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Contrasting patterns of local adaptation along climatic gradients between a sympatric parasitic and autotrophic tree species

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Abstract

Sympatric tree species are subject to similar climatic drivers, posing a question as to whether they display comparable adaptive responses. However, no study has explicitly examined local adaptation of co-occurring parasitic and autotrophic plant species to the abiotic environment. Here we test the hypotheses that a generalist parasitic tree would display a weaker signal of selection and genomic variation would associate with fewer climatic variables (particularly precipitation) but have similar spatial patterns to a sympatric autotrophic tree species. To test these hypotheses, we collected samples from 17 sites across the range of two tree species, the hemi-parasite *Nuytsia floribunda* (*n*=264) and sympatric autotroph *Melaleuca rhaphiophylla* (*n*=272). We obtained 5,531 high-quality genome-wide single nucleotide polymorphisms (SNPs) for *M. rhaphiophylla* and 6,727 SNPs for *N. floribunda* using DArTseq™ genome scan technology. Population differentiation and environmental association approaches were used to identify signals of selection. Generalized dissimilarly modelling was used to detect climatic and spatial patterns of local adaptation across climatic gradients. Overall, 322 SNPs were identified as putatively adaptive for the autotroph, while only 57 SNPs were identified for the parasitic species. We found genomic variation to associate with different sets of bioclimatic variables for each species, with precipitation relatively less important for the parasite. Spatial patterns of predicted adaptive variability were different and indicate that co-occurring species with disparate life history traits may not respond equally to selective pressures (i.e. temperature and precipitation). Together, these findings provide insight into local adaptation of sympatric parasitic and autotrophic tree species to abiotic environments.

Keywords

Comparative genomics, generalist parasite, genome scans, genotype environment association, landscape genomics, parasitic species

1. Introduction

Patterns of local adaptation emerge from the interplay between the evolutionary processes of natural selection and gene flow (Kawecki & Ebert, 2004), and these can vary greatly between species (Savolainen, Lascoux, & Merilä, 2013). Local adaptation can enable species' persistence under changing climates (Franks & Hoffmann, 2012), facilitate range expansion or contraction (Davis & Shaw, 2001), and enable adaptation to biotic environments (Kaltz & Shykoff, 1998). Therefore, understanding local adaptation of species is essential for informing landscape conservation and management practises (Bragg, Supple, Andrew, & Borevitz, 2015; Flanagan, Forester, Latch, Aitken, & Hoban, 2018), particularly given that species respond to drivers of local adaptation in different ways (Aitken, Yeaman, Holliday, Wang, & Curtis-McLane, 2008). Information on patterns of local adaptation can be integrated into management approaches to improve the likelihood of species long-term survival and persistence under variable future climates (Flanagan et al., 2018); for instance in the design of assisted migration in animals and climate appropriate seed sourcing in plants (Allendorf, Hohenlohe, & Luikart, 2010; Breed et al., 2019; Hoffmann et al., 2015).

Detecting signals of selection and identifying climatic drivers is crucial for understanding local adaptation within species (Kawecki & Ebert, 2004). Traditionally, this has been studied using common garden and reciprocal transplant experiments (Blanquart, Kaltz, Nuismer, & Gandon, 2013; Kawecki & Ebert, 2004), but more recently using genomic detection methods (Savolainen et al., 2013; Stapley et al., 2010); for example by using genome scans that discover putatively adaptive loci in a reverse-ecology approach (Li, Costello, Holloway, & Hahn, 2008;

Tiffin & Ross-Ibarra, 2014). Genomic methods have also been combined with spatial modelling to further quantify and map patterns of allelic turnover along climatic gradients, which can reveal climatic relationships that traditional genomic methods would otherwise overlook (Fitzpatrick & Keller, 2014). As species within the same landscape can display different signals of selection to climatic variables (e.g. Hopley & Byrne, 2019; Shryock et al., 2017), comparing patterns between sympatric species would provide a better understanding of the common climatic pressures that facilitate selection.

Comparative genomic studies between multiple plant species can provide insight into landscape genomic variation and adaptation (Bragg et al., 2015; Wang & Bradburd, 2014). To date, studies on comparative adaptive genomics have focussed predominantly on species with differing pollination strategies (e.g. Shryock et al., 2017), species occurring in specific habitat niches (e.g. Hopley & Byrne, 2019), or species within the same family (e.g. Nadeau, 2014; Steane, Potts, et al., 2017). Few studies have considered the life history of parasitism and it is not known whether analogous signals of adaptation are observed between plants with a parasitic life history and co-occurring autotrophic species.

Parasitic plants have evolved to utilise a variety of host species and inhabit a diverse range of abiotic environments, with many considered keystone resources within ecosystems (Press & Phoenix, 2005; Watson, 2001). Generally, these taxa rely on a specialised haustorium to penetrate the roots or stems of hosts to obtain water and nutrients (Calder, 1983). As parasitic plants are reliant on occurrence of host species for survival, the distribution of hosts may shape adaptive genetic diversity in parasitic species, providing a unique opportunity for studying local adaptation (Gandon & Van Zandt, 1998; Kawecki & Ebert, 2004). Furthermore, many studies have assessed local adaptation of parasites to their hosts (see meta-analysis by

Greischar & Koskella, 2007), but few have quantified local adaptation to the abiotic environment (Gorter, Scanlan, & Buckling, 2016).

Generalist plant parasites utilise multiple host species for survival either through parasitising single individuals at a time (e.g. Barney, Hawksworth, & Geils, 1998) or multiple individuals simultaneously (e.g. Woodall & Robinson, 2003). Many generalists, particularly perennial species, are relatively widespread across landscapes, and it has been suggested that heterogeneous host communities promote occurrence of generalist parasites (Norton & Carpenter, 1998). Consequently, perennial generalist parasites may have a wider tolerance to climatic conditions in comparison to sympatric autotrophic plants, but we are not aware of any study that explicitly compares local adaptation along climatic gradients in sympatric parasitic and autotrophic species.

Comparing signals of selection between plant species with different life history traits is important to increase our understanding of local adaptation and provide material to inform conservation strategies, such as seed sourcing for restoration (Breed et al., 2019). Here, we focus on a widespread sympatric generalist parasite and an autotrophic plant species that have experienced similar abiotic selective pressures (e.g. climatic conditions). Parasitic plants have been previously found to have faster rates of molecular evolution compared to autotrophic relatives (Bromham, Cowman, & Lanfear, 2013), although an increased mutation rate does not necessarily result in adaptive substitution (Weissman & Barton, 2012). However, increased mutation rates are generally associated with shorter generation times and higher reproductive rates (Gandon & Michalakis, 2002; Kaltz & Shykoff, 1998), whereas our generalist parasitic species is long-lived, clonal and rarely reproduces by seed (Hocking & Fineran, 1983).

Temperature and precipitation have been found to be important drivers of adaptive genomic diversity in autotrophic plants (Hopley & Byrne, 2019; Shryock et al., 2017; Steane, Mclean, et al., 2017; Supple et al., 2018), but no study has quantified the importance of climatic drivers on adaptive genomic diversity in parasitic species. As generalist parasites acquire water from numerous host species that include deep-rooted perennial plants (Hocking & Fineran, 1983), they may have different tolerances to climatic conditions than autotrophs that acquire water from the abiotic environment. Additionally, parasitic plants are known to have higher transpiration rates, which are influenced by temperature, than their hosts in order to access nutrient and water flows (Ehleringer & Marshall, 1995; Kuijt, 1969). Therefore, temperature, rather than precipitation, may be a more important driver of adaptation in parasitic plants. Further, acquisition of water from multiple host plants may provide a buffer between generalist parasites and climatic conditions, thus creating a more uniform environment with reduced selection pressures compared to that experienced by autotrophic species in the same habitat.

Detecting concordant spatial patterns of local adaptation across the landscape is important for identifying environmental drivers of adaptation and informing conservation and management strategies (Bragg et al., 2015; Richardson, Brady, Wang, & Spear, 2016). While parasitic and autotrophic plants have different water and nutrient acquisition strategies, and thus may have different responses to selective pressures, it is not known whether spatial patterns of adaptation would be similar across the landscape. Identifying geographical regions where adaptation to climate occurs in multiple species may provide important information for future conservation and/or seed sourcing strategies (e.g. climate-adjusted provenancing; Prober et al., 2015).

