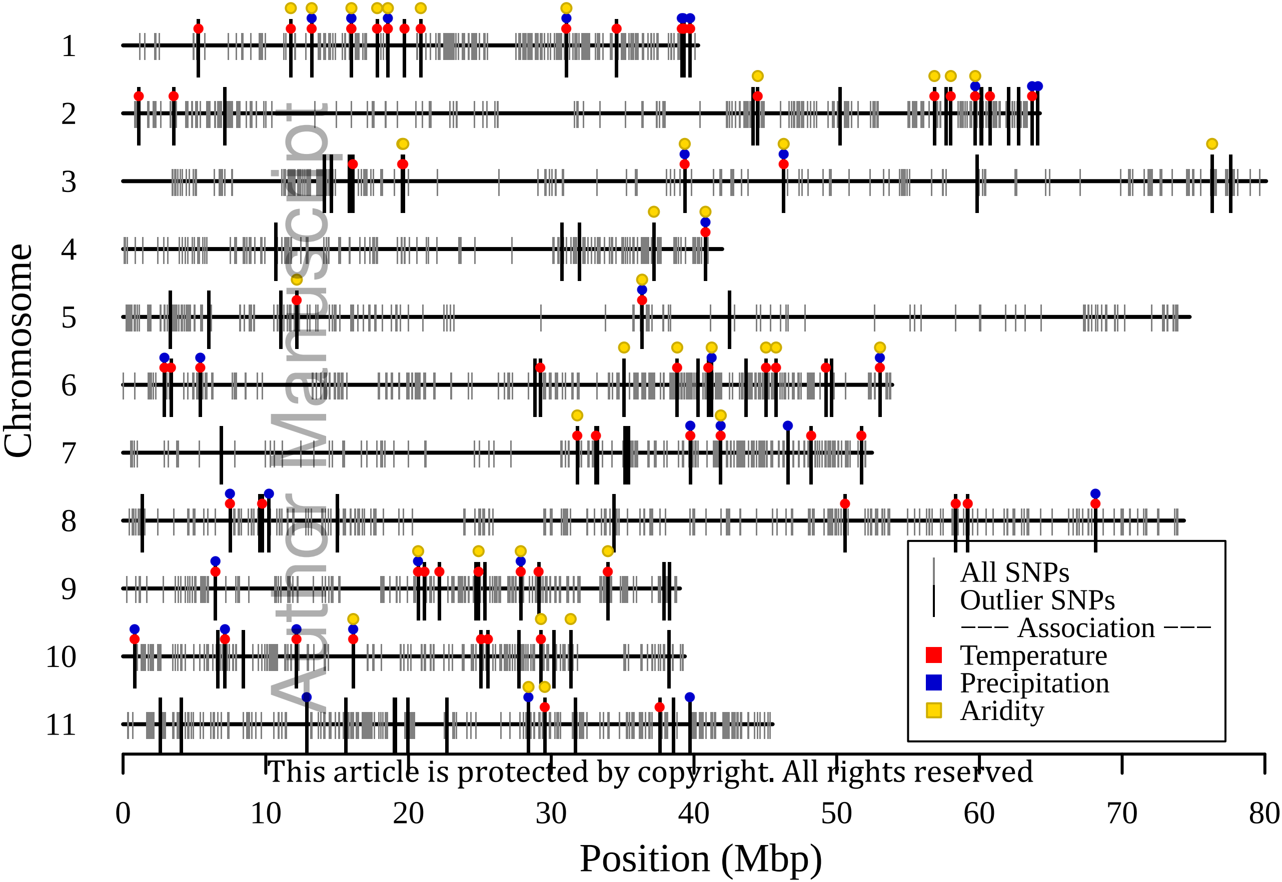
[2] Black shading = very strong association (BF > 150), grey shading = strong association (20 < BF < 150). Ann. Aridity = Mean annual aridity index (ratio precipitation to potential evaporation), Max. Aridity = Maximum month aridity index (ratio precipitation to potential evaporation), Ann. Prec. = Annual precipitation (mm), Winter Prec. = Winter precipitation (mm), Summer prec. = Summer precipitation (mm), Driest Prec. = Driest period precipitation (mm), MAT = Mean annual temperature (ºC), Warmest Max. Temp. = Warmest period maximum temperature (ºC). See Supporting Information, Table S1 for climate variable definitions.