In this study, we applied a landscape genomics approach to compare patterns of adaptive genomic variation between a generalist parasitic tree (*Nuytsia floribunda*, family Loranthaceae) and a sympatric autotrophic tree species (*Melaleuca rhaphiophylla*, family Myrtaceae) widespread in south-western Australia. *Nuytsia floribunda* is a long-lived root hemiparasite with over 100 known host species, including *M. rhaphiophylla* (Calladine, Pate, & Dixon, 2000). Both species are long-lived, have the same form and life history in terms of gene flow (i.e. pollination and seed dispersal) but have different water and nutrient acquisition strategies. Our aims were to quantify and compare for each species: (a) genomic signal of selection measured as the number of loci under putative selection; (b) the association of adaptive genomic variation with climatic variables and the relative importance of temperature, precipitation and geographic distance with allelic turnover; and (c) the predicted spatial pattern of local adaptation to climatic variables across the species' range.

We hypothesized that the parasitic plant (*N. floribunda*) would have a weaker genomic signal of selection as utilisation of multiple host species may buffer climatic conditions and create a more uniform environment with reduced selection pressures. As the parasitic plant acquires nutrients and water from other species, we expect adaptive genomic variation in *N. floribunda* to associate with fewer climatic variables, and have a lower magnitude of allelic turnover along significant climatic gradients, in comparison to the autotroph (*M. rhaphiophylla*). Further, we expect precipitation to be less important in predicting allelic turnover in the parasitic plant than for the autotrophic species that acquires water from the abiotic environment. Finally, we hypothesized that the predicted spatial pattern of local adaptation to climatic variables across the landscape would be similar between the two species because sympatric species in the same environment likely experience similar selective pressures.

2. Materials and Methods

2.1 Study species and sample collection

Melaleuca rhaphiophylla Schauer and *N. floribunda* (Labill.) R.Br. ex G.Don have widespread distributions in south-western Australia spanning *c*. 700 km north-south and *c*. 600 km east-west (**Figure 1**). The region has a Mediterranean climate with warm, dry summers and cool, wet winters with temperature and precipitation varying across the species' distribution. Both species are trees up to 10 m tall, insect-pollinated and with seed dispersal by wind and/or gravity. South-western Australia is an isolated, relatively stable landscape and phylogeographic patterns in many species indicate persistence throughout the range as a primary response through the climatic oscillations of the Pleistocene (Byrne et al., 2014). Hence, we consider both species are likely to have persisted in their current range during this time with minimal range contraction or expansions.

Species' distributional data was obtained from Florabase records of specimens lodged at the Western Australian Herbarium (https://florabase.dpaw.wa.gov.au). Sampling sites for each species were selected using random sampling to ensure sites were independent (>50 km separation) and captured the entire geographic and climatic space the species occupy. *Melaleuca rhaphiophylla* generally occurs along sandy waterways and swamps while *N. floribunda* occurs across sandplains, sandy swamps and at the base of rocky outcrops, and the two species frequently co-occur. Leaf tissue was collected from 272 adult *M. rhaphiophylla* and 265 adult *N. floribunda* plants with 12–16 individuals sequenced from each of 17 populations per species (**Tables S1, S2**). As *N. floribunda* is a clonal species (Pate, 1995) a minimum sampling distance of 50 m between individuals was set to avoid collecting the same,

or a related, individual, although in small populations this distance was reduced to 30 m. *Melaleuca rhaphiophylla* does not have a clonal habit, therefore a minimum sampling distance of 20 m was used. Samples were stored on silica gel and the location of each individual sampled was recorded using a GARMIN eTrex® 10 GPS.

2.2 Climatic data assemblage

Fifteen bioclimatic variables covering mean annual temperature, annual precipitation totals, seasonality, quarterly temperature means and quarterly precipitation totals were downloaded in raster format with 1 km cell resolution from the Worldclim 2.0 database (Fick & Hijmans, 2017; Hijmans, Cameron, Parra, Jones, & Jarvis, 2005). Point information for all 15 variables was extracted using the coordinates of each sampled individual for both species and the Spatial Analyst toolbox in ArcMap v10.7.1 (ESRI, 2019). To reduce redundancy, Spearman rank correlation tests were performed between the point data for climatic variables using the R *stats* package (R Core Team, 2019) and variables were split into temperature and precipitation groups. Variables that had pairwise correlation coefficients of $|r| < 0.8$ within each group and varied across the study area were selected to create an uncorrelated subset of climatic data, to avoid inclusion of highly correlated factors and achieve the most parsimonious model (Rellstab, Gugerli, Eckert, Hancock, & Holderegger, 2015).

Four temperature variables (isothermality, temperature seasonality, mean temperature of wettest quarter and mean temperature of the warmest quarter) and three precipitation variables (precipitation seasonality, precipitation of the wettest quarter and precipitation of the driest quarter) were selected for the uncorrelated dataset. Uncorrelated bioclimatic variables were plotted in the R package *raster* v3.0-12 (Figure S1; Hijmans, 2020) and mean values for each population are given in **Tables S1, S2**.

2.3 Genomic data generation and bioinformatics

Approximately 8 mg of silica-dried leaf material for *N. floribunda* individuals and *c*. 10 mg silica-dried leaf material *M. rhaphiophylla* individuals were sent to Diversity Arrays Technology Pty. Ltd. (Canberra, Australia) for DNA extraction using an in-house extraction protocol and individual genotyping using DArTseq™ technology (Sansaloni et al., 2011). Leaf material from a small number of individuals (3.5% for *M. rhaphiophylla* and 5% for *N. floribunda*) were replicated across multiple plates, but processed independently, to ensure between-plate continuity of genotyping. DArTseq™ combines double digest complexity reduction with high throughput sequencing to assay millions of markers for single nucleotide polymorphisms (SNPs) across the genome (Kilian et al., 2012; Sansaloni et al., 2011).

Reduction of the genome was performed using a combination of methylation-sensitive restriction enzymes, *Pst*I/*Hpa*II for *M. rhaphiophylla* and *Pst*I/*Mse*I for *N. floribunda,* with the digestion and adaptor ligation process described by Kilian et al. (2012). High-density sequencing was carried out on the Illumina HiSeq 2500 platform and, as reference genomes or transcriptomes were not available for either of these non-model species (or related species), sequences generated were aligned *de novo* using Diversity Array Technology's propriety analytical pipeline. Poor-quality sequences with a Phred score <30 (probability of incorrect base is 1 in 1,000) were removed and identical sequences collapsed to obtain *c*. 2.5 million sequences per individual. SNP marker calling was performed in the propriety DArTsoft14 pipeline with approximately 25% of samples re-genotyped as technical replicates, which allowed a measure of reproducibility to be calculated for each locus. Barcode sequences were trimmed and split into individual organism data.

To ensure only high-quality data were used for downstream analysis, further quality control filtering was performed using the package *dartR* v1.1.11 (Gruber, Unmack, Berry, & Georges, 2018) in R (R Core Team, 2019) with replicates of individuals first removed from the SNP datasets. We filtered the dataset to retain: 1) loci with less than 5% missing data; 2) high reproducibility (DArTseq™ reproducibility score > 0.98); 3) minor allele frequency (MAF) greater than 5%; and 4) individuals with <20% missing data. As many genomic analysis programs assume loci are not linked (see Hoban et al., 2016), closely linked loci were excluded from the dataset by randomly selecting one SNP per fragment to be retained in the dataset. Filtered datasets were converted to csv format using the *write.csv* function (R Core Team, 2019).

2.4 Population structure

Population differentiation (pairwise F_{ST}) was estimated according to Weir and Cockerham (1984) using the R package *hierfstat* v0.04-22 (Goudet, 2005). To investigate population structure for both species, we first analysed the filtered datasets at an individual level using principal coordinate analysis (PCoA) in the R package *adegenet* v2.1.1 (Jombart, 2008; Jombart & Ahmed, 2011) and plotted the first two PCoA axes using R package *ggplot2* v3.2.1 (Wickham, 2016).

Secondly, we estimated the number of ancestral population groups using the sparse non-negative matrix factorization (SNMF) algorithm in the R package *LEA* v2.4 (Frichot et al. 2015), which estimates the most likely number of ancestral genetic clusters (*K*). We tested values of *K* between *K*=1 and *K*=20 with ten replicates run for each species using the default alpha regularization parameter of 100 with 5% of genotypes masked for calculation of the cross-entropy error. Average cross-entropy values were plotted for each value of *K* and we selected

the optimum value of *K* in predicting masked genotypes at the point of inflection where the additional loss of cross-entropy becomes minimal (François, 2016).

Finally, to examine the influence of geography on genomic structure (i.e. isolation-by-distance), we ran Mantel tests in the R package *vegan* v2.5-6 (Oksanen et al., 2019) to assess significance of the correlation between the pairwise population-level genetic distances measured as $F_{ST}/(1-F_{ST})$ and the natural logarithm of geographic distance. Geographical matrices were calculated in the R package *fossil* v0.3.7 (Vavrek, 2011) and Mantel tests were run using Pearson's correlation coefficient with 9999 permutations.

2.5 Analytical approaches

2.5.1 Genomic signals of selection

To test whether the parasitic species had a weaker genomic signal of selection than the autotrophic species, we used population differentiation (PD) tests and environmental association (EA) analyses to identify loci under putative selection. PD methods identify putatively adaptive loci with significantly higher genetic differentiation between populations than expected under neutral evolutionary processes (e.g. gene flow, genetic drift). In contrast, EA approaches identify significant correlations between allele frequencies and environmental variables to identify loci that may be associated with climatic variables (i.e. temperature and precipitation; Hoban et al., 2016). PD tests were performed using OutFLANK (Whitlock & Lotterhos, 2015) and PCAdapt (Luu, Bazin, & Blum, 2017), while the EA approaches used were latent factor mixed modelling (LFMM; Caye, Jumentier, Lepeule, & François, 2019; Frichot, Schoville, Bouchard, & François, 2013), and redundancy analysis (RDA; Forester, Lasky, Wagner, & Urban, 2018).

OutFLANK uses a modified Lewinton-Krakauer method to infer a null F_{ST} distribution of loci not affected by spatially diversifying selection to detect loci under positive selection, assuming F_{ST} follows a chi square distribution (Whitlock & Lotterhos, 2015). Corrections for false discovery rate (FDR) were applied and outlier loci were differentiated as those that fell outside the null F_{ST} distribution as likely to be experiencing evolutionary processes, such as local adaptation (Storfer, Patton, & Fraik, 2018). PCAdapt uses principal component analysis and assumes loci with excessive association to population structure are candidates for loci under selection. The approach uses a robust Mahalanobis distance as the test statistic to detect outlier loci as those for which the ʐ-scores do not follow the same distribution as those of the larger dataset while using the *q*-value procedure to control the FDR (Luu et al., 2017).

Analyses were run using the R packages *OutFLANK* v0.2 (Whitlock & Lotterhos, 2015) and *pcadapt* v4.1.0 (Luu et al., 2017) with the FDR set to 5% and the number of populations set to 17 for both species. OutFLANK was run with 5% left and right trims for the null distribution of F_{ST} and a minimum heterozygosity for loci of 0.1. PCAdapt was initially run with K=20 to identify the optimum number of principal components (PCs) for each species using Cattell's rule (see Cattell 1966) to interpret the scree plot. Then, PCAdapt was run again with the optimum number of PCs and a minimum MAF threshold of 0.05 to calculate the Mahalanobis distance and *p*-values for each locus. For both approaches, *p*-values were transformed into *q*-values using the R package *qvalue* v2.18.0 (Storey, Bass, Dabney, & Robinson, 2019) and all loci with a *q*-value below 0.05 were identified as outliers (i.e. putative signals of selection).

LFMM 2.0 is a univariate EA method that was implemented in the R package *lfmm* v0.0 (Caye et al., 2019) using least-squares estimation approach to control for confounding variables. LFMM uses allele frequency data and the imputed number of latent factors (i.e. ancestral

population groups) determined by SNMF to calculate an exact solution for latent factor regression models. Missing data was imputed using mode data for *K* ancestral genetic clusters and climatic variables were scaled such that each variable had a standard deviation of 1. Parameters for LFMM analysis were obtained through ridge estimates using *K* latent factors and LFMM tests were run for each climatic variable. Genomic inflation factors (λ) were calculated from the z-scores for each climatic variable and were used to adjust *p*-values based on a chi-squared (χ2) distribution (François, Martins, Caye, & Schoville, 2016). Histograms of *p*-values and λ value were examined to assess model fit as histograms should have a uniform distribution with a peak near 0 and λ should be close to 1, to ensure SNP data fits the LFMM model. We then applied a Benjamini-Hochberg *p*-value correction according to Frichot and François (2015) and SNP-environment variable associations were considered significant when $q<0.05$.

To further assess SNP-environment associations, constrained RDA was performed using the R package *vegan* v2.5-6 (Oksanen et al., 2019) as described in Forester et al. (2018). RDA is a multivariate EA method that projects the variation in genomic data explained by predictor variables (i.e. climatic data) onto a reduced space, assuming a linear relationship between genetic and predictor data (Capblancq, Luu, Blum, & Bazin, 2018). Missing genotypes were imputed in the same process as for LFMM analysis and we constrained the individuals by climatic variables to identify the relationship between genotypes and climatic data. Significance of the overall model was assessed using the *anova.cca* function, and the first three RDA axes were selected for further analysis. We extracted the SNP loadings for all selected RDA axes and identified SNPs that had RDA scores ±3 standard deviations from the mean as those considered to have significant environmental association. The climatic variable

that had the greatest absolute SNP loading was the predictor variable with which the significant SNP was associated.

Genomic signals of selection were compared between the two species with the total number of significant SNPs across each method plotted in Venn diagrams using the R package *VennDiagram* v1.6.20 (Chen, 2018). Secondly, we used Pearson's Chi-Square test with Yates' continuity correction (Yates, 1934) in R (R Core Team, 2019) to test whether the number of adaptive and non-adaptive SNPs was significantly associated with species.

2.5.2 Patterns of local climatic adaptation

Generalized dissimilarity modelling (GDM; Ferrier, 2002; Ferrier, Watson, Pearce, & Drielsma, 2002) was used to test whether: (i) genomic variation in the parasite associated with fewer climatic variables and that magnitude of allelic turnover would be higher in the autotrophic plant, using the method described by Fitzpatrick and Keller (2014); and (ii) preciptiation variables would be relatively less important in predicting allelic turnover than either temperature or geographic distance for the parasitic plant but not for the autotrophic species, using variation partitioning (Borcard, Legendre, & Drapeau, 1992). For each species we first estimated pairwise F_{ST} matrices (Weir & Cockerham, 1984) using the R package *hierfstat* v0.04-22 (Goudet, 2005). Separate F_{ST} matrices were created for i) reference SNPs (neutral SNPs not detected by any genomic method) and ii) candidate SNPs, and scaled each F_{ST} matrix to between 0 and 1 prior to running the GDM. SNPs in the candidate dataset were those identified by two or more genomic methods, which can be a useful strategy to ensure false-positive rates are kept low (Forester et al., 2018). However, due to the limited number of SNPs identified by multiple methods for *N. floribunda* and the challenge for genetic distance calculation, we also included SNPs in the candidate dataset that were identified singularly by

one of the EA methods. Population-level climatic datasets were compiled with the geographic coordinates of each population and the seven climatic variables used in the EA analysis as predictors.

We implemented GDM analysis in the R package *gdm* v1.3.11 (Manion et al., 2018), which removed variation attributed to geographic distance and compared the remaining genomic variation to climatic variables to identify the importance of climate in allelic turnover. Initially, we used the default of three splines and a backwards elimination procedure with 500 permutations at each step to measure the significance (at a 5% significance level) of each climatic variable (Ferrier, Manion, Elith, & Richardson, 2007; Fitzpatrick et al., 2013). These procedures were run separately for each dataset and only significant bioclimatic variables were retained. The three spline coefficients were summed for each remaining predictor variable (e.g. subset of geographic distance and the seven climatic variables) to quantify the relative importance (Fitzpatrick et al., 2013; Yates et al., 2019).