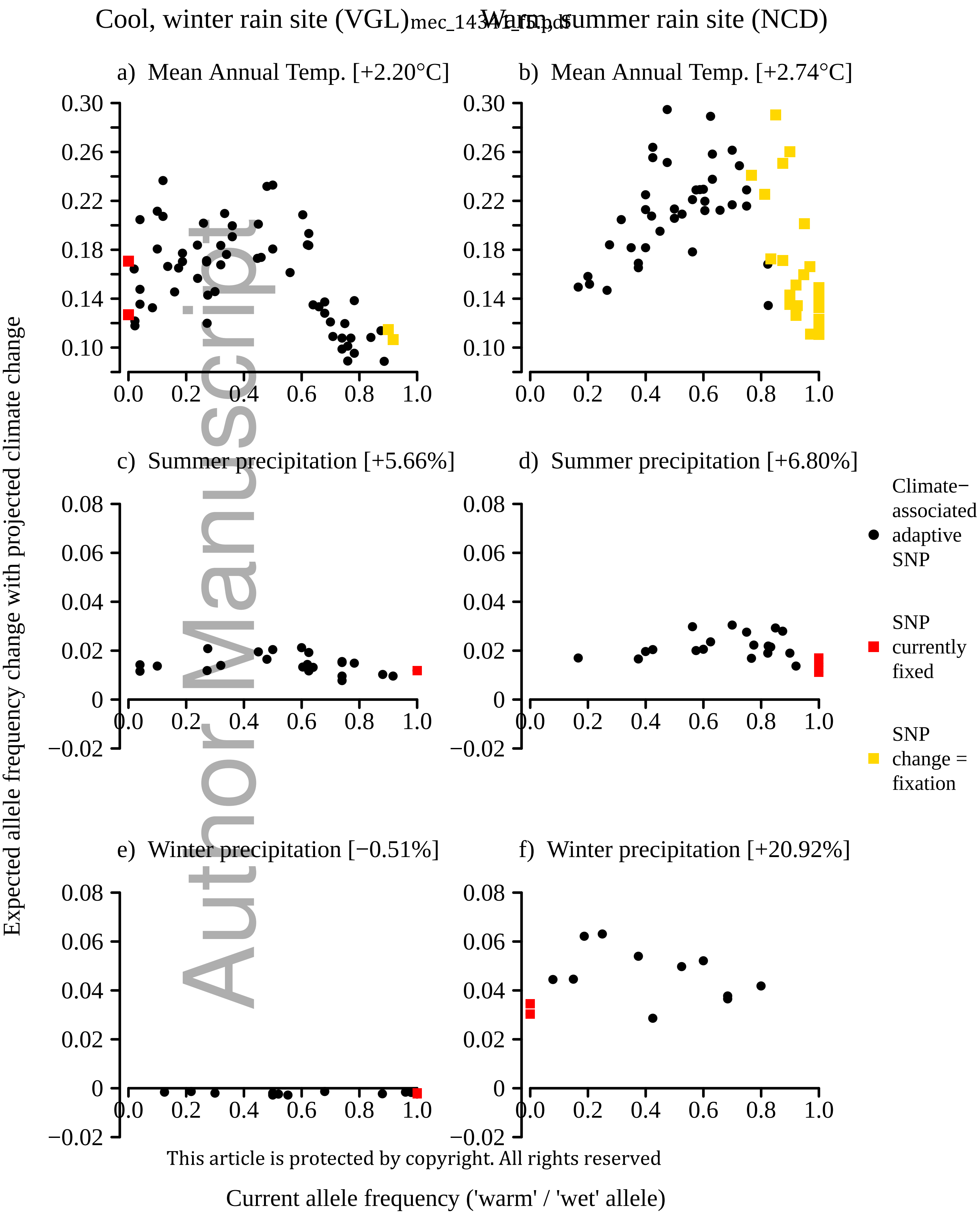

Elevation
1380 m
0 m
Genetic region
● Central NSW
● Southern NSW
● Central Vic
● Western Vic
NAL
NDU
NBG
NCD
EWN
NBY
NHI
NGR
NCR
NEW SOUTH WALES
NNA
NBJ
NCB
NTA
Canberra
ACT
NFN
NTR
VICTORIA
NMV
NCN
VMJ
VTT
VDB
VMA
VRH
VMC
VBU
VGL
VWG
Melbourne
N
0
100
200 km