To evaluate the contributions of temperature and precipitation, as captured by multiple climatic variables, and geographic distance in explaining allelic turnover in the model, we partitioned the deviance resulting from GDMs using geographic distance, all significant temperature variables and/or all significant precipitation variables (Borcard et al., 1992; Yates et al., 2019). The partioned deviance values were plotted in Venn Diagrams using the R package *eulerr* v6.0.0 (Larsson, 2019).

Finally, monotonic I-spline turnover functions were calculated for all remaining predictor variables where spline height represented the amount of explained genomic variation, when holding all other variables constant, and spline slope indicated the rate of genetic differentiation across the range of the predictor (Fitzpatrick & Keller, 2014; Fitzpatrick et al.,

2013). These functions were mapped using *ggplot2* to visualise the relationship between allelic turnover and climatic variables.

2.5.3 Spatial patterns of local adaptation

To assess whether the predicted spatial patterns of local adaptation to climatic variables across the landscape were similar for both the parasitic and autotrophic species, we followed the approach by Fitzpatrick and Keller (2014) to visualise genomic variation. Spatial interpolations of genomic distance were derived for the two datasets (reference SNPs; candidate SNPs) of both species using the fitted GDMs to perform biologically-informed transformations for raster datasets of significant climatic variables into genetic importance values. Geographic distance, even if a significant predictor of genomic variation, was not included in the fitted model for GDM transformation as it could not be similarly included as a raster dataset. We used principal component analysis (PCA) in R (R Core Team, 2019) to reduce the transformed climatic variables into three principal components that were converted into raster grids. An RGB colour palette was assigned to each of the raster grids and mapped in geographical space with similarity in colour corresponding to similarity in predicted patterns of adaptive genomic variability.

To measure the similarity of multivariate configuration between the two datasets (reference SNPs; candidate SNPs) for each species, we ran a PCA on extracted values of the GDM-transformed climatic variables for each dataset following Fitzpatrick and Keller (2014) and performed Procrustes Analysis on the resulting PCA ordinations based on Peres-Neto and Jackson (2001). The Procrustes residuals reflect the absolute distance in spatial genetic predictions between the two datasets and these were mapped in geographical space to allow identification of areas with large differences in predicted genomic variability where local

adaptation may be occurring. Prior to mapping, we scaled the residuals by the largest and smallest value observed across both species to allow direct comparison of the differences in predicted genomic variability between the parasitic and autotrophic species.

3. Results

3.1 SNP generation and population structure

DArTseq™ technologies produced datasets of 52,450 SNPs for *M. rhaphiophylla* (*n*=272) and 36,881 SNPs for *N. floribunda* (*n*=265). Nine of the ten biological replicates for *M. rhaphiophylla* had greater than 98% genetic similarity with the tenth replicate having 82.4% similarity. Similarly, fifteen of the sixteen biological replicates for *N. floribunda* had greater than 95% genetic similarity with the sixteenth replicate having 88.5% similarity. Following further quality control filtering described in the methods, the working datasets for each species comprised 5,531 SNPs for *M. rhaphiophylla* and 6,727 SNPs for *N. floribunda* (Walters, Robinson, Byrne, Wardell-Johnson, & Nevill, 2020) with global missing data of 1.30% and 1.01%, respectively. No individuals were removed from the *M. rhaphiophylla* dataset, and one individual with a low marker call rate from the Yanchep population was removed from the *N. floribunda* dataset.

There was moderate population differentiation across the range of both species with a global F_{ST} of 0.111 for *M. rhaphiophylla* and 0.178 for *N. floribunda*. The PCoA of population structure showed discrete clustering of individuals by population with overlap in populations at the centre of the species' distributions (**Figure 1**). Populations in the northern and eastern areas separated from the remaining populations in both species, reflecting their spatial distribution at the edge of the range. In general, the distribution of populations was more

continuous for *N. floribunda*. Overall, the two PCoA axes explained 14.9% and 16.2% of the genomic variation between individuals for *M. rhaphiophylla* and *N. floribunda*, respectively.

SNMF analysis indicated the presence of five main ancestral population groups for *M. rhaphiophylla* and four main ancestral population groups for *N. floribunda*, with these values representing the number of latent factors included in LFMM analysis. Mantel tests indicated presence of statistically significant isolation-by-distance (IBD) patterns for *M. rhaphiophylla* (p=0.017) and *N. floribunda* (p<0.001). Geographic distance explained 36.8% of the variation in genetic divergence for *M. rhaphiophylla* and 58.2% of the variation for *N. floribunda*. Patterns of IBD were even stronger for *M. rhaphiophylla* when the two populations that were most separated along the first PCoA axis (**Figure 1a**) were removed. Geographic distance explained 69.0% of the variation in genetic divergence for the fifteen remaining *M. rhaphiophylla* populations (p<0.001).

3.2 Genomic signals of selection

The numbers of adaptive and non-adaptive SNPs were significantly associated with species (χ^2= 249.02, df=1, p<0.001). Overall, 322 putatively adaptive SNPs (5.28%) were identified by at least one analytical method for *M. rhaphiophylla*, while only 57 SNPs (0.85%) were identified by those same methods for *N. floribunda* (**Figure 2**). Of these putatively adaptive SNPs, only 21.1% and 1.75% for *M. rhaphiophylla* and *N. floribunda*, respectively, were identified by multiple methods. While OutFLANK overlapped the least with other methods (**Figure 2**), excluding OutFLANK results still produced a significant association between SNPs and species (χ^2= 48.09, df=1, p<0.001).

OutFLANK identified 4.36% of *M. rhaphiophylla* SNPs (mean F_{ST}=0.10, df=7.57) and 0% of *N. floribunda* SNPs (mean F_{ST}=0.19, df=8.48) as under putative positive selection across the 17 sampling sites. In contrast, PCAdapt identified 1.70% of *M. rhaphiophylla* SNPs (mean χ^2=4.99, df=4) and 0.37% of *N. floribunda* SNPs (mean χ^2=2.19, df=2) as outlier loci.

LFMM identified 36 and 11 significant SNP-environment associations involving 16 and 10 SNPs for *M. rhaphiophylla* and *N. floribunda*, respectively. Genomic inflation factors varied from 3.10–6.29 for *M. rhaphiophylla* and 4.15–8.12 for *N. floribunda*. The number of significant SNPs varied between climatic variables for both species and no SNP for either species was significantly associated across all variables (**Table 1**).

Lastly, RDA identified 51 and 23 SNP-environment associations for *M. rhaphiophylla* and *N. floribunda*, respectively. Climatic data explained 14.2% and 17.7% of the variation in genomic data for *M. rhaphiophylla* and *N. floribunda*, adjusted based on the number of predictors. The overall RDA model was significant for both *M. rhaphiophylla* ($F_{7,264}$=7.40, *p*=0.001) and *N. floribunda* ($F_{7,256}$=9.08, *p*=0.001) and the first three axes explained over 75% of the variation in genomic data. The number of SNPs identified as demonstrating signatures of selection identified also varied across the seven climatic variables for both species (**Table 1**).

3.3 Patterns of local climatic adaptation

Candidate SNP datasets identified by two or more genomic methods, or singularly by one of the EA methods, comprised 98 and 33 SNPs for *M. rhaphiophylla* and *N. floribunda*, respectively. Three significant predictor variables were retained in the GDM model of reference SNPs for *M. rhaphiophylla*, while four variables were retained for the candidate SNPs dataset. In contrast, five significant predictor variables were retained in the GDM model

of reference SNPs for *N. floribunda* while six variables were retained for the candidate SNPs dataset. The best GDM models (i.e. containing only significant predictor variables) explained a similar deviance in turnover in genetic composition for both species (**Table 2**).

Temperature and precipitation variables, together, explained 74.53% of GDM model deviance for the *M. rhaphiophylla* candidate SNP dataset, with majority of allelic turnover explained by both temperature and precipitation (**Figure 3a**). Similarly, temperature and precipitation variables, together, explained 69.28% of GDM model deviance for the *N. floribunda* candidate SNP dataset, although 59.6% of allelic turnover was explained by temperature alone (**Figure 3b**). Geographic distance contributed the least to explaining GDM model deviance for both species, and was almost completely nested within the other two variables.

Patterns of allelic turnover varied by both climatic variable and species (**Figure 4**). The most important predictor variable for both *M. rhaphiophylla* datasets was isothermality with the largest change in allelic turnover predicted to occur above 51% (**Figure 4a**), which corresponds to the southern area of the species range (**Figure S1**). In contrast, the most important predictor for the *N. floribunda* reference SNP dataset was geographic distance, with a gradual change in allelic turnover across the geographic range (**Figure 4h**). The most important predictor for the *N. floribunda* candidate SNP dataset was temperature seasonality (**Table 2**), with the largest change in allelic turnover predicted to occur above 425% (**Figure 4b**), corresponding with inland areas (**Figure S1**).

3.4 Spatial patterns of local adaptation

The spatial patterns of predicted genetic composition were different for each species and dataset (**Figure 5**). Rapid turnover in genetic composition was predicted for *M. rhaphiophylla*

in the eastern region of its range and comparatively little elsewhere. In constrast, the turnover in genetic composition for *N. floribunda* occurred throughout the species range, with more rapid turnover in the centre. Differences in multivariate configuration between reference and candidate SNP datasets for *M. rhaphiophylla* were strongest (i.e. Procrustes residuals >0.7) in isolated areas along the south-western coast with moderate differences (i.e. Procrustes residuals between 0.4-0.7) detected throughout the western coast region (**Figure 5c**). In contrast, the strongest differences for *N. floribunda* were found in isolated areas along the western coast with moderate differences along the southern coast and in the northern region (**Figure 5f**).

4. Discussion

Our analysis of patterns of local adaptation confirms our expectation of a weaker genomic signal of selection in the long-lived, generalist parasitic species *N. floribunda*, compared to the sympatric autotrophic species *M. rhaphiophylla*. Further, while we observed that the overall magnitude of allelic turnover was stronger in the autotrophic species, we did not find adaptive genomic variation in the parasite to associate with fewer climatic variables as hypothesized. In fact, the parasitic species had more climatic associations and these were predominantly with temperature variables, as we had expected due to the species' water acquisition strategy (Hocking & Fineran, 1983). Finally, we observed a differing spatial pattern of local adaptation between the parasitic and autotrophic species, suggesting that co-occurring species with varying life histories may respond differently to landscape-scale selective pressures.

4.1 Genomic signals of selection

Our investigation found a weaker genomic signal of selection in the parasitic plant *N. floribunda*, despite both study species occurring across similar geographical and climatic gradients. In particular, *N. floribunda* had fewer SNP-environment associations than autotroph *M. rhaphiophylla*, which may reflect the reliance on multiple host species for water and nutrient acquisition (Hocking & Fineran, 1983). Reliance on multiple host plants, rather than the abiotic environment, could provide a buffer to climatic conditions and create a more uniform environment with reduced selection pressures. Although these study species are not closely related, differences at the genomic level have previously been recorded between parasitic plants and autotrophic relatives (Bromham et al., 2013), yet few studies have compared genomic signals of selection between parasitic plants and their autotrophic hosts.

Performance of genomic approaches vary under differential statistical frameworks (Lotterhos & Whitlock, 2015) and the limitations of each approach have been well-documented (Ahrens et al., 2018; de Villemereuil, Frichot, Bazin, François, & Gaggiotti, 2014; Hoban et al., 2016; Lotterhos & Whitlock, 2015; Rellstab et al., 2015). Consequently, we opted to utilise multiple methods as recommended by De Mita et al. (2013) to select candidate SNPs using a consensus approach, allowing for robust identification of adaptive loci independent from assumptions (and limitations) of a single model (Rellstab et al., 2015). In our study, numbers of loci identified with signals of selection varied by species, climatic variable and method; this has also been observed in other genomics studies (e.g. Ahrens, Byrne, & Rymer, 2019; Andrew, Jensen, Hagen, Lundregan, & Griffith, 2018; Hopley & Byrne, 2019). Further, the overall proportion of SNPs identified as putatively adaptive in this study were similar to those observed in plant (e.g. Ahrens et al., 2019; Hopley & Byrne, 2019; Shryock et al., 2017)

and other landscape genomic studies (e.g. Dudaniec, Yong, Lancaster, Svensson, & Hansson, 2018).

Stronger population structure was observed in the generalist parasite *N. floribunda*, which can make it more difficult to accurately detect signals of selection (Flanagan et al., 2018). Specifically, where population genetic structure correlates with selection gradients, loci under selection may be missed due to false negatives, particularly in F_{ST} outlier methods (e.g. Andrew et al., 2018; Bernatchez, Laporte, Perrier, Sirois, & Bernatchez, 2016). F_{ST} outlier methods such as OutFLANK can only detect loci under selection when they are substantially more differentiated than neutral loci (Whitlock & Lotterhos, 2015). As *N. floribunda* had greater neutral differentiation between populations, adaptive loci would need to be more differentiated than they would for *M. rhaphiophylla* in order to be detected as outliers, which could explain why no outlier loci were detected by OutFLANK for the parasite. Further, OutFLANK had proportionally the lowest overlap of outlier loci for *M. rhaphiophylla* compared to the other three methods, which could be due to different statistical approaches (Hoban et al., 2016; Rellstab et al., 2015). Methods using similar statistical approaches would likely have more outlier loci in common, although agreement between methods with different approaches can still identify true outliers (Benestan et al., 2016).

Our results show that both species displayed strong IBD, which can affect the power of genomic methods to detect signals of selection and potentially exclude true positives (Forester et al., 2018). In an IBD scenario, outlier methods such as PCAdapt have been found to outperform univariate EA methods of LFMM and Bayenv, which lose power when correcting for neutral population structure (Forester et al., 2018; Lotterhos & Whitlock, 2015). However, multivariate EA methods such as constrained RDA have been found to retain

greater statistical power in IBD scenarios, particularly for loci under weak selection that are often missed in PD and univariate EA approaches (Forester et al., 2018; Whitlock & Lotterhos, 2015). As we have done in this study, combining multivariate EA methods with other genomic detection methods (e.g. univariate EA methods) to assess climatic variability can be advantageous in detecting SNP-environment associations when selection gradients are weakly correlated with population structure (Capblancq et al., 2018).

Advances in genome scanning technology have enabled many previously understudied, non-model organisms, such as those examined here, to be assessed in landscape genomics studies (Ahrens et al., 2018; Haasl & Payseur, 2016). Both species in this study have not previously been assessed using genomic tools and, consequently, reference genomes and transcriptomes were not available for either these, or related species. Availability of annotated reference genomes and transcriptomes allows the chromosomal position of putatively adaptive loci to be identified (Bragg et al., 2015) and mapped against known gene function (Tiffin & Ross-Ibarra, 2014), enabling inference of the potential underlying mechanisms of adaptation (Breed et al., 2019; Tiffin & Ross-Ibarra, 2014). However, even in the absence of reference genomes, quantification of adaptive genomic variation can still be obtained (Kawecki & Ebert, 2004; Savolainen et al., 2013) and used, as we have in this study, to compare patterns between co-occurring species; offering valuable insight into local adaptation across the landscape (Bragg et al., 2015).

4.2 Patterns of local climatic adaptation

Climatic variables of temperature and precipitation have previously been identified as important drivers of genetic variation in tree species (Ahrens et al., 2019; Gauli, Vaillancourt, Bailey, Steane, & Potts, 2015; Manel et al., 2012; Poelchau & Hamrick, 2012; Steane, Mclean,

et al., 2017; Supple et al., 2018), and our results further support these findings. Our results show the parasitic plant to have fewer associations with precipitation variables than the autotrophic species. This could be the result of *N. floribunda* acquiring water through haustorial connections to numerous host plants (Hocking & Fineran, 1983), rather than the abiotic environment as for *M. rhaphiophylla*. However, this pattern was reversed for temperature variables. Increased association with temperature could be due to the effect that temperature has on transpiration rates and the need for the parasitic plant to maintain a water potential gradient with the host (Kuijt, 1969).