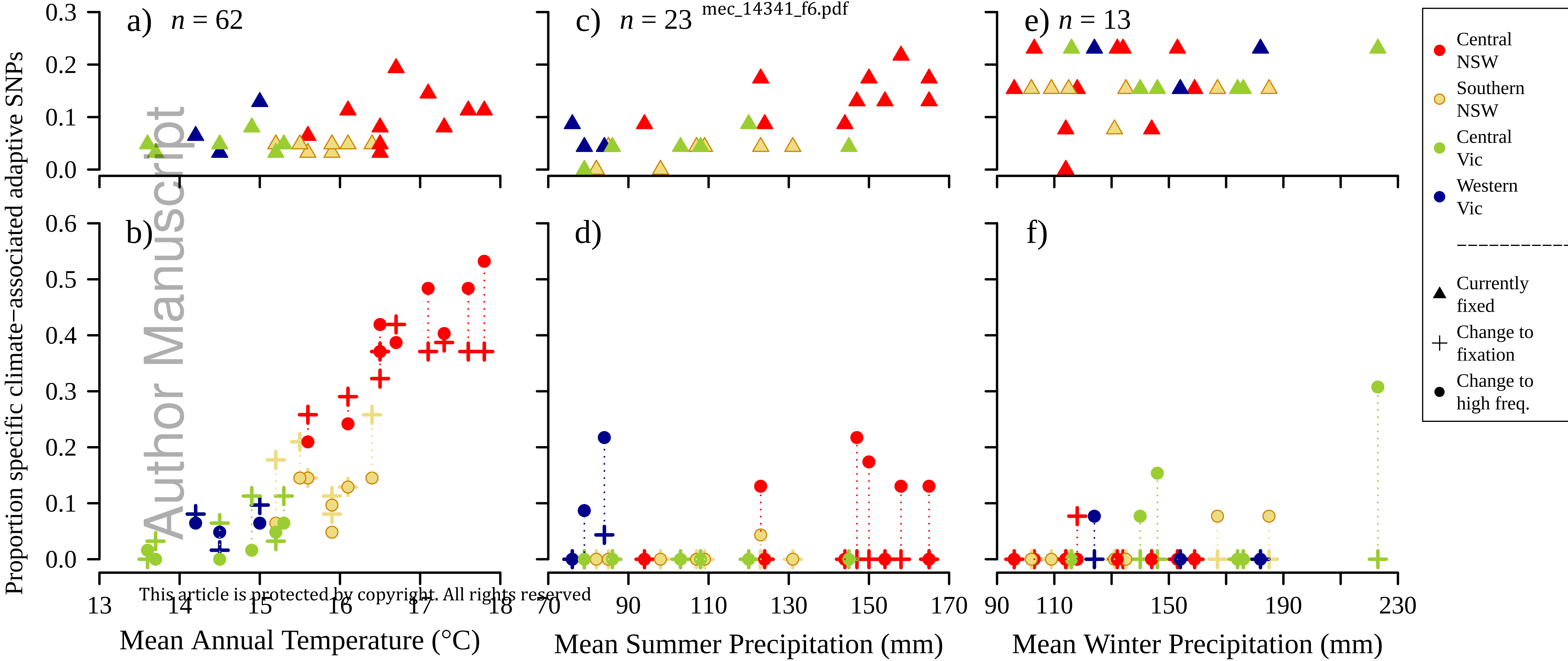

a)
PC2 (0.53%)
2
0
-2
-4
-6
-4
-3
-2
-1
0
1
2
3
4
PC1 (0.99%)
b)
PC2 (1.08%)
2
1
0
-1
-2
-2
-1
0
1
2
PC1 (1.2%)
c)
PC2 (2.6%)
2
1
0
-1
-2
-2
-1
0
1
2
PC1 (7.41%)
Central NSW
● EWN
● NAL
● NBG
● NBY
● NCD
● NCR
● NDU
● NGR
● NHI
● NNA
Sth NSW
○ NBJ
○ NCB
○ NCN
○ NFN
○ NMV
○ NTA
○ NTR
Central Vic
▲ VDB
▲ VGL
▲ VMC
▲ VRH
▲ VTT
▲ VWG
Western Vic
△ VBU
△ VMA
△ VMJ