GDM analysis showed that the magnitude of allelic turnover was greater in the autotrophic species, particularly for measures of isothermality and seasonality. Isothermality has also been recorded as a predictor of genomic distance for *Eucalyptus melliodora* (also in the Myrtaceae family; Supple et al., 2018), and linked to increased allelic variability in other tree species (Bradbury, Smithson, & Krauss, 2013; Hopley & Byrne, 2019). Similarly, increased allelic turnover has also been linked to temperature (Bradbury et al., 2013; Shryock et al., 2017) and precipitation seasonality (Hopley & Byrne, 2019; Shryock et al., 2017), and could be a greater driver of adaptation in autotrophic plants that must develop adaptation mechanisms to persist during resource-limited seasons. In comparison, generalist root parasites such as *N. floribunda* could develop haustoria on additional host plants with higher water potentials or have haustoria die off on plants with lower water potentials in response to seasonal climatic variations (Hocking & Fineran, 1983), thereby reducing the selective pressures posed by seasonality.

In this study, climatic variables identified as significant predictors by GDM were not always aligned with the most significant SNP-environment associations identified by either LFMM or

RDA analysis, and this variation is likely due to assumptions of the underlying models. For example, genomic methods such as LFMM and RDA can only assess linear relationships between genomic data and predictor variables (Capblancq et al., 2018; Caye et al., 2019; Forester et al., 2018), whereas GDM analysis can also reveal non-linear relationships. Nonetheless, even with the capability of GDM to detect both linear and non-linear relationships, multicollinearity among environmental variables may conceal true drivers of local adaptation by associating genetic-environment relationship with a correlated variable that does not itself drive adaptation (Hoban et al., 2016). While we cannot discount the influence of other environmental factors (e.g. solar radiation; Garnier-Géré & Ades, 2001) in these species, the climatic variables that we did include collectively explained over 70% of GDM model deviance, enabling us to quantify and compare patterns of local adaptation to climatic variables.

4.3 Spatial patterns of local adaptation

Determining whether co-occurring species exhibit concordant spatial patterns of local adaptation is crucial for our understanding of evolutionary and environmental drivers of adaptation (Bragg et al., 2015). This study indicates that local adaptation occurs in different geographic regions for sympatric root-parasitic and autotrophic plant species, possibly because of species responding differently to selective pressures within those environments (i.e. temperature or precipitation). Procrustes Analysis has previously been used to compare two genomic datasets by Fitzpatrick and Keller (2014) who demonstrated how GDMs can be applied to genomic data. It has also been used in landscape genomics analyses to access model uncertainty of bootstrapped GDMs (Shryock et al., 2015, 2017). However, we are

aware of no empirical study that has utilised Procrustes Analysis on genomic data in a comparative species study.

As Procrustes residuals only give the absolute difference between multivariate configurations (Peres-Neto & Jackson, 2001), we suspect that there are multiple possible interpretations of this data. For example, regions with moderate-high residuals could indicate areas where local adaptation is occurring due to current selective pressures. However, it could also indicate areas where historical events (e.g. range expansions) have influenced the contemporary population structure and genetic diversity, but are not currently driving local adaptation. Combining these landscape genomic results with information from other genetic studies (e.g. population genetics and/or phylogeography), could assist in further understanding the pattern of genomic variation in natural populations, with applications for landscape conservation and restoration (Breed et al., 2019; Hoffmann et al., 2015).

5. Conclusion

Our study has provided insight into contrasting patterns of local adaptation along climatic gradients between a generalist root parasite and sympatric autotrophic tree species, which have different water and nutrient acquisition strategies. Recently, other landscape genomic studies have also identified contrasting patterns of local climatic adaptation in sympatric plant species with different life history traits (e.g. Hopley & Byrne, 2019; Shryock et al., 2017), but this is the first study to explicitly examine local adaptation to climatic gradients in co-occurring parasitic and autotrophic plants. Our findings that adaptive genomic variation in *N. floribunda* associates with fewer precipitation variables, but more temperature variables, could be the result of reliance of the generalist parasite on multiple host species for water acquisition,

rather than the abiotic environment as for autotroph *M. rhaphiophylla*. Further, we found differing spatial patterns of local adaptation between the parasitic and autotrophic species, suggesting that co-occurring species with varying life histories may respond differently to landscape-scale selective pressures (e.g. temperature and precipitation). Together, these findings provide evidence for differing patterns of local climatic adaptation between a generalist parasitic plant and sympatric autotrophic species, and extending this work to examine other parasitic plants (e.g. host-specific species) would further expand our knowledge of local adaptation across landscapes (Bragg et al., 2015). Further, this study presents information on signals of local adaptation in these two species along climatic gradients that, combined with neutral genetic data, can provide important information for designing landscape conservation and restoration strategies (Bragg et al., 2015; Breed et al., 2019), such as identification of genetically diverse seed sources (Broadhurst et al., 2008; Prober et al., 2015).

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Data Accessibility

Data generated and analysed in this study are available in the DRYAD archives under accession https://doi.org/10.5061/dryad.0p2ngf1xn.

Author Contributions

All authors conceptualised the study design. S.J.W. collected field samples and analysed the data with input from co-authors. S.J.W., P.N., T.P.R. and M.B. interpreted the results. S.J.W. wrote the manuscript. All authors read, edited and approved the final manuscript.

Table and Figures

Table 1. Number of loci under putative selection across seven climatic variables. Environmental association analyses, LFMM and RDA, were run on *Melaleuca rhaphiophylla* and *Nuytsia floribunda* datasets of 5,531 and 6,727 SNPs respectively. The total number of unique SNPs identified by either, or both, approach are shown for each of the seven bioclimatic variables (IT: isothermality; TS: temperature seasonality; MTWQ: mean temperature of the wettest quarter; MTHQ: mean temperature of the warmest quarter; PS, precipitation seasonality; PWQ, precipitation of the wettest quarter; PDQ, precipitation of the driest quarter).

Species	Approach	IT (BIO3)	TS (BIO4)	MTWQ (BIO8)	MTHQ (BIO10)	PS (BIO15)	PWQ (BIO16)	PDQ (BIO17)
Melaleuca rhaphiophylla	LFMM	7	8	2	1	11	2	5
	RDA	1	9	3	34	0	3	1
	Total	8	15	4	35	11	5	6
Nuytsia floribunda	LFMM	2	0	2	1	3	0	3
	RDA	0	6	13	1	1	2	0
	Total	2	6	15	2	4	2	3

Table 2. Model fit and relative importance of predictor variables in generalized dissimilarity modelling. Reference datasets contained 5,209 neutral SNPs for *Melaleuca rhaphiophylla* and 6,670 neutral SNPs for *Nuytsia floribunda.* Candidate SNP datasets contained only SNPs identified as putatively adaptive in prior genomic analyses (98 and 33 SNPs for each species, respectively). The 'best' models contain only significant predictor variables (p<0.05). Relative importance values were obtained from the summations of the three spline coefficient for each significant predictor variable (Geo: geographic distance; IT: isothermality; TS: temperature seasonality; MTWQ: mean temperature of the wettest quarter; MTHQ: mean temperature of the warmest quarter; PS: precipitation seasonality; PWQ: precipitation of the wettest quarter; PDQ: precipitation of the driest quarter).

	Melaleuca rhaphiophylla		***Nuytsia floribunda***	
Best model	**Reference SNPs**	**Candidate SNPs**	**Reference SNPs**	**Candidate SNPs**
Model	IT + PS + PDQ	IT + TS + PS + PDQ	Geo + IT + TS + PS + PDQ	Geo + IT + TS + MTWQ + MTHQ + PWQ
Model deviance	10.55	13.08	4.81	9.83
Percentage	76.69	74.53	75.02	70.86
p-value	0.005	0.000	0.000	0.000
Relative importance				
Geographic distance	-	-	0.73	0.22
IT (BIO3)	1.08	1.56	0.21	0.42
TS (BIO4)	-	0.46	0.60	0.70
MTWQ (BIO8)	-	-	-	0.24
MTHQ (BIO10)	-	-	-	0.29
PS (BIO15)	0.51	0.62	0.18	-
PWQ (BIO16)	-	-	-	0.52
PDQ (BIO17)	0.29	0.60	0.25	-

Figure 1. Sampling sites and principal coordinate analysis (PCoA) of genomic distance between individuals. Maps show the geographical location of the 17 sampling sites for (**a**) *Melaleuca rhaphiophylla* and (**b**) *Nuytsia floribunda* in south-western Australia with species distributions shown in grey (distributional data obtained from Florabase: https://florabase.dpaw.wa.gov.au). Samples for each species are colour-coded by site. The percentage on each PCoA axis indicates how much genomic variation between individuals was explained by the axis.