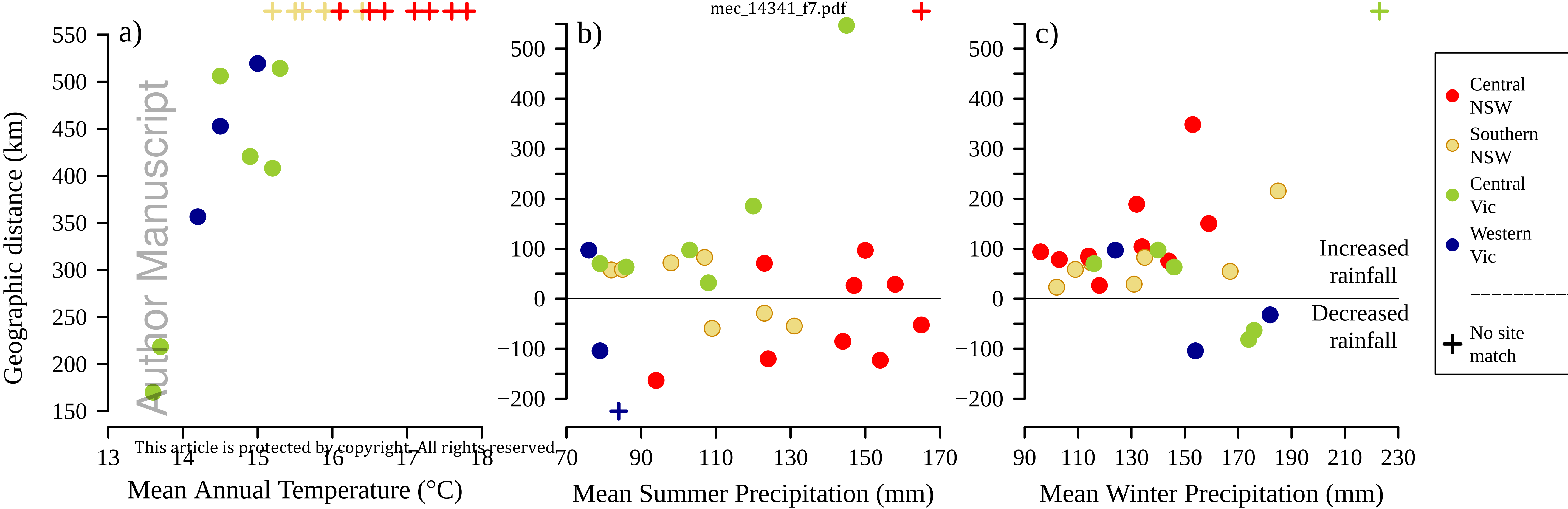

FDIST2 (223)
h-FDIST2 (65)
BayeScan (39)
Bayenv $X^T X$ (173)
89
8
0
6
1
0
0
16
44
34
0
73
0
5
0

mec_14341_f4.pdf




Cool, winter rain site (VGL)
Warm, summer rain site (NCD)
a) Mean Annual Temp. [+2.20°C]
b) Mean Annual Temp. [+2.74°C]
c) Summer precipitation [+5.66%]
d) Summer precipitation [+6.80%]
e) Winter precipitation [−0.51%]
f) Winter precipitation [+20.92%]
0.30
0.26
0.22
0.18
0.14
0.10
0.08
0.06
0.04
0.02
0
−0.02
0.0
0.2
0.4
0.6
0.8
1.0
Expected allele frequency change with projected climate change
Current allele frequency ('warm' / 'wet' allele)
● Climate−associated adaptive SNP
■ SNP currently fixed
■ SNP change = fixation


Proportion specific climate−associated adaptive SNPs
a) n = 62
c) n = 23
e) n = 13
b)
d)
f)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
13
14
15
16
17
18
70
90
110
130
150
170
230
190
Mean Annual Temperature (°C)
Mean Summer Precipitation (mm)
Mean Winter Precipitation (mm)
● Central NSW
● Southern NSW
● Central Vic
● Western Vic
▲ Currently fixed
+ Change to fixation
● Change to high freq.


a)
b)
c)
Geographic distance (km)
550
500
450
400
350
300
250
200
150
13
14
15
16
17
18
Mean Annual Temperature (°C)
500
400
300
200
100
0
−100
−200
70
90
110
130
150
170
Mean Summer Precipitation (mm)
90
110
130
150
170
190
210
230
Mean Winter Precipitation (mm)
Increased rainfall
Decreased rainfall
● Central NSW
● Southern NSW
● Central Vic
● Western Vic
+ No site match