Figure 2. Venn diagrams of outlier SNPs for (**a**) *Melaleuca rhaphiophylla* and (**b**) *Nuytsia floribunda*. The diagrams show a comparison between the outliers obtained from OutFLANK, PCAdapt, LFMM and RDA methods. For the univariate environmental association method, LFMM, SNPs that were significant for multiple environmental variables were only included once in the Venn diagram.

Figure 3. Partitioning of generalized dissimilarity model deviance by predictor variables. Three sets of predictor variables were used (geographic distance, temperature and precipitation variables) for (**a**) *Melaleuca rhaphiophylla*; and (**b**) *Nuytsia floribunda* adaptive genomic datasets containing only SNPs identified as putatively adaptive in prior genomic analyses.

Figure 4. Generalized dissimilarity model-fitted I-splines showing allelic turnover across predictor variables. Reference datasets contained neutral SNPs and candidate datasets contained SNPs identified as putatively adaptive in prior genomic analyses. Allelic turnover was plotted only if the dataset had a significant relationship with the predictor variable: (**a**) Isothermality (BIO3); (**b**) Temperature seasonality (BIO4); (**c**) Mean temperature of the wettest quarter (BIO8); (**d**) Mean temperature of the warmest quarter (BIO10); (**e**) Precipitation seasonality (BIO15); (**f**) Precipitation of the wettest quarter (BIO16); (**g**) Precipitation of the driest quarter (BIO17); and (**h**) Geographic distance. Height of the curve indicates the total amount of allelic turnover associated with that predictor variable, when holding all other variables constant, and the shape indicates the rate of allelic turnover along the gradient.

Figure 5. Spatial patterns of predicted genomic composition and differences in multivariate configuration (Procrustes residuals). Genomic compositions were derived using fitted generalized dissimilarity models to perform biologically-informed transformations of significant climatic variables for *Melaleuca rhaphiophylla* (**a**) reference and (**b**) candidate datasets; and *Nuytsia floribunda* (**d**) reference and (**e**) candidate datasets. Principal component analysis (PCA) was used to reduce transformed climatic variables into three principal components that were each assigned an RGB colour. The RGB maps do not have a scale bar but similarity of colours within each frame indicate similarity in predicted patterns of genetic composition. Differences in multivariate configuration between reference and candidate datasets were measured by Procrustes Analysis for (**c**) *M. rhaphiophylla* and (**f**) *N. floribunda*. Procrustes residuals were scaled to allow direct comparisons between species.

Figure 1

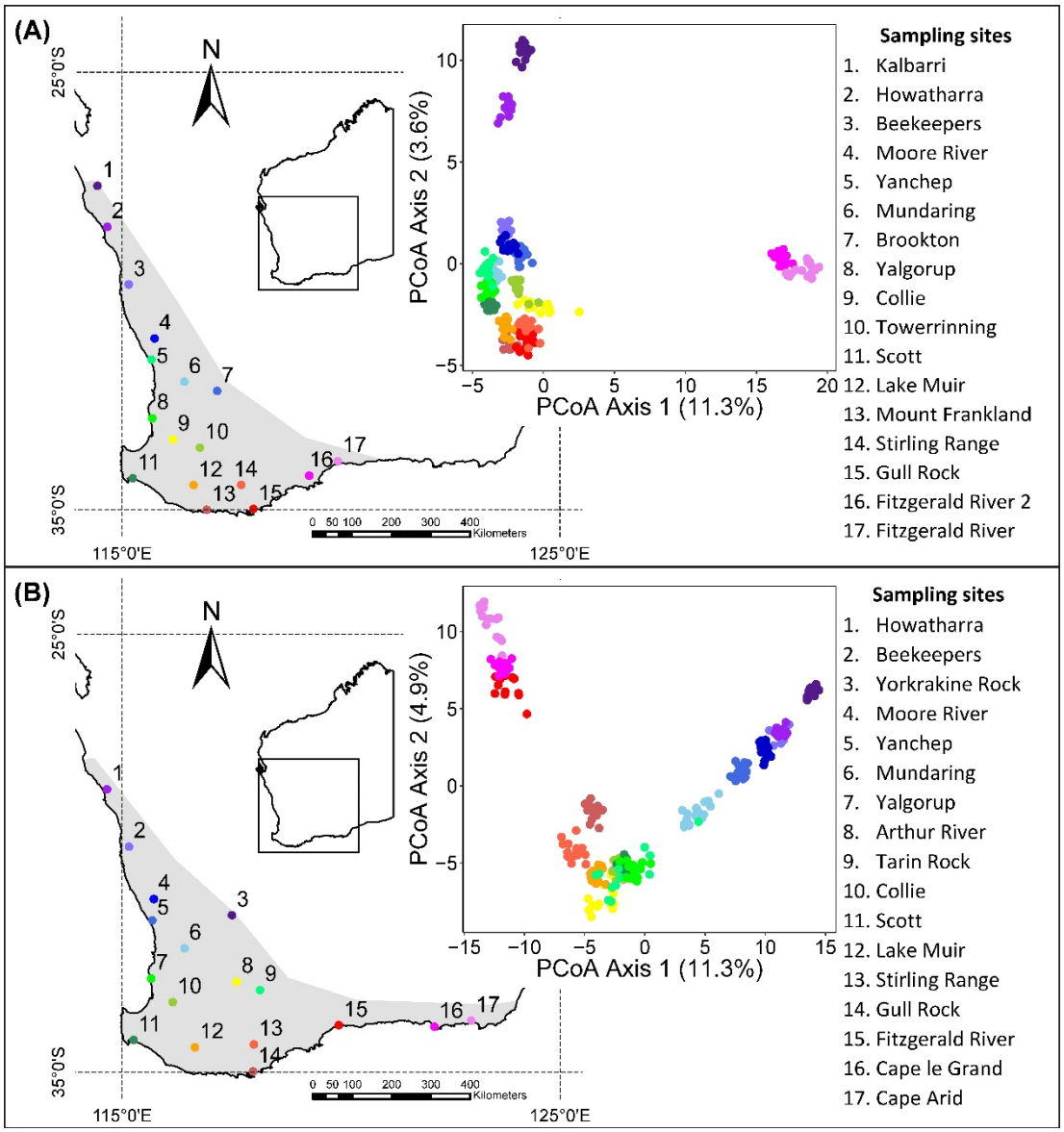
(A)
N
25°0'S
35°0'S
115°0'E
125°0'E
0 50 100 200 300 400
Kilometers
PCoA Axis 2 (3.6%)
PCoA Axis 1 (11.3%)
Sampling sites
1. Kalbarri
2. Howatharra
3. Beekeepers
4. Moore River
5. Yanchep
6. Mundaring
7. Brookton
8. Yalgorup
9. Collie
10. Towerrinning
11. Scott
12. Lake Muir
13. Mount Frankland
14. Stirling Range
15. Gull Rock
16. Fitzgerald River 2
17. Fitzgerald River
(B)
N
25°0'S
35°0'S
115°0'E
125°0'E
0 50 100 200 300 400
Kilometers
PCoA Axis 2 (4.9%)
PCoA Axis 1 (11.3%)
Sampling sites
1. Howatharra
2. Beekeepers
3. Yorkrakine Rock
4. Moore River
5. Yanchep
6. Mundaring
7. Yalgorup
8. Arthur River
9. Tarin Rock
10. Collie
11. Scott
12. Lake Muir
13. Stirling Range
14. Gull Rock
15. Fitzgerald River
16. Cape le Grand
17. Cape Arid

Figure 2

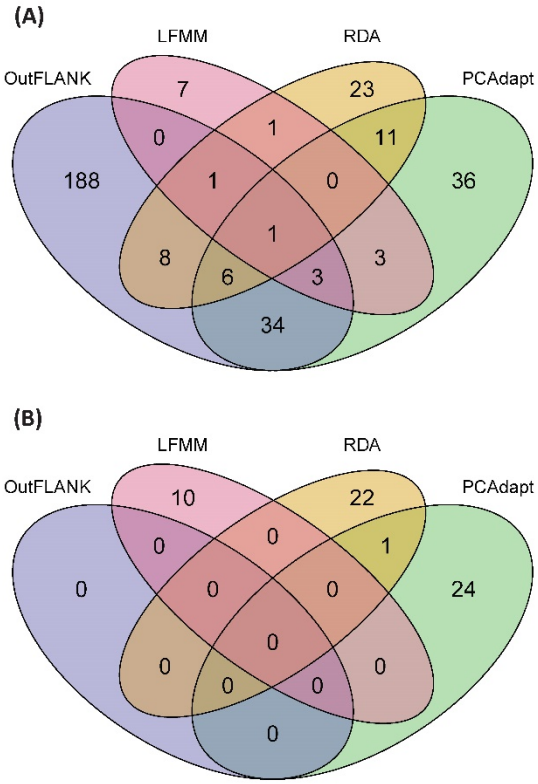
(A)
LFMM
RDA
OutFLANK
PCAdapt
7
23
0
1
11
188
1
0
36
1
8
3
6
3
34
(B)
LFMM
RDA
OutFLANK
PCAdapt
10
22
0
0
1
0
0
0
24
0
0
0
0
0
0
0

Figure 3

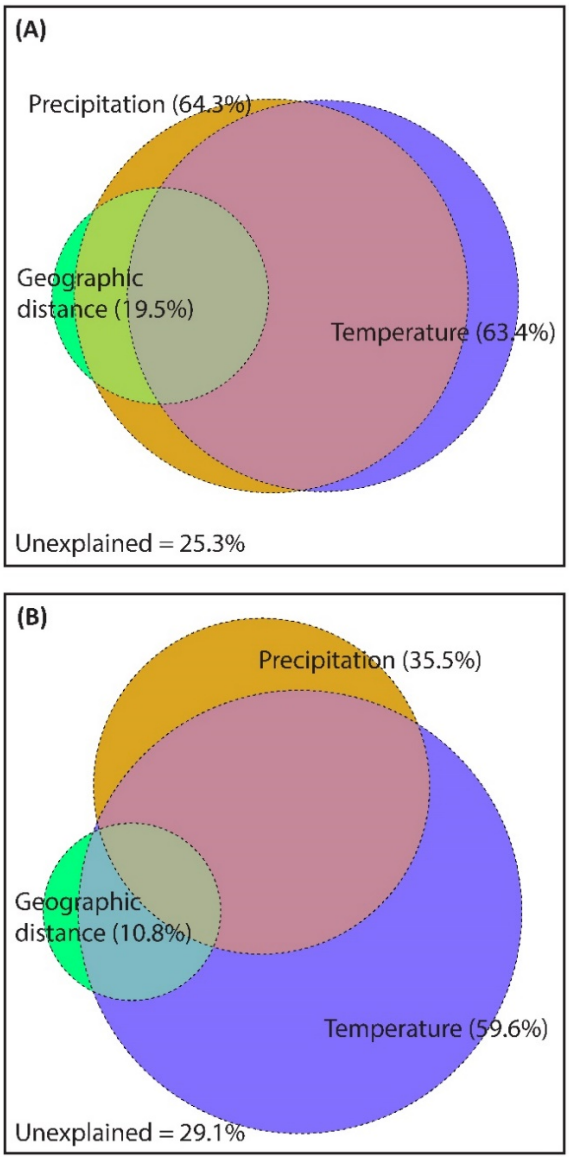
(A)
Precipitation (64.3%)
Geographic distance (19.5%)
Temperature (63.4%)
Unexplained = 25.3%
(B)
Precipitation (35.5%)
Geographic distance (10.8%)
Temperature (59.6%)
Unexplained = 29.1%

Figure 4

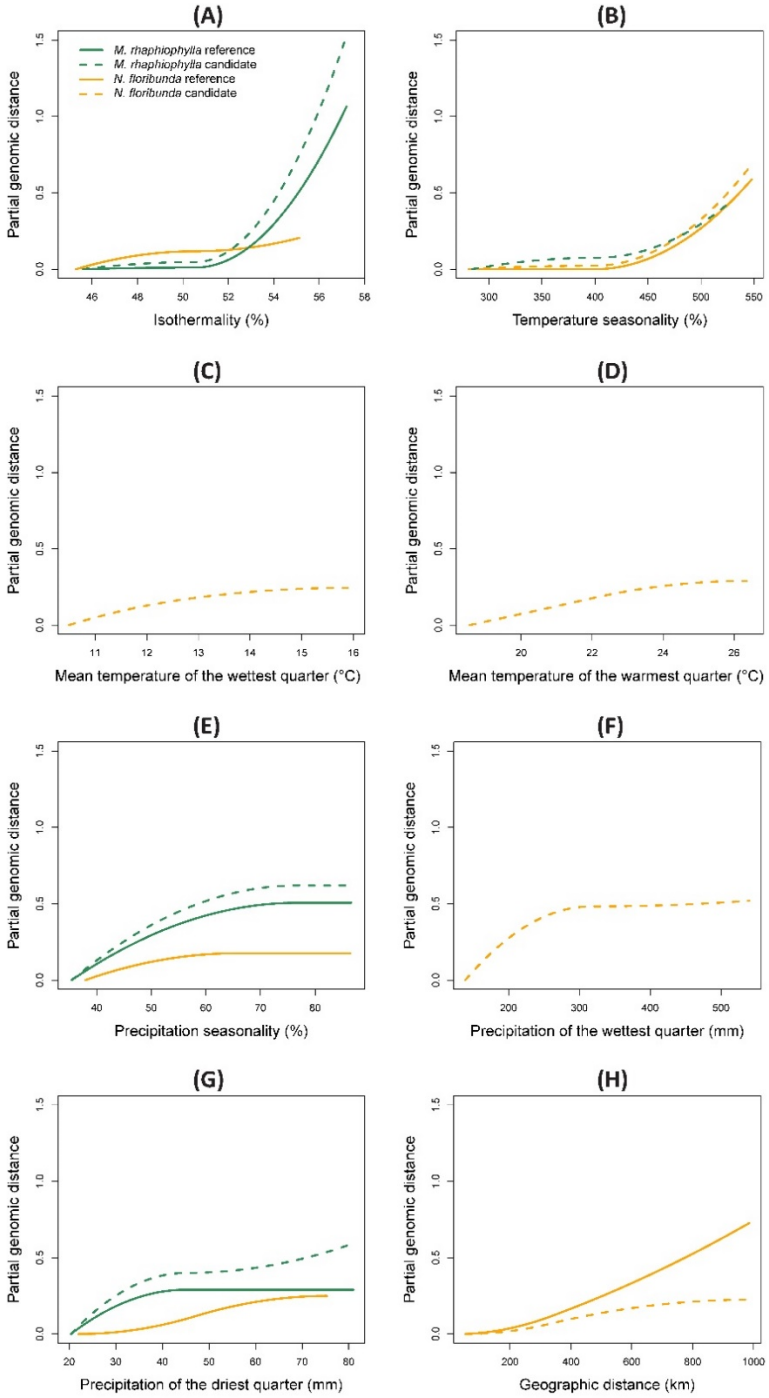
(A)
M. rhaphiophylla reference
M. rhaphiophylla candidate
N. floribunda reference
N. floribunda candidate
Partial genomic distance
Isothermality (%)
(B)
Partial genomic distance
Temperature seasonality (%)
(C)
Partial genomic distance
Mean temperature of the wettest quarter (°C)
(D)
Partial genomic distance
Mean temperature of the warmest quarter (°C)
(E)
Partial genomic distance
Precipitation seasonality (%)
(F)
Partial genomic distance
Precipitation of the wettest quarter (mm)
(G)
Partial genomic distance
Precipitation of the driest quarter (mm)
(H)
Partial genomic distance
Geographic distance (km)

Figure 